Assessing the population biology of Black Jewfish (*Protonibea diacanthus*) in Queensland

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Executive Summary

Researchers from Queensland's Department of Agriculture and Fisheries evaluated the population biology and stock structure of the Black Jewfish (*Protonibea diacanthus)* in Queensland waters. The project was focused around high catch areas in central Queensland which account for the majority of harvest from the Queensland East Coast fishery. Whole specimens were collected during 2020 and 2021, through a complimentary approach of sampling commercial fishery catches during the open fishing season (January and February), and through representative fishery independent sampling (March to December). The collection of additional samples for genetics was undertaken across the species' distribution in Queensland and part of the Northern Territory, and was heavily supported by assistance from the commercial, recreational, charter and Indigenous fishing communities. The research was able to characterise key aspects of the species' population biology and population structure which have been used to directly inform the stock assessment and fishery management in Queensland waters.

Aims and objectives

Throughout Australian waters *P. diacanthus* are considered of high value and importance to recreational, traditional Indigenous, charter and commercial fisheries. The commercial fishery for *P. diacanthus,* off the East Coast of Queensland has recently experienced increases in targeted commercial fishing effort, with harvest levels climbing from the historical level of ~20 tonnes per year (prior to 2017), to over ~130 t per year in 2019. The increased harvest of *P. diacanthus* was driven by high demand and high value for their swim bladders within the Chinese wellness market. This species also has a long history of harvest by Indigenous, recreational and charter fishers. The overlap in fishing interests between these sectors potential for cross-sector conflict to occur due to increasing commercial harvest. Due to the potential risk posed by increasing harvest levels, new management measures were implemented in 2019 which resulted in the fishery being effectively closed for around 10 months of the year. The current study evaluated key aspects of the species' population biology, as well as the stock's structure across its distribution in North-eastern Australia to inform development of a stock assessment and inform management arrangements within the Queensland fishery. The specific objectives of the project were to:

1) Determine the stock structure and connectivity of Black Jewfish throughout Queensland.
2) Assess the age structure, fecundity, and size-at-maturity for Black Jewfish populations on the East Coast of Queensland.

Methodology

Genetic samples were collected throughout the Queensland distribution of *P. diacanthus,* extending from Gladstone in the southeast, through to the Northern Territory border in the Gulf of Carpentaria. The sample collection was designed to allow for comparison among management regions within Queensland waters (Appendix 7), as well as across the Queensland and Northern Territory jurisdictional boundary. Genome-wide sequencing of the samples was undertaken to characterise and identify single nucleotide polymorphisms (SNPs), which were analysed to resolve the genetic population structure.

Monthly collections of whole specimens were undertaken during 2020 and 2021, which comprised both commercial catch sampling and fishery independent sampling to ensure that temporal patterns in age, growth and reproduction could be evaluated. To ensure that the samples were representative of the

fishery, biological samples were collected across two management regions of the fishery in Central Queensland which together account for over 95% of the total harvest on the Queensland East Coast (Figure 8).

Key biological characteristics related to age, growth and reproduction were determined from the collected samples. Age was estimated using sectioned sagittal otoliths. Where data was sufficient, length and age frequency data were used to describe regional and sex-specific population demography. Length and maturity information were used to estimate the size at 50% and at full maturity. Growth was estimated using the Von Bertalanffy growth function fit to length at age data. Gonads were staged macroscopically and validated using histology to determine reproductive condition and maturity, as well as seasonality of spawning by examining monthly trends in the gonadosomatic index (GSI). Batch fecundity was estimated for a subset of reproductively active ovaries from a range of different size classes.

Key findings

A total of 330 samples from 14 locations were submitted for genomic sequencing, resulting in the identification of 33,000 single nucleotide polymorphisms (SNPs) in total; 11,446 SNPs were retained after filtering to inform population structure analyses. The outputs of analyses revealed the presence of genetic population structure for *P. diacanthus* across north-eastern Australia. Two primary clusters were resolved and consisted of (1) samples from the Queensland East Coast (Townsville to Gladstone); and (2) samples from the Cape York Peninsula and Gulf of Carpentaria, including both Northern Territory and Queensland waters. The only unresolved location was the grouping of Lockhart River samples which appeared to form a third independent population, but further work is needed to confirm their connectivity with the other populations.

The evaluation of Gonadosomatic Index (GSI) values and histological staging of ovaries indicated a single major spawning period from October to February, which coincided with the peak of the wet season. Spawning periodicity was not found to vary latitudinally along the East Coast but was found to differ from other regions in northern Australia including Cape York Peninsula (Queensland) and the Northern Territory.

Clearly identifiable annuli were observed in sectioned sagittal otoliths, with edge type analysis indicating that opaque zones were generally laid down during the austral winter-spring period (May to October). Growth rates were in the range observed by other *P. diacanthus* studies, but the East Coast population was found to have a higher maximum age of fish (up to 15 years old) and a greater proportion of older fish than has been reported elsewhere for the species.

Size at 50% maturity (L50) was found to be 83 cm and size at full maturity (L100) was 105 cm, both of which are greater than the current minimum legal-size limits in Queensland of 75cm. The present study also determined no significant differences in maturity or growth rates between sexes and provided robust estimates of fecundity for *P. diacanthus.*

Implications for relevant stakeholders

- The presence of a single population in the Gulf of Carpentaria, which spans multiple fisheries, fishing sectors and jurisdictions (Northern Territory and Queensland) means that considerable cooperation will be required between managing agencies and stakeholder groups to effectively manage this stock.

- The age composition and observation of strong recruitment in the present study suggest that periods of increased fishing pressure on the East Coast were unlikely to have significantly impacted the health of the East Coast stock. However, the strong age-structure observed in the East Coast population raises some concerns around age-truncation and the sustainability of other *P. diacanthus* stocks throughout Australian waters such as those in the Northern Territory.

- The observed timing of spawning provides evidence to support implementation of a seasonal closure for the fishery, to shift peak harvests outside of the peak spawning period.

- The identification of a single population and determination of key biological parameters spanning the two main East Coast Inshore Fishery management regions provides for a clearly definable stock. Up-to-date biological inputs for this population were directly integrated into developing the first *P. diacanthus* stock assessment for the East Coast of Queensland, the outputs of which have been used to implement management change in the fishery. The full stock assessment report can be found at: https://era.daf.qld.gov.au/id/eprint/8870/

Recommendations

- We recommend ongoing biological monitoring of the East Coast *P. diacanthus* population through the collection of age and length information. Routine biological monitoring is particularly important given the impacts of fishing on the age structure of *P. diacanthus* populations elsewhere in the world, as well as the aggregating behaviour of the species which suggests that commercial catch rates are likely to be affected by hyperstability.

- We recommend that peak harvest levels should be shifted from the *P. diacanthus* spawning period. Seasonal spawning closures should be sufficiently long (i.e. Nov to Feb) to encompass interannual variability in peak spawning activity.

- Further work is needed to understand the status of the Gulf of Carpentaria population. Monitoring and assessment of *P. diacanthus* from the Gulf of Carpentaria should be jointly supported by Queensland and Northern Territory fisheries agencies and use standardised monitoring and data collection techniques.

- Additional information on the fecundity of large specimens (> 130cm) as well as movement, connectivity, identifying essential habitats for all stages of the life cycle, and post-release survival of *P. diacanthus* would be valuable for improving the stock assessment and informing best management practices for the fishery.

- Improving catch data collection for the Indigenous, charter and recreational fishing sectors is recommended, given the aggregatory behaviour of this species and the potential for localised depletion.

Keywords

Sciaenidae, croaker, black spotted croaker, jewfish, fecundity, spawning strategy, reproduction, stock structure, genetic populations, age, growth, otolith

Introduction

The Family Sciaenidae includes a diverse range of species which are commonly known as ‘Croakers’ or ‘Drums’ due to their ability to vocalise (Ramcharitar et al. 2006). The Black Jewfish (*Protonibea diacanthus*) is one of the largest Sciaenid species and occurs throughout the Indo-west Pacific region. The species has experienced population declines throughout its range, with localised depletion of some populations being associated with large increases in targeted fishing practices (Phelan 2002; Sadovy and Cheung 2003). In most cases, intensification of fishing pressure on *P. diacanthus* populations has been driven by increasing prices and demand for their swim bladder which are dried and sold as ‘maw’ for the Chinese wellness market. As a result, the global status of the species was recently listed as Near Threatened by the International Union for the Conservation of Nature (IUCN) Red List (Sadovy et al. 2020). Although generally characterised by fast growth and early maturation, *P. diacanthus* are also known to form discrete populations which have been shown to exhibit variation in their reproductive characteristics including size and age at maturity, fecundity, spawning season, spawning frequency and growth rates (Phelan 2008; Taillebois et al. 2017). The observed variability in the population biology among regions and continued increase in demand for their swim bladders highlights the need for characterisation of their biology at a local level.

In Australia, *P. diacanthus* are found in estuaries and coastal waters from approximately the Burnett River in Central Queensland, across northern Australia to Broome in Western Australia. Recent research using a range of methods including genetics, otolith chemistry, and parasitology focused on resolving the population structure of *P. diacanthus* across north-western Australia. The research indicated that *P. diacanthus* can form genetically distinct populations over spatial scales as little as 10s of km and as far as 1000 km (Taillebois et al., 2017). The study identified four populations across 11 locations between Roebuck Bay in Western Australia and the Vanderlin Islands in the Southern Gulf of Carpentaria. The study did not include any sites within Queensland waters and therefore the population structure of the species across North-Eastern Australia remains unresolved.

Protonibea diacanthus form predictable year-round or seasonal aggregations which make them particularly susceptible to targeted fishing practices (Phelan 2002). This aggregating behaviour not only allows for easy targeting, but can also mask population declines through the effect of hyperstability in catch indices (Mitcheson and Erisman 2012; Erisman et al. 2017). One of the clearest examples of this occurred in the Cape York Peninsula Area (Queensland, Australia) where in the early 1990s subsistence fishing of nearshore aggregation sites by traditional owners resulted in localised stock depletion. The signs of depletion in the area were best observed through truncation of age structures, with catches becoming dominated by immature fish under 59 – 69 cm total length (TL) (Phelan et al. 2008). This is in contrast to historical catch records from the region indicating that fish have been observed reaching upwards of 150 cm TL, with the local aggregation previously dominated by mature fish > 90 cm (Phelan et al. 2008). The clear signs of depletion in the size and age composition for *P. diacanthus,* contrasting with consistent fishery catch data due to hyperstability, highlights the importance of characterising and monitoring change in age demographics for this species.

Within Australian waters, spawning of *P. diacanthus* occurs from the austral spring in October to the end of autumn in April in the Northern Territory (NT), and is suspected to occur between April and September in Cape York (Phelan 2008). In north-western India, the spawning season for *P. diacanthus* (or ghol as they are referred to locally) commences at the start of the monsoon period in June, and continues until August (Rao, 1963). Variation in other reproductive characteristics such as size at maturity has also been observed among populations, with estimates of length at 50% maturity varying from 80-84 cm in Cape York (Phelan 2002) and India (Rao 1963), to 89 cm in the Northern Territory (NT) (Phelan 2008), and 98 cm in the Gulf of Carpentaria (McPherson 1997).

No literature on the fecundity of *P. diacanthus* currently exists from Australian waters, and as a result, the most recent stock assessment from the NT recommended a detailed study into the reproductive biology of *P. diacanthus*, with an emphasis on fecundity (Grubert et al., 2013). Several fecundity estimates exist from Northern Hemisphere populations, with estimates from India ranging from 1,743,010 to 6,868,368 eggs (Rao 1963; Kizhakudan and Kizhakudan 2017), and 3,883,840 eggs reported in a single fish sampled in Taiwan (Mok et al. 2009). Given the high variability in current fecundity estimates, there remains a need for greater information on their reproductive biology that is directly applicable to population models of *P. diacanthus* from Australian waters.

Need

In the state of Queensland, Australia, *P. diacanthus* have a long history of harvest by not only commercial fishers, but also Indigenous, charter and recreational fishers. Harvest by Indigenous communities is recognised as being important throughout Cape York and along the eastern side of the Gulf of Carpentaria, but the exact level of harvest is not well quantified (Phelan 2002). Similarly, the recreational fishery in Queensland has a history of targeting *P. diacanthus* throughout central and north Queensland waters, as well as in Cape York and along the eastern side of the Gulf of Carpentaria. Although *P. diacanthus* were targeted by recreational fishers initially for food, there has been a more recently shift in their recognition as a prized sportfish. The multi-sector value of this species means there is potential for cross-sector conflict to occur due to recent increases in commercial harvest.

Along the East Coast of Australia *P. diacanthus* was historically considered a commercial byproduct species within the inshore net fishery, with very little targeted fishing effort on the species. From 2017, the fishery saw large increases in catch and within two years total harvest in the fishery increased from 20 tonnes to over 130 t (Figure 1). The increase was stimulated by growing demand for *P. diacanthus* swim bladders which is sold as 'maw' within the Chinese wellness market. This increase in harvest raised concerns over the sustainability of the stock and as a result new management measures were implemented in 2019, which effectively reduced the harvest of all fishing sectors back to historical catch levels (Jacobsen et al., 2019). The absence of biological information to inform an assessment of the population status meant that the conservative management measures were set to remain until updated biological information became available (Saunders et al. 2020).

Basic biological and genetic information including stock structure, fecundity, age, growth, and maturity were required to enable a robust assessment and to support evidence-based management of this species in Queensland. While biological information had previously been obtained for this species across several regions of Australia, the observed variability in the population biology among these regions and the fine-scale population structure found in Northwestern Australia highlighted the need for population-specific information (Taillebois et al. 2017). Furthermore, given that the distribution of *P. diacanthus* in Queensland extends over 2,500 km, it is likely that multiple genetic stocks exist throughout the state. The current project was designed to evaluate the population structure throughout Northeastern Australia, building on the work by Taillebois et al. (2017), and provide information relevant to intra- and inter- jurisdictional management arrangements. It was also established to characterise the population biology of *P. diacanthus* on the East Coast, to allow for updated assessment and management of the species within Queensland waters.

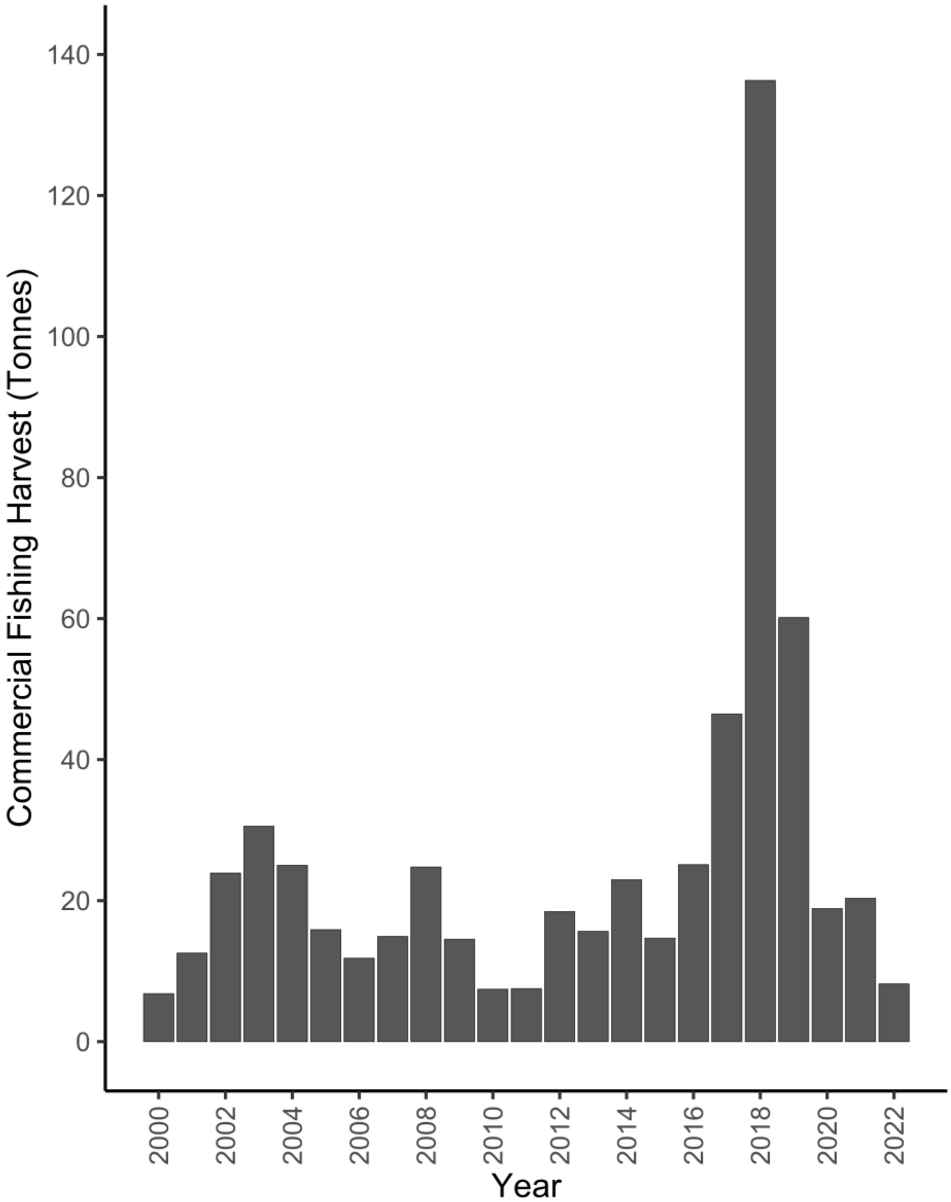

Figure 1. Commercial fishing harvest of *Protonibea diacanthus* from the Queensland east coast inshore fishery showing an increase in catches from 2016 to 2018, followed by a sharp reduction in catch after management intervention in 2019.

Objectives

The objectives of these study were to:

1) Determine the stock structure and connectivity of Black Jewfish throughout Queensland.

2) Assess the age structure, fecundity, and size-at-maturity for Black Jewfish populations on the East Coast of Queensland.

Objective 1: Determine the stock structure and connectivity of Black Jewfish throughout Queensland.

Method

Sample collection and study sites

A total of 329 samples of *P. diacanthus* were collected from 14 locations for genetic sequencing (Figure 2). Muscle tissue samples or fin clips (n = 293) were obtained from *P. diacanthus* from Queensland (QLD) waters between July 2020 and July 2022 through targeted sampling assisted by commercial, indigenous, charter and recreational fishers. These samples were complimented by collections (n = 36) from the Northern Territory (NT), including samples collected by Taillebois et al. (2017) from the Pellow islands in the Central Southern Gulf of Carpentaria, and samples collected during a 2021 trawl survey in the Central Gulf of Carpentaria (Knuckey et al. 2021). Fish total length, weight and sex were recorded for all landed individuals. Genetic samples were stored in 90% ethanol at -20°C until DNA extraction. Tissue samples from dedicated research sampling were obtained under General Fisheries Permit #208082 (QLD), Animal Ethics permit #CA2020/04/1368 and Great Barrier Reef Marine Park Permit #G20/44421.1.

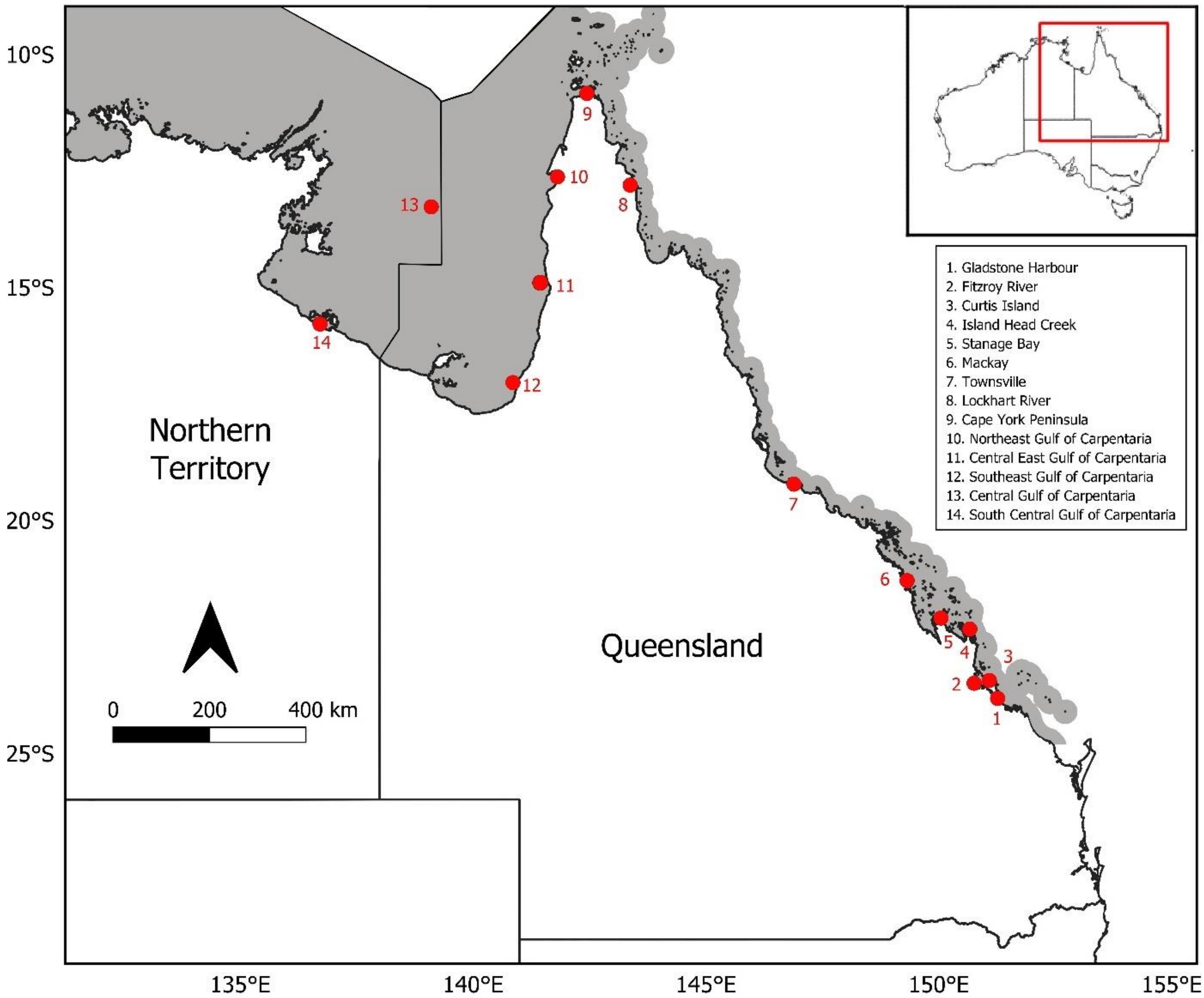

Figure 2. Genetic sample collection locations throughout Queensland and Northern Territory waters. Numbers correspond to the following locations (1) Gladstone Harbour (GH, n = 35), (2) Fitzroy River (FR, n = 8), (3) Curtis Island (CI, n = 55), (4) Island Head Creek (IHC, n = 28), (5) Stanage Bay (SBAY, n = 6), (6) Mackay (MKY, n = 55), (7) Townsville (TVL, n = 11), (8) Lockhart River (LHR, n=22), (9) Cape York Peninsula (CYP, n = 11), (10) North-eastern Gulf of Carpentaria (NEGOC, n = 24), (11) Central-eastern Gulf of Carpentaria (CEG, n=6), (12) South-eastern Gulf of Carpentaria (SEGOC, n = 32), (13) Central Southern Gulf of Carpentaria (CSGOC, n = 26), and 1(4) Central Gulf of Carpentaria (CGOC, n = 10) collected in 2022. Grey shading indicates the theorised distribution of *Protonibea diacanthus*. See appendix 7 for a full map of east coast inshore management regions.

Sample extraction, sequencing and filtering

DNA extraction and quality assurance checking was performed by Diversity Arrays Technology (DArT). SNP loci were discovered and genotyped following the DArTseq™ protocol (Kilian et al., 2012) for all individuals from across the 14 collection locations. Quality control, read assembly and SNP calling were undertaken using proprietary DArTseq™ analytical pipelines (DArTsoft14) described in detail by Georges et al. (2018).

Genetic loci returned from DArT were further filtered using R (R-Core-Team, 2018) package '*DartR*' (Gruber et al. 2018). The filters were applied to remove samples with: greater than or equal to 35% missing data, loci with reproducibility less than 95%, loci where the minor allele frequency (MAF) were less than 0.01 (< 1%), missing data were greater than 20%, coverage outside of five and 80, and secondary SNPs on the same locus to allow for short distance linkage-disequilibrium. To ensure that sex ratios among collection locations did not influence population structure analysis, we also tested for the presence of sex-linked markers and identified the presence of putative loci under selection using the R package '*Outflank*'.

Population genetic analysis

FST values were calculated using the R package '*DartR'* to understand the level of genetic differentiation among collection locations (Keenan et al. 2013) with significant pairwise comparisons tested by 20,000 permutations via bootstrapping (R Core Team 2014). We explored the SNP dataset across all collection locations using a sparse non-negative matrix factorization (SNMF) approach (Frichot and François 2015), which is comparable to STRUCTURE (Pritchard et al. 2000), but produces a least-squares estimate of ancestral (source) populations given *K* ancestral populations. This method estimates the number of ancestral populations (K) that were proposed to have existed at some point in the past and assumes that modern individuals were produced by recent mixing and interbreeding of these ancestral populations. We estimated ancestry coefficients for K = 1 to 10, using 10 repetitions per K, and default parameters. To explore the appropriate number of ancestral populations, we plotted the minimum entropy criterion against K, searching for the inflection point where additional cross-entropy loss was minimal as suggested in Forester et al. (2018). The '*TESS3R'* package (Caye et al. 2016) was then used to develop admixture maps by adding UTM coordinates for each individual, and we ran 20 replicates of the admixture model for each value of the maximal number of clusters (K). The analysis was processed for K-values from 2 to 5, although only the results for K = 2 and K= 3 are presented, as only these clustering results warrant a biological interpretation.

Genetic population analyses were performed to determine the relationships among individuals from each region through principal components analysis (PCA) with the package ADEGENET 2.1.1 in R (Jombart and Ahmed 2011). Group membership for PCA was defined by region and Bayesian Information Criterion (BIC) scores were used to assess the optimal number of genetic clusters across a range of K values. Models with the lowest BIC scores were identified as representing the optimal number of K for use in PCA.

Results

Sequencing and quality control

A total of 37,485 SNPs were scored and filtered, resulting in 26,039 SNPs which were deemed not suitable for downstream analyses as features such as call rate, monomorphism, coverage, Minor Allele Frequency (MAF), Linkage Disequilibrium (LD) and heterozygosity, diverged outside of suitability thresholds. From the filtering steps applied, the filters on MAF (7,291 SNPs excluded), monomorphic loci (8,069 SNPs excluded), and call rate (3,522 SNPs excluded) had the strongest influence when detecting non-conforming markers. An additional 23 loci were identified and removed due to their identification as putatively under selection. The final dataset after filtering included 11,446 neutral SNPs which were used for further evaluation of genetic population structure. The quality assurance and filtering of sequence data also resulted in the removal of 34 specimens which either were unable to be genotyped due to low quality genomic DNA, large amounts of missing data or containing irregular minor allele frequencies, resulting in a dataset of 295 individuals progressed for analysis.

Genetic population structure

Bayesian Information Criterion values (BICs) generated as part of the DAPC analysis indicated the greatest confidence around the K = 3 cluster scenario. The DAPC and PCA outputs indicated the presence of three clusters which aligned with the geographic position of collection locations (Figures 3 and 4). The first cluster contained all the samples from East Coast collection locations between Townsville and Gladstone, the second cluster contained samples from Gulf of Carpentaria locations as well as Cape York Peninsula, and the third cluster contained three samples collected from the Lockhart River. Except for samples from the Lockhart River, which were split between the second and third clusters, the assignment of individuals conforms with the geographic locality of samples.

Ancestry analysis using SNMF allowed the exploration of alternate drivers of genetic population structure of *P. diacanthus*. Using a genetic clustering approach and the SNP dataset, the inflection point of the minimum cross entropy plot was K = 3 (Figure 5). We explored assignment plots for $1 \leq K \leq 6$, and presented assignment plots showing the assignment of individual *P. diacanthus* to 2 and 3 hypothesized ancestral populations as these were the most informative (Figure 6). The K = 2 assignment plot showed strong differentiation in the ancestral entity of the Gulf of Carpentaria and East Coast collection locations. The same overall trend was found for the K = 3 assignment plots, however, there was some subtle variability among individuals from particular collection locations. Although the assignment of individuals from the Central Gulf of Carpentaria and the Lockhart River were dominated by the same primary ancestral entity as observed in other Gulf of Carpentaria locations, they were found to have different proportions of assignment. Similar patterns were found within East Coast collection locations, where the ancestral entity of samples was dominated by the same primary, but the overall proportion of ancestral entity differed. No clear assignment of individuals or locations to additional groupings were identified in plots for K = 4 or greater. For the regional dataset, the TESS3 admixture map for K = 2 and K=3 both showed similar outputs, with the formation of two clusters which intersected spatially between Lockhart River and Townsville in North Queensland (Figure 7).

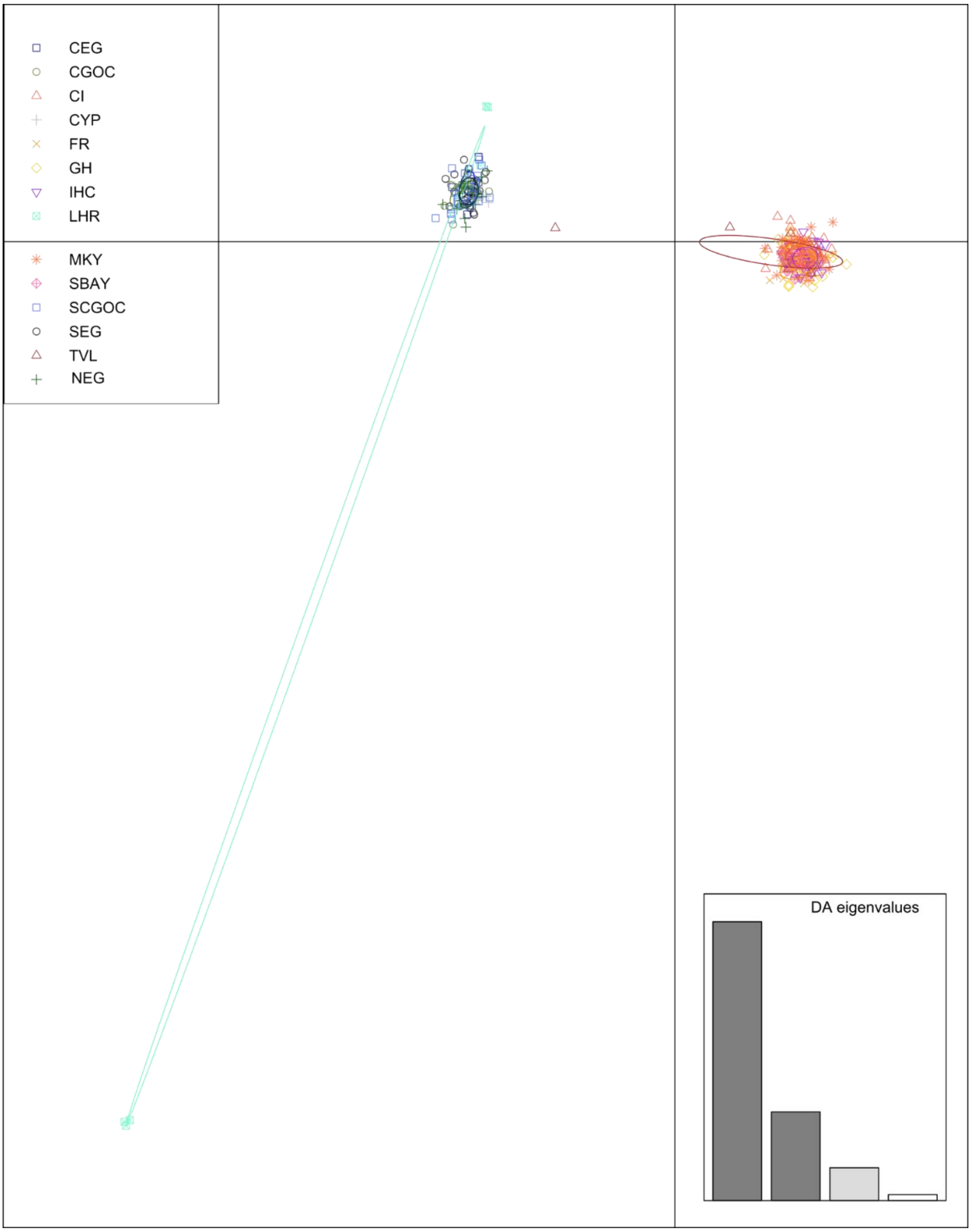

Figure 3. Discriminant Analysis of Principal Components (DAPC) between *Protonibea diacanthus* samples. Acronyms for collection locations are as follows: Gladstone Harbour (GH), Fitzroy River (FR), Curtis Island (CI), Island Head Creek (IHC), Stanage Bay (SBAY), Mackay (MKY), Townsville (TVL), Lockhart River (LHR), Cape York Peninsula (CYP), North-eastern Gulf of Carpentaria (NEGOC), Central-eastern Gulf of Carpentaria (CEG), 12) South-eastern Gulf of Carpentaria (SEGOC), Central Southern Gulf of Carpentaria (CSGOC), and 14) Central Gulf of Carpentaria (CGOC)

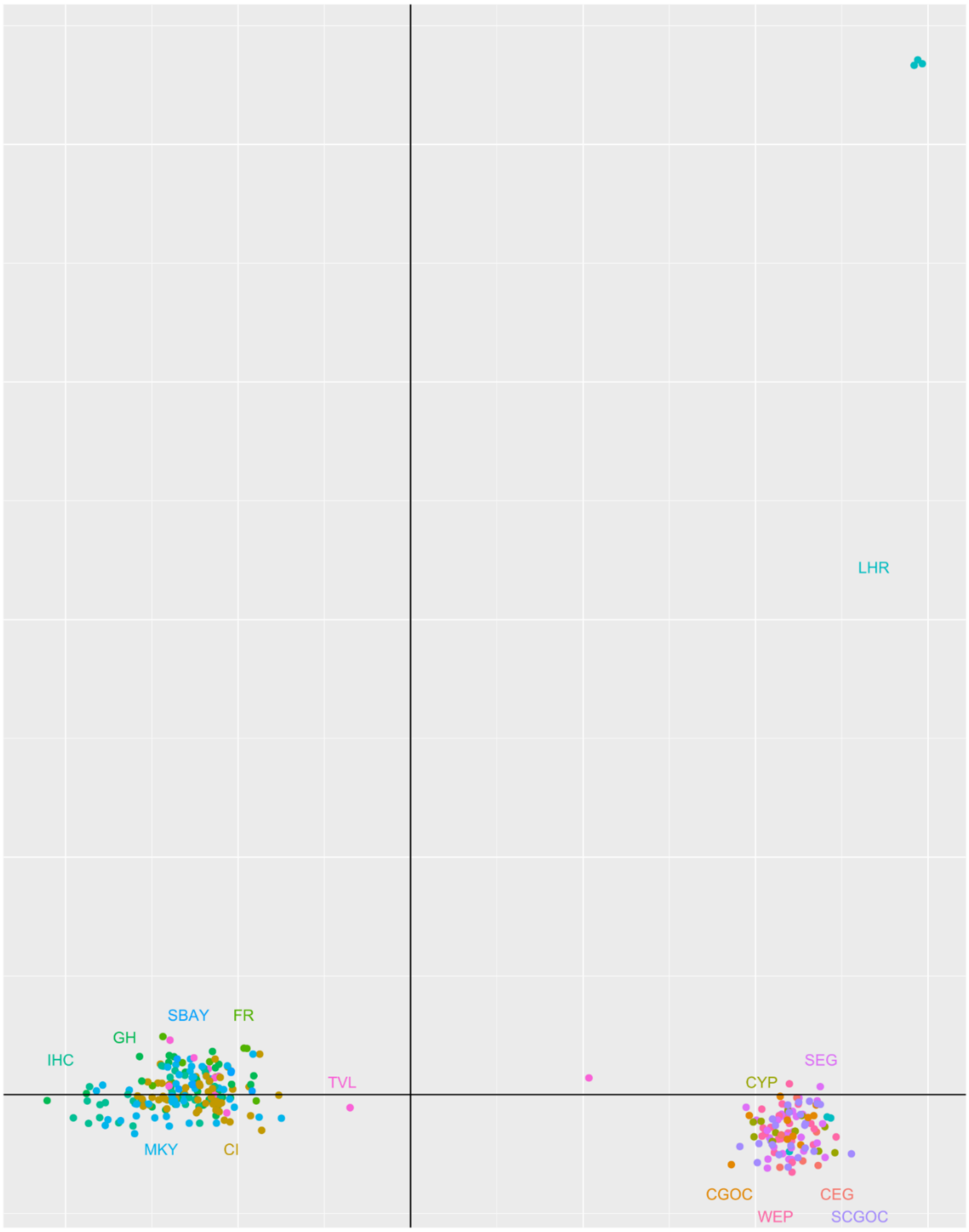

Figure 4. Principal Components Analysis (PCA) between *Protonibea diacanthus* samples. Acronyms for collection locations are as follows: Gladstone Harbour (GH), Fitzroy River (FR), Curtis Island (CI), Island Head Creek (IHC), Stanage Bay (SBAY), Mackay (MKY), Townsville (TVL), Lockhart River (LHR), Cape York Peninsula (CYP), North-eastern Gulf of Carpentaria (NEGOC), Central-eastern Gulf of Carpentaria (CEG), 12) South-eastern Gulf of Carpentaria (SEGOC), Central Southern Gulf of Carpentaria (CSGOC), and 14) Central Gulf of Carpentaria (CGOC)

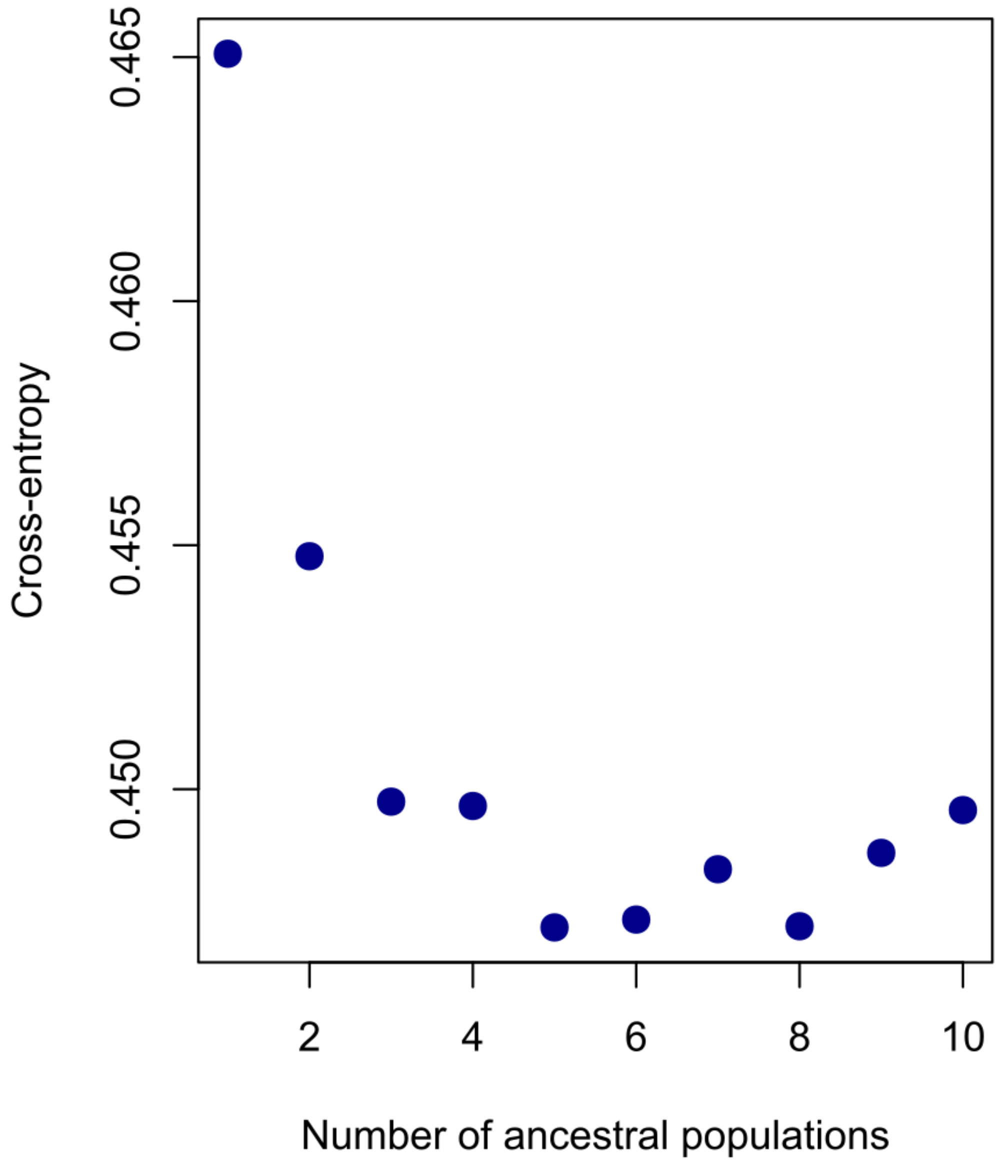

Figure 5. Cross-entropy plot to determine the most likely number of populations (K)

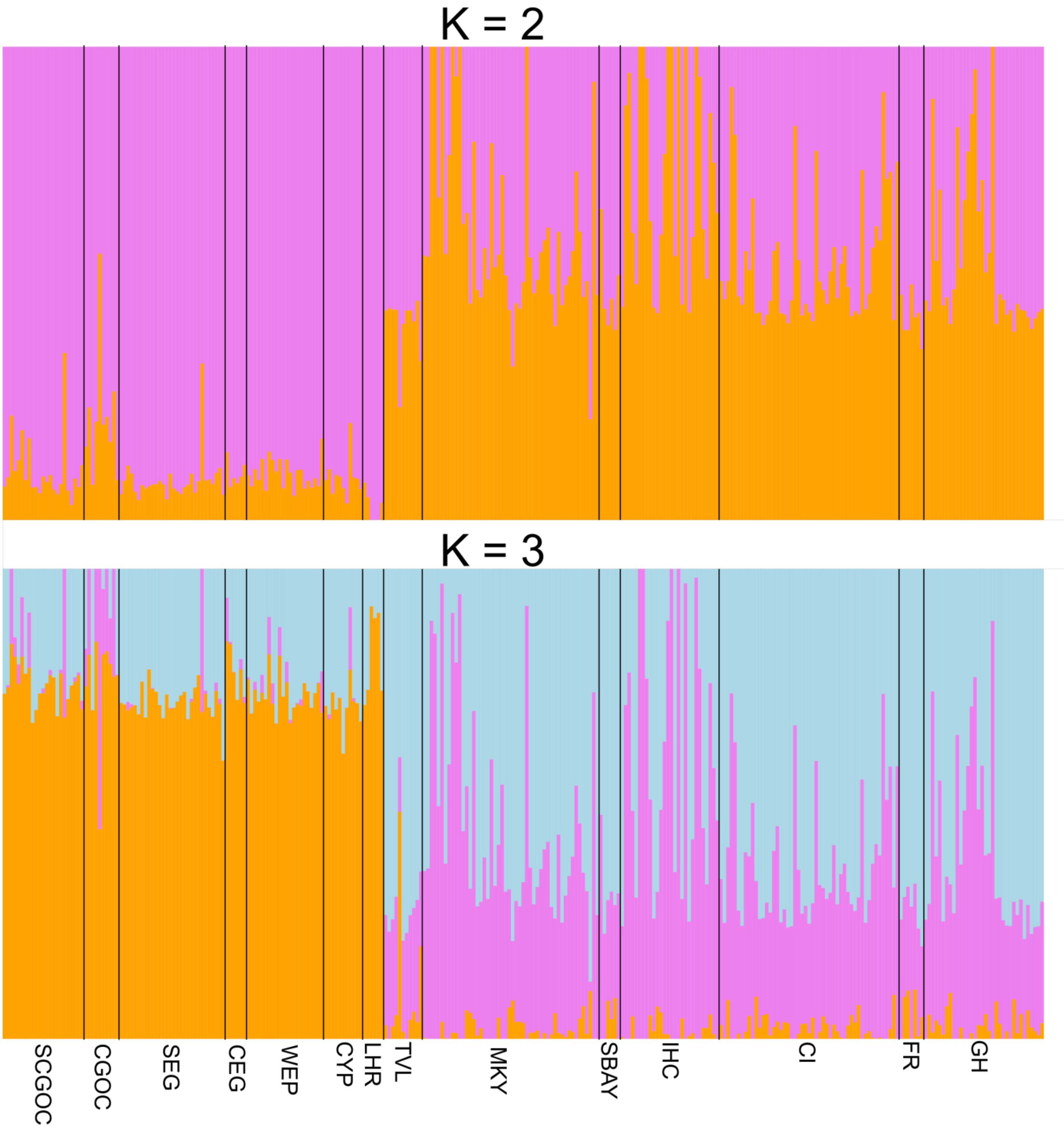

Figure 6. SNMF analysis of *Protonibea diacanthus* sampled from collection locations across northern Australia showing individual ancestry coefficients to the ancestral genetic cluster (K) for K = 2 and K = 3 using SNP loci. Acronyms for collection locations are as follows: Gladstone Harbour (GH), Fitzroy River (FR), Curtis Island (CI), Island Head Creek (IHC), Stanage Bay (SBAY), Mackay (MKY), Townsville (TVL), Lockhart River (LHR), Cape York Peninsula (CYP), North-eastern Gulf of Carpentaria (NEGOC), Central-eastern Gulf of Carpentaria (CEG), 12) South-eastern Gulf of

Carpentaria (SEGOC), Central Southern Gulf of Carpentaria (CSGOC), and 14) Central Gulf of Carpentaria (CGOC).

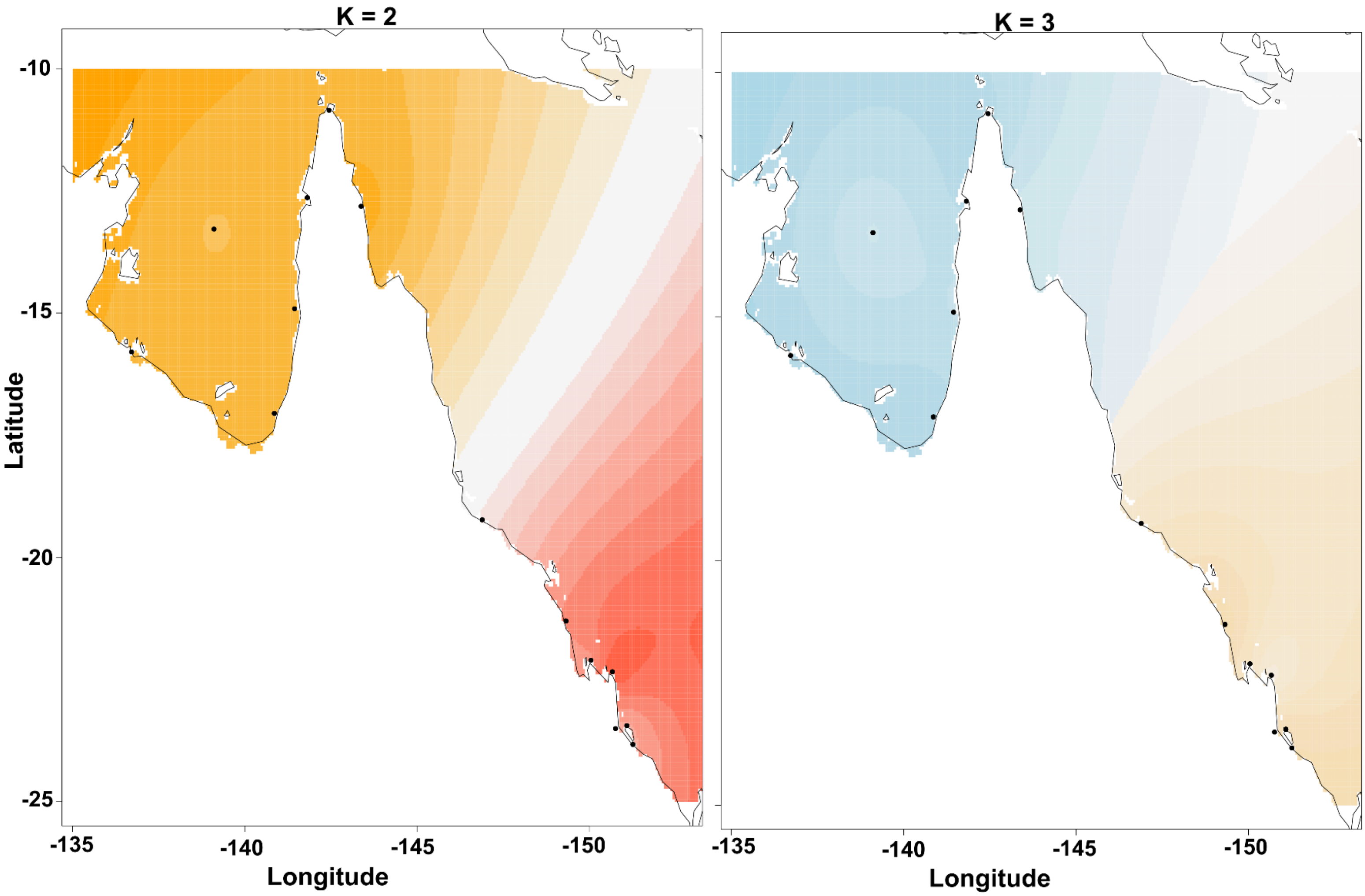

Figure 7. TESS3 admixture maps showing geographic clustering based on the assignment signature of sampling locations from throughout northern Australia for K=2 and K=3 scenarios. Black spots correspond to latitude/longitude of sampling locations.

Genetic differentiation and fixation indices

All pairwise comparisons between collection locations, except one, were significantly different at α = 0.05, and all but five comparisons were significantly different at the α = 0.001 level. Evaluation of F_{ST} strength showed that pairwise comparison fell into three different groupings: (1) high pairwise difference value $F_{ST} > 0.05$; (2) moderate pairwise difference value F_{ST} 0.05-0.02); or (3) very low pairwise difference value ($F_{ST} < 0.02$).

Overall, the F_{ST} estimates demonstrated very comparable trends to those observed in the clustering analyses. The pairwise F_{ST} estimate among all locations from the Gulf of Carpentaria and Cape York Peninsula showed low pairwise differences ($F_{ST} < 0.02$). Similarly, low pairwise F_{ST} estimates were also observed among all locations from the East Coast South of Townsville ($F_{ST} < 0.02$). Pairwise comparisons with moderate F_{ST}'s occurred between the locations from the Gulf of Carpentaria or Cape

York Peninsula with all locations on East Coast South of Townsville (0.05 < F_{ST} < 0.02). The pairwise comparisons with high pairwise difference values (F_{ST} > 0.05) were found between Lockhart River samples and those from all other locations, as well as between samples from the Central Eastern Gulf of Carpentaria and all East Coast locations (Table 1).

Table 1. *Protonibea diacanthus* pairwise F_{ST} values are below the diagonal and associated p-values are above the diagonal. F_{ST} values are highlighted based on their level of variance as follows: green = high pairwise difference value (F_{ST} > 0.05); yellow = moderate pairwise difference value (F_{ST} 0.05-0.02); orange = very low pairwise difference value (F_{ST} < 0.02).

	CI	FR	LHR	CYP	GH	MKY	WEP	SEG	CEG	SCGOC	TVL	SBAY	IHC	CGOC
CI		0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
FR	0.0035		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.6136	0.0040	0.0000	0.0000
LHR	0.0977	0.1050		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CYP	0.0393	0.0400	0.0690		0.0000	0.0000	0.0234	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0000
GH	0.0030	0.0040	0.0974	0.0399		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MKY	0.0028	0.0049	0.0956	0.0403	0.0039		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
WEP	0.0371	0.0370	0.0639	0.0015	0.0377	0.0383		0.0084	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SEG	0.0377	0.0379	0.0631	0.0031	0.0388	0.0387	0.0010		0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
CEG	0.0560	0.0586	0.0970	0.0215	0.0573	0.0559	0.0207	0.0203		0.0000	0.0000	0.0000	0.0000	0.0000
SCGOC	0.0384	0.0379	0.0653	0.0025	0.0394	0.0385	0.0018	0.0024	0.0211		0.0000	0.0000	0.0000	0.0000
TVL	0.0033	-0.0003	0.0981	0.0332	0.0040	0.0046	0.0319	0.0324	0.0513	0.0332		0.0483	0.0000	0.0000
SBAY	0.0053	0.0045	0.1101	0.0412	0.0053	0.0068	0.0372	0.0378	0.0617	0.0382	0.0023		0.0000	0.0000
IHC	0.0084	0.0127	0.0997	0.0447	0.0081	0.0067	0.0430	0.0434	0.0616	0.0428	0.0112	0.0151		0.0000
CGOC	0.0395	0.0414	0.0705	0.0050	0.0395	0.0390	0.0036	0.0034	0.0252	0.0042	0.0351	0.0423	0.0421	

Discussion

Our study was able to detect the presence of genetic population structure for *P. diacanthus* across north-eastern Australia. The presence of moderate to high level fixation indices and heterogeneous clustering of geographically distinct locations indicate that genetic connectivity among the Queensland East Coast and the Gulf of Carpentaria (including Cape York Peninsula) is low. The results of this study contribute to our understanding of *P. diacanthus* population structure within Australian waters by building on the work by Taillebois et al. (2017). The outcomes of this work provide a baseline for developing biologically informative management units and informing stock assessments for *P. diacanthus* throughout its Australian range.

East coast population structure

The genetic data supported the presence of a separate population of *P. diacanthus* on the Queensland East Coast spanning from Townsville in the north to Gladstone in the south. This range corresponds directly to the area defined by the East Coast Inshore Management as Regions 3 and 4 which account for ~95% of the commercial harvest within the fishery (QDAF 2021). Connectivity of fish in this region is supported by continuous distribution of *P. diacanthus* throughout coastal waters, as well as preliminary results from acoustic tagging research which has shown movements in excess of 200 km throughout central Queensland waters (Barnett et al. unpublished data). The connectivity of fish which are found in waters north of Townsville and south of the Lockhart River remains unresolved. While *P. diacanthus* are caught in Management Region 2 (Hinchinbrook to Cooktown), catches are characterised as being irregular and appear to reflect fish that are transient through the area. As part of this study, we made numerous attempts to collect samples of *P. diacanthus* between Cardwell and Port Douglas, however, we were unable to collect a sufficient number for inclusion in the analysis. Future attempts using acoustic tagging and/or through analysis of genetic samples of fish from Management Region 2 (Cooktown to Hinchinbrook) would be valuable in understanding their relationship with fish from Management Region 1 and Management region 3. Similarly, the use of additional complimentary methods for evaluating population structure such as otolith chemistry and parasite assemblage analyses could aid in resolving fine scale population connectivity.

Gulf of Carpentaria and Cape York population structure

The genetic outputs also provided important insights into the population structure within the Gulf of Carpentaria and Cape York Peninsula areas, indicating the likelihood of a single distinct population, which spans the QLD and NT border. Previous evaluation of *P. diacanthus* population structure across Northwestern Australia by Taillebois et al. (2017) included only a single location within the Gulf of Carpentaria which was found to be genetically distinct from all other locations, including *P. diacanthus* from the Arafura Sea. Our work expands on this by defining the spatial extent of the Gulf of Carpentaria population to include all QLD and NT waters of the Gulf, extending around the tip of Cape York to the Escape River in the North-Eastern Gulf. Genetic connectivity within this region is likely maintained through the presence of considerable shallow water rocky rubble habitat allowing for ease of movement throughout the Gulf of Carpentaria, along with circular currents which would allow for mixing of larvae throughout the Gulf.

The association of Lockhart River fish to the Gulf of Carpentaria population remains uncertain. While several Lockhart River samples were found to cluster with the Gulf of Carpentaria samples, and overall the Lockhart River appeared to be more closely related to Gulf of Carpentaria than the East Coast population, the results consistently indicated that samples from the Lockhart River held a different genetic signal. Most notably, the pairwise F_{ST}s between Lockhart River and all other locations were found in many cases to be orders of magnitude higher than all other observed FST values. This indicates that the Lockhart River may indeed be a third distinct population within Queensland waters. This outcome was surprising given that only ~200 km of coastline separated collections from Cape York Peninsula and the Lockhart River, while the other populations (East Coast and Gulf of Carpentaria) were found to span locations separated by over 1,000 km. However, the recent evaluation of *P. diacanthus* population structure in Northwestern Australia suggested the presence of populations on scales as little as 100s of km may be justified (Taillebois et al. 2017). They also found that Roebuck Bay in the Northwest of Australia is a discrete population and that fish from this region showed strong differences to all other locations including the adjacent sampling location at Camden Sound.

Conclusions

Resolving the population structure of *P. diacanthus* provides a basis for implementing assessments and management of the species throughout Queensland waters, as well as its complete Australian distribution. The identification of separate populations between the East Coast and Gulf of Carpentaria highlights the importance of evaluating population structure in species of high fisheries value. Additional work that explores the movement or provenance of fish in Management Region 2 and the Lockhart River is warranted to understand the contribution of these areas to each of the resolved stocks.

While the presence of a discrete East Coast population south of Townsville allows for straightforward adoption of results into Queensland fisheries management, the Gulf of Carpentaria population spans multiple fisheries and multiple jurisdictions and will require considerable cooperation between management agencies and stakeholders to support its effective management. We recommend that fisheries biologists and managers from NT and Qld work together to establish coordinated data collection protocols for monitoring this shared population, including representative biological sampling and use of consistent otolith ageing protocols, as well as collaborating closely on future stock assessments. Within the Gulf of Carpentaria, commercial, recreational and indigenous fishers all have a strong interest in the resource and meeting the needs of each through management should be an important consideration.

Objective 2: Assess the age structure, fecundity, and size-at-maturity for Black Jewfish populations on the East Coast of Queensland.

Methods

Sample collection and study sites

Between September 2020 and October 2021, biological samples were collected monthly with the assistance of commercial fishers in Central Queensland. Sampling within the *P. diacanthus* fishing season (January and February) occurred via the collection of a representative subset of samples from the commercial fishery. Outside of the fishing season (March to December), sampling was undertaken by contracting commercial fishers to collect samples indicative of normal fishing activity. To ensure the greatest possible length range of *P. diacanthus* was sampled, specimens smaller than the minimum legal length (75 cm TL) were also targeted in each region by means of scientific collection permits. Samples were collected from two regions, corresponding to the Queensland Inshore Fishery Management Regions: Region 3 (19°00’ S to 22°00’ S) and Region 4 (22°00’ S to 24°30’ S) (Figure 8). Sampling was designed to collect at least 20 specimens per month per management region for the purpose of biological data collection and were obtained under General Fisheries Permit #208082 (QLD), Animal Ethics permit #CA2020/04/1368 and Great Barrier Reef Marine Park Permit #G20/44421.1. A small sub-set of samples were also collected from Northern Territory (NT) waters for fecundity estimation. Biological data recorded for each sampled fish included total length (TL, ±0.5 cm), body weight (±1 g), sex, gonad weight (±0.1 g) and gonad stage. Whole gonads and otoliths were also extracted and stored for future processing. Monthly male-female sex ratios were characterised and compared between regions using a one-way ANOVA.

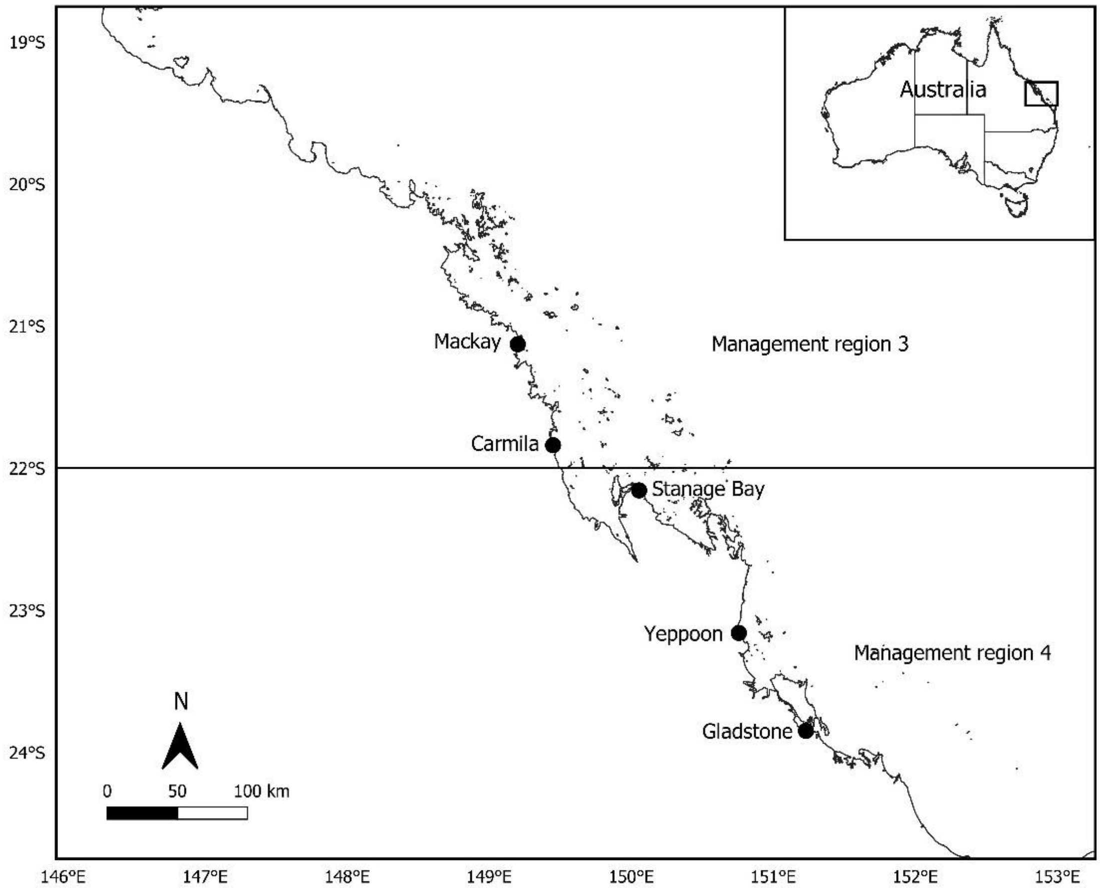

Figure 8. Map of the Central Queensland coast showing the locations where *Protonibea diacanthus* were sampled from within Management region 3 and Management region 4. See appendix 7 for a full map of east coast inshore management regions.

Gonad development

Gonads were assigned to a developmental stage based on macroscopic appearance as outlined in the Black Jewfish staging guide (Randal 2020). The gonadosomatic index (GSI) was calculated based on the following equation:

$$\mathrm{GSI} = \frac{\text{gonad weight (in g)}}{\text{fish total weight (in g)}} \cdot 100 \qquad \text{(Eq. 1)}$$

Mean monthly GSI was calculated separately for females and males. Only fish that were mature (stage 2 or greater) were included to reduce bias due to immature fish.

Histology

Histological analysis was undertaken to validate macroscopic staging and was completed on a sub-sample of five female gonads from each gonad stage across the sampling period. Representative gonads from a range of lengths, stages and sampling sites were kept in a fixative solution of 10% neutral buffered formalin (NBF) for histology and fecundity estimates. After 10 days, the gonad samples were rinsed in water before being transferred into 70% ethanol for storage. The fixed gonad tissue was embedded in paraffin wax and three 6 µm cross-sections were taken through the middle of each gonad and stained with haematoxylin and eosin. Histological sections were examined under a compound microscope and a microscope mounted camera was used to collect images of each gonad stage.

Maturity

Maturity was based on macroscopic staging of gonads (validated by histology) and categorized into either immature (Stages 1) or mature (Stages 2, 3, 4 and 5). The lengths at maturity were calculated by determining the proportion of mature fish in 1 cm TL classes and fitting a logistic curve for each sex as well as for pooled sex data in R. Immature fish with indeterminate sex were assigned to both sexes to help fit the bottom of the maturity curve. The logistic curves for maturity for each sex were compared using a Wald's *F*-test in the R package "*aod*" to test the null hypothesis that male and female lengths at maturity did not differ.

Fecundity and oocyte development

Batch fecundity (BF) was estimated for a subset of stage 3 and 4 ovaries, which were selected to ensure that a complete length range was included. Formalin-fixed ovaries were weighed whole and by lobe to the nearest 0.1 g, after which sub-samples of 0.4 g of tissue were removed from each lobe. An image of the oocytes collected was taken by placing oocytes in a petri dish filled with 70% ethanol. The eggs in each sub-section of ovary were counted by capturing an image of the sub-section with a Leica M205A microscope-mounted camera. Image analysis software (Image J Version 1.381) was employed to automatically count oocytes for a gravimetric estimate of BF (as described in Klibansky & Juanes, 2008).

To account for possible differences in the number of oocytes in different lobes and different sections of the ovary, a series of sub-samples were taken. For each ovary, four sub-samples were collected from each of the left and right lobes to account for potential non-uniform bias in the oocyte content. The subsamples collected from each gonad were then averaged to produce a representative estimate of the number of eggs per gram which could be upscaled to determine the BF per gonad using the gravimetric method where:

$$\mathbf{F = n\ G\ /\ g} \qquad \text{(Eq. 2)}$$

F = fecundity, n = number of eggs in sub-sample, G = weight of ovary and g = weight of sub-sample. Mean estimates were then used to evaluate the relationship between fecundity and other biological parameters (gonad weight, total length and whole weight) in R.

Otolith processing

Estimation of age was undertaken by blocking, sectioning and mounting of sagittal otoliths. The left otolith was embedded in clear casting resin to form resin blocks. A single 400 µm section was cut through the centre of each otolith using a low-speed diamond saw. Sections were then cleaned and mounted on glass slides before being observed using reflected LED light at 16 × magnification on a Leica MZ6 microscope and captured on a Leica DFC295 camera (Leica-Microsystems, Germany). Age estimation was completed by counting the number of opaque zones (annuli), starting from the primordium and counting to the outer edge of the central lobe (Figure 9). Marginal edge types were assigned by readers based on the following criteria: new = opaque visible on edge of otolith; intermediate = translucent material past the last opaque zone is less than 2/3 of the previous completed zone; wide = translucent material past the last opaque zone greater than 2/3 of the previous completed zone. A subset of 150 otolith images were then interpreted by a second reader to assess reader accuracy.

Estimation of Age

A year class was established to include all individuals from the same spawning season (i.e. October to February) off Eastern Queensland. The relative frequency of otolith edge types was assessed to confirm the timing of formation of the slow growth opaque band. To validate the age estimation, the mean size of the cohort estimated at 1 year of age was compared to the estimated size at age from the Von Bertalanffy Growth Function (VBGF)

Growth and longevity

Growth was estimated by fitting the length-at-age data to the VBGF separately for males and females, and also for both sexes combined, using the equation:

$$L_t = L_{inf} \left[1 - e^{-k(t-t0)}\right] \quad \text{(Eq. 3)}$$

Where L_t is the length (TL) at age t, L_{ind} is the asymptotic length, k is the rate at which the curve approaches L_{inf}, t0 is the theoretical age (years) at length zero. Immature fish with indeterminate sex were assigned to both sexes to help fit the bottom of the growth curve. The growth function was fit in R using the package "*FSA*", and the package "*stats*" was used to determine the nonlinear least-squares estimates of the model parameters. The midpoint of year classes was used for the analysis of growth as it better reflects the real age that a fish may be, due to the protracted spawning season of the species.

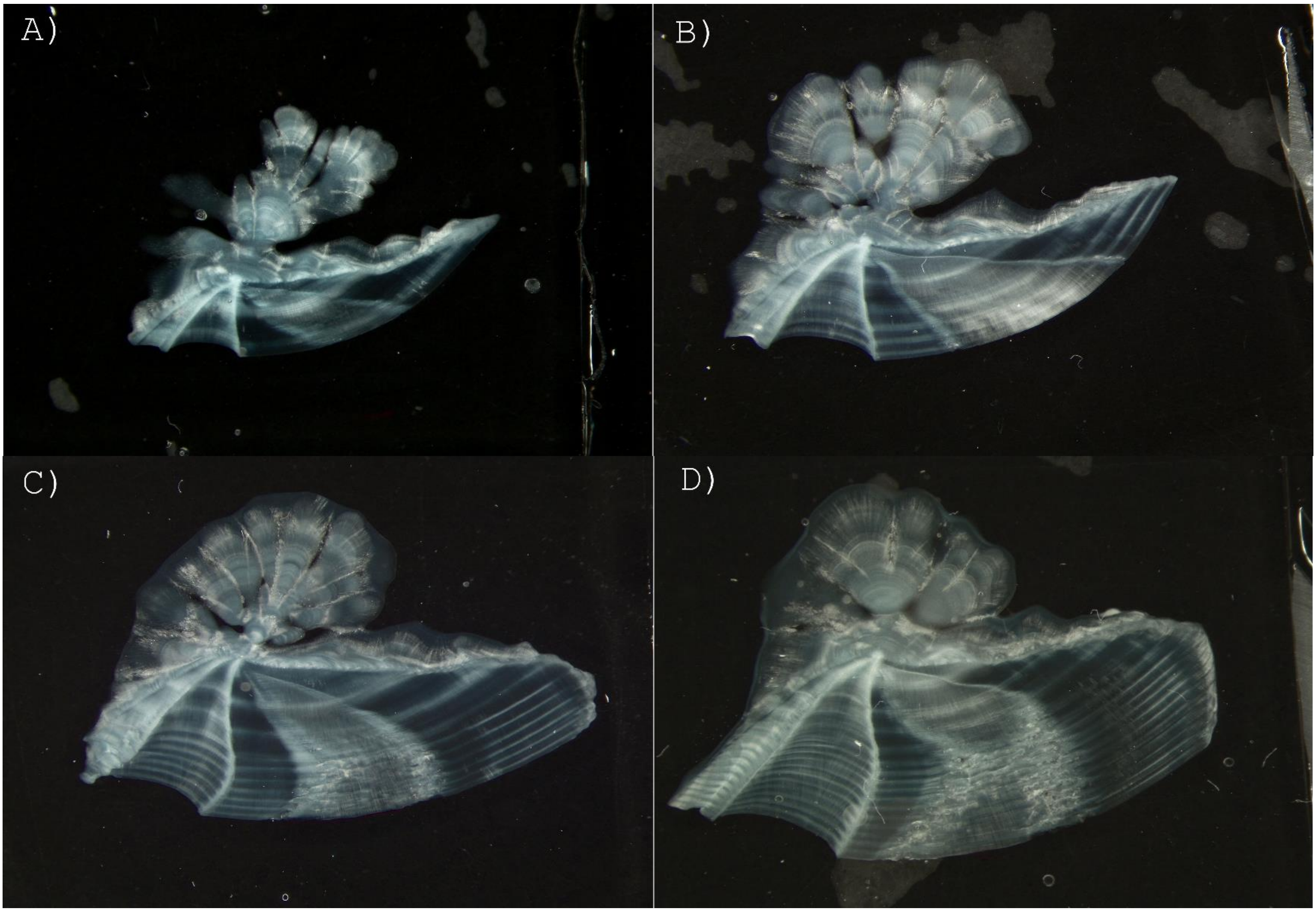

Figure 9. Sectioned otoliths of *Protonibea diacanthus* viewed using reflected light at 16x magnification: (A) 72.5 cm TL female age 1+, (B) 114 cm TL female age 5, (C) 119 cm TL female age 9, (D) 121 cm TL female age 15.

Results

A total of 574 fish were sampled throughout two management regions of the fishery (females: 225, males: 268, immature with indeterminate sex: 42) (Figure 10). Gonads of juveniles below ~70 cm in TL were visually very similar between male and female fish, which prohibited the macroscopic identification of sex for most immature specimens. Samples collected during the open fishing season spanned the entire fishery areas, while samples collected under a scientific permit outside of the fishing season were focused around the main fishing ports. Fish total length ranged from 43 cm to 140 cm, with the majority of specimens being between 105 cm and 125 cm. From the representative commercial catch sampling 49% of fish were in the 106 cm - 126 cm length classes and the 11 kg – 16 kg weight range (Figure 11). Access to large numbers of sub-legal fish was easier within management region 4 due to the presence of several large nursery areas. There was no significant difference in monthly sex ratios by region (one-way ANOVA; *p = 0.378*).

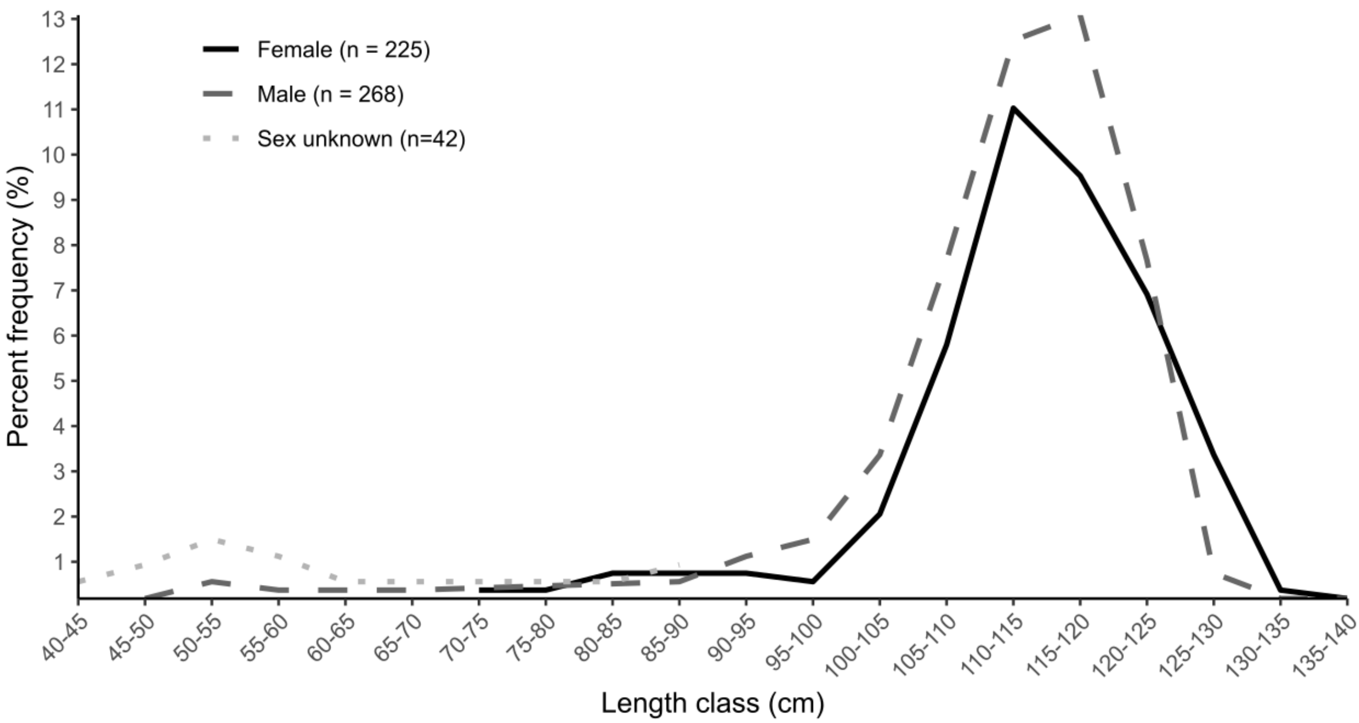

Figure 10. Length-frequency distributions of male, female and indeterminate-sex *Protonibea diacanthus* samples collected.

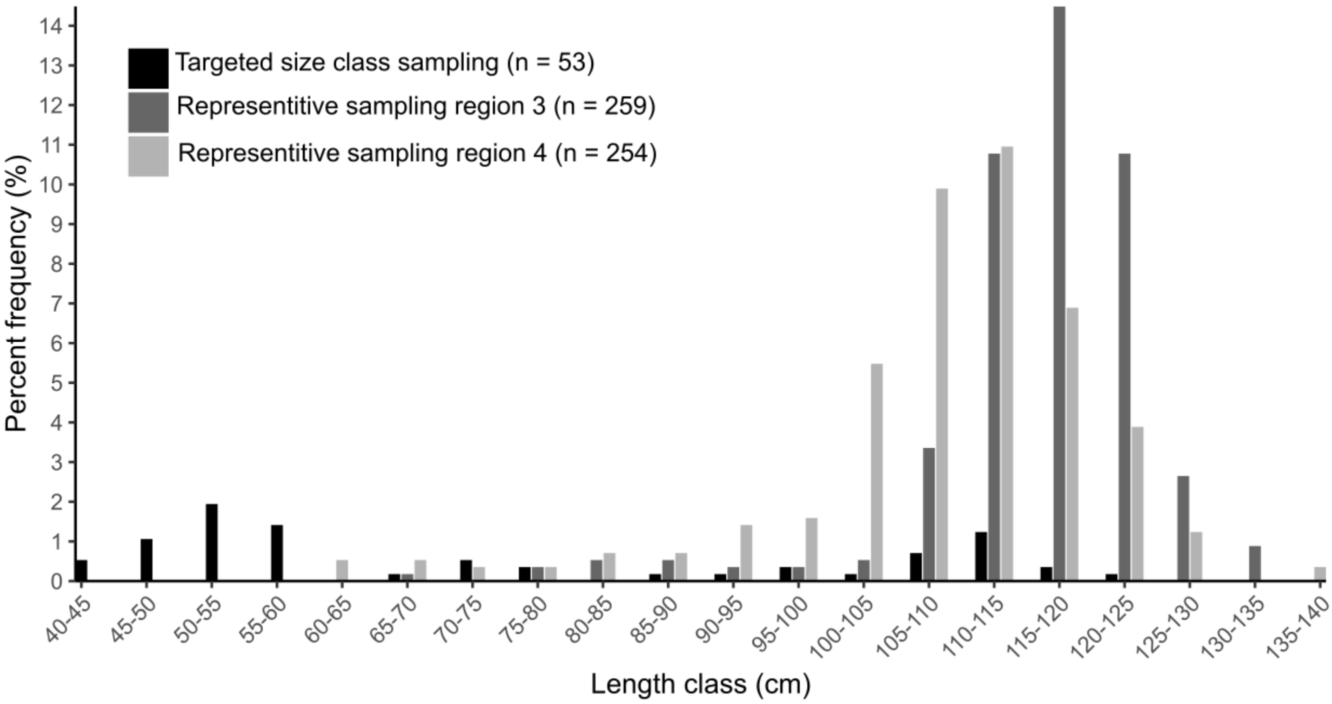

Figure 11. Length-frequency distributions of *Protonibea diacanthus* samples from (1) targeted size class sampling, (2) representative sampling from management region 3, or 3) representative sampling from management region 4.

Spawning period

Monthly mean GSI and gonad staging identified clear seasonal transitions in reproductive condition which was consistent among management regions. Monthly mean GSI showed peak reproductive condition occurred during September and October (females ~3.4%, males ~0.8%, Figure 12), coinciding with a high proportion of stage 4 males and females (Figure 13). GSI value steadily declined from December to April for both sexes (Figure 12) as the proportion of Stage 5 (spent) and Stage 2 (resting) fish increased (Figure 13). Mean GSI remained at its lowest point (females ~0.5%, males ~0.2%) between March and June (Figure 12), which was characterised by high proportions of resting and spent fish (Figure 13). Reproductive condition increased during June, July, and August (Figure 12), with large numbers of ripe gonads present in both sexes (stage 3) (Figure 13).

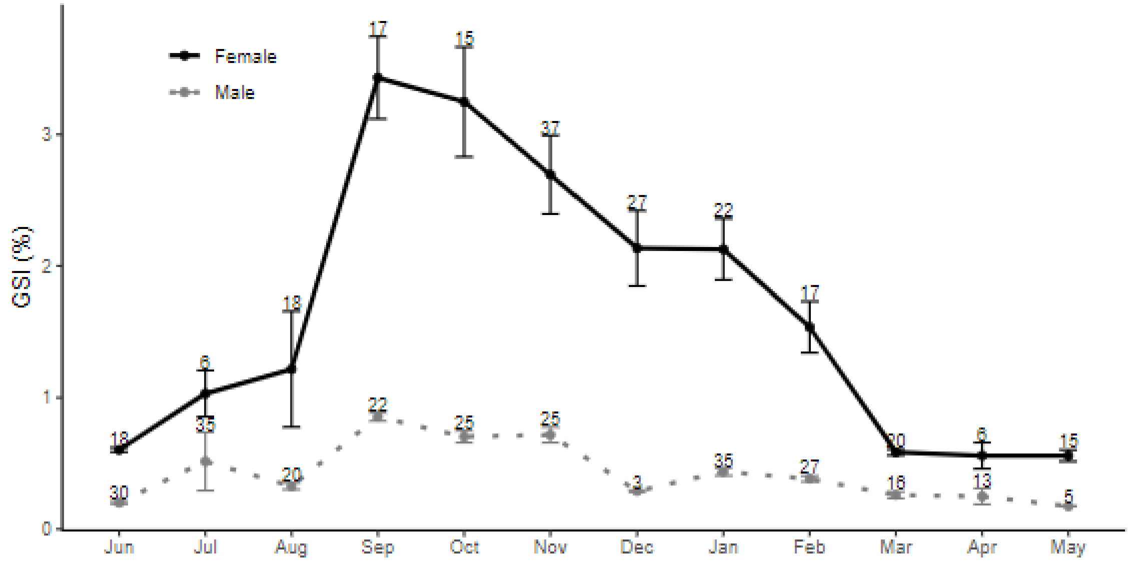

Figure 12. Mean GSI (±SE) by month of male and female *Protonibea diacanthus*. Numbers indicate sample sizes.

Three fish had degenerate gonads, which may have been caused by large urinary bladder stones that appeared to obstruct key reproductive organs, or due to the fish being in noticeably poor condition (Figure 14-f). In three fish, the presence of large urinary bladder stones was observed to affect gonad development, with stones reaching 8.8 cm in diameter and 88 g weight. While the presence of degenerate gonads was rare, these specimens did have the potential to downward bias maturity estimates and so were excluded from subsequent analyses. However, in most cases, small urinary bladder stones did not appear to obstruct the reproductive biology.

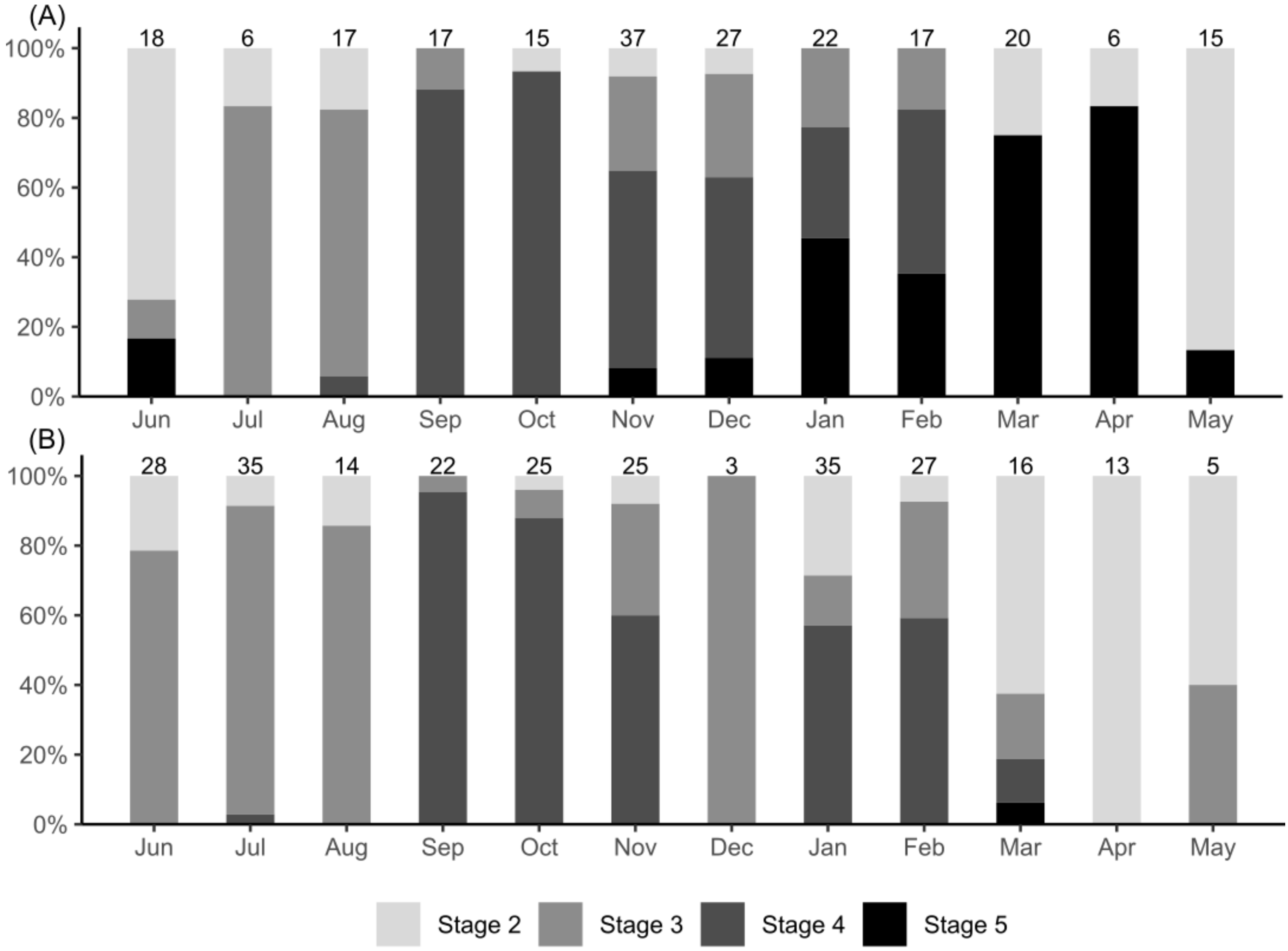

Figure 13: Change in the relative occurrence of stage 2-5 gonads in female (A) and male (B) *Protonibea diacanthus* from Central Queensland waters. Numbers indicate sample sizes.

Gonad histology

Histology was able to validate macroscopic staging, with clear differences in oocyte development among macroscopically staged ovaries. Female immature stage 1 and female stage 2 gonads were dominated by previtellogenic oocytes (Figure 14-a) and large primary cortical alveolar oocytes (Figure 14-b). Stage 3 gonads were characterised by vitellogenic oocytes in addition to smaller previtellogenic oocytes (Figure 14-c). Ripe stage 5 gonads contained hydrated oocytes (Figure 14-d). Stage 5 spent gonads were similar to stage 2 gonads with the exception of remnant yolk granules undergoing atresia and less densely packed cortical alveolar oocytes and primary growth oocytes (Figure 14-e). Degenerate gonads were found to have large sections of unformed oocytes (Figure 14-f).

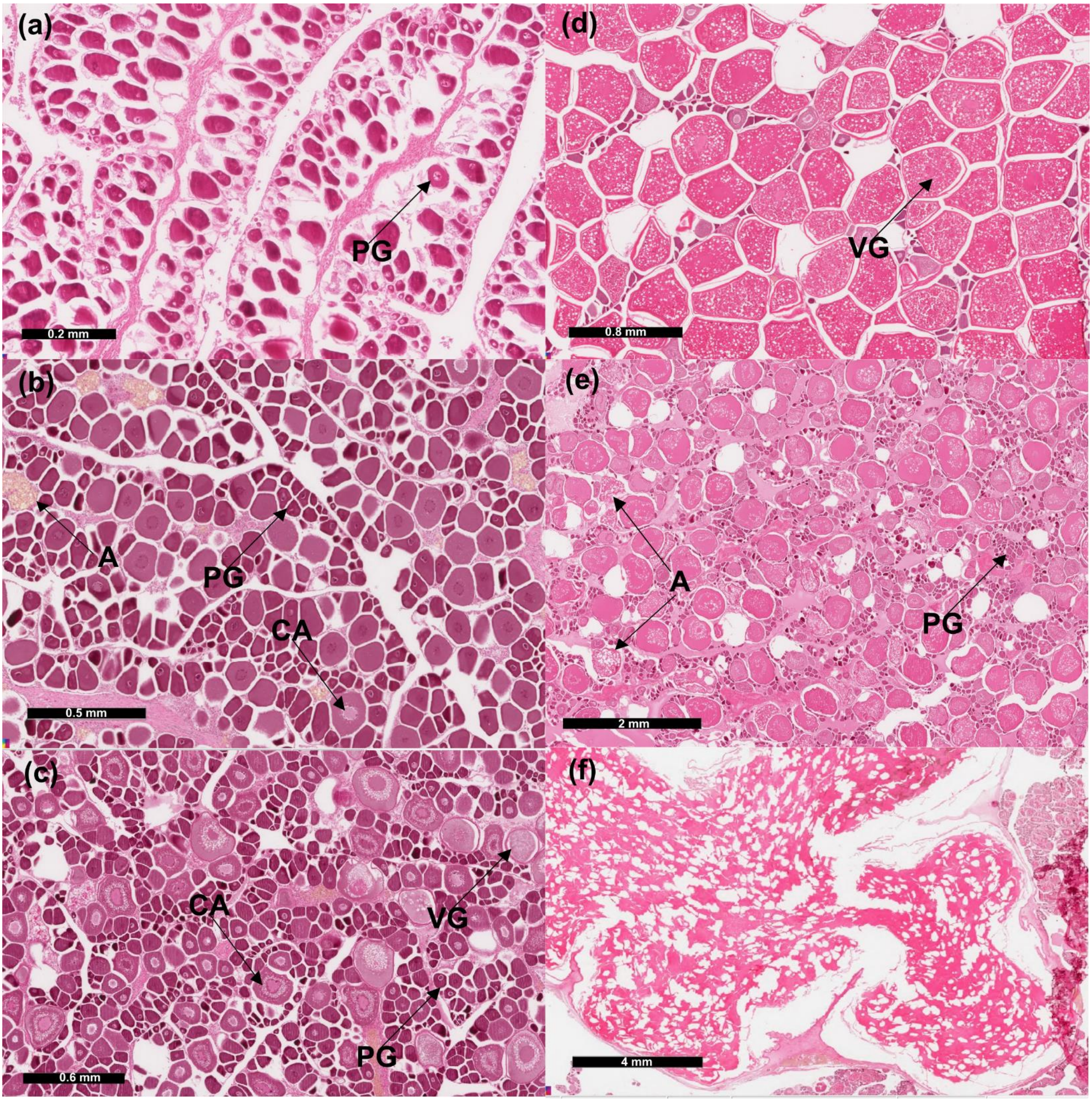

Figure 14: Histological sections of *Protonibea diacanthus* ovarian tissue illustrating (a) Stage 1 (immature); (b) Stage 2 (developing); (c) Stage 3 (ripe); (d) Stage 4 (running ripe); (e) Stage 5 (spent); (f) Stage 6 (degenerate). A, atretic oocyte; CA, cortical alveolar oocyte; PG, primary growth oocyte; VG, vitellogenic oocyte

Maturity

The lengths at which 50% (L50) of females and males reached sexual maturity were not significantly different (Wald's test, $p = 0.16$) and so maturity data for both sexes were combined (Figure 15). The combined L50 was 83 cm TL, and the length at full maturity (L100) was estimated at 105 cm TL.

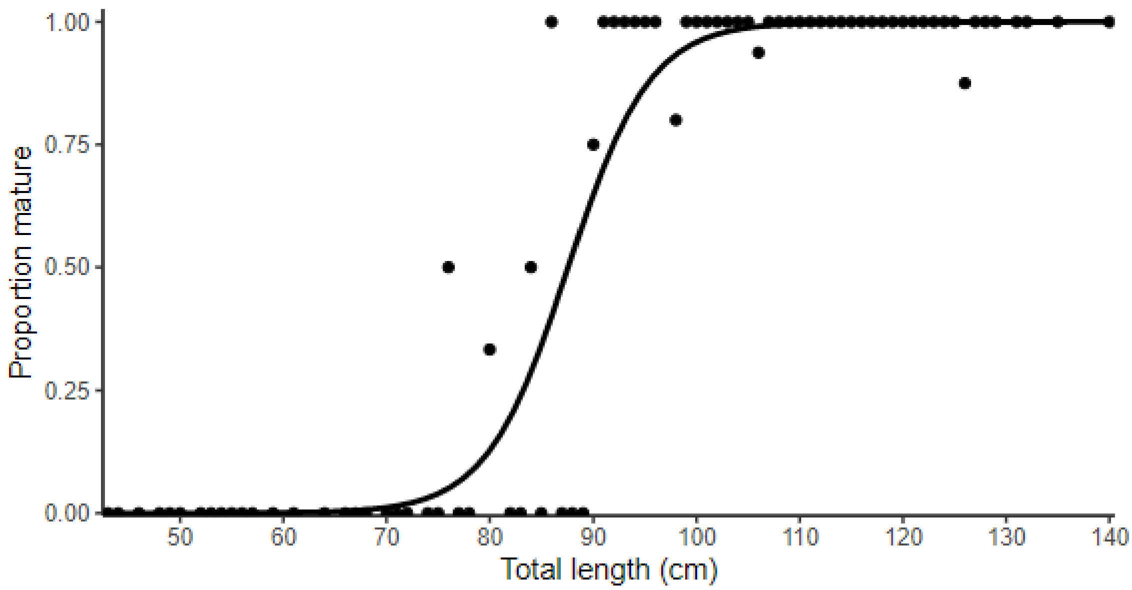

Figure 15: Length at maturity with a fitted logistic curve for *Protonibea diacanthus* from Queensland East Coast waters.

Fecundity

Mean gravimetric fecundity (GF) was estimated at 4,495,172 ± 1,415,862 oocytes (mean ± SE), with a range from 489,738 oocytes to 27,893,709 oocytes for samples collected in Queensland. By comparison, GF has been estimated for fish from the NT at 6,291,035 ± 1,780,922, with a maximum estimate of 8,297,831 eggs. The relationship between GF and TL was best described by the exponential relationship:

$$Gravimetric\ fecundity = 32.3\ \cdot e^{0.09 \cdot TL}$$

With an R^2 of 0.78 (Figure 16, n = 19). Relative fecundity (RF) ranged from 31 to 910 oocytes g^{-1} body mass. The relationship was observed to be strongly influenced by the largest individual within the dataset (140 cm TL, ~28 million oocytes). After removal of data related to this single specimen, the fit of the data reflected a flat, non-significant linear relationship (R^2 = 0.003).

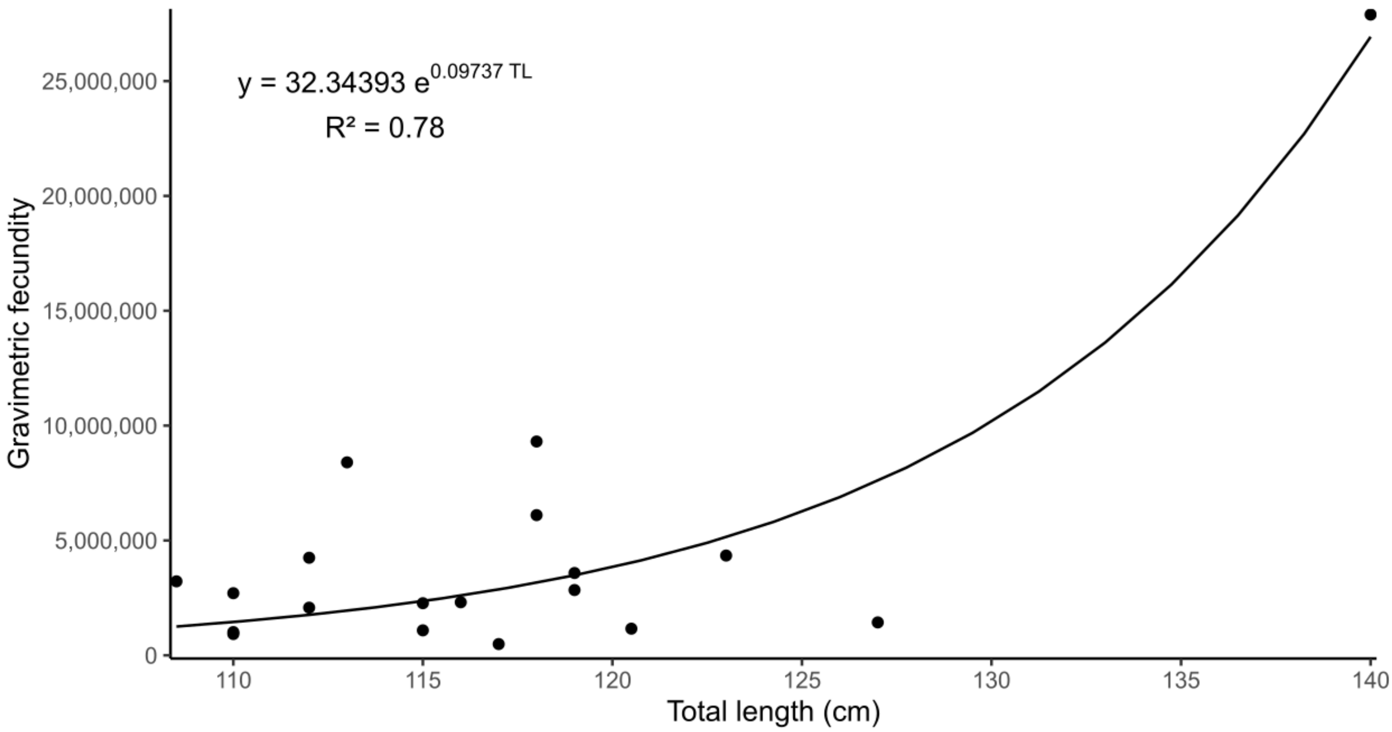

Figure 16: Exponential relationship between total length (cm) and batch fecundity for *Protonibea diacanthus* from Queensland East Coast waters.

Age, growth and longevity

The formation of new opaque bands (i.e. narrow edge type, classified as newly formed) was observed as early as May and accounted for 21% of fish sampled during that month. Narrow edge types increased in frequency during winter months before accounting for 100% of edge types in October (Figure 17). Intermediate edge types were identified in samples from November to March, peaking in January, while edge types classified as wide were present in sampled collections between March and September. Evaluation of the variability in edge types between regions revealed very little difference among Management Region 3 and Management Region 4.

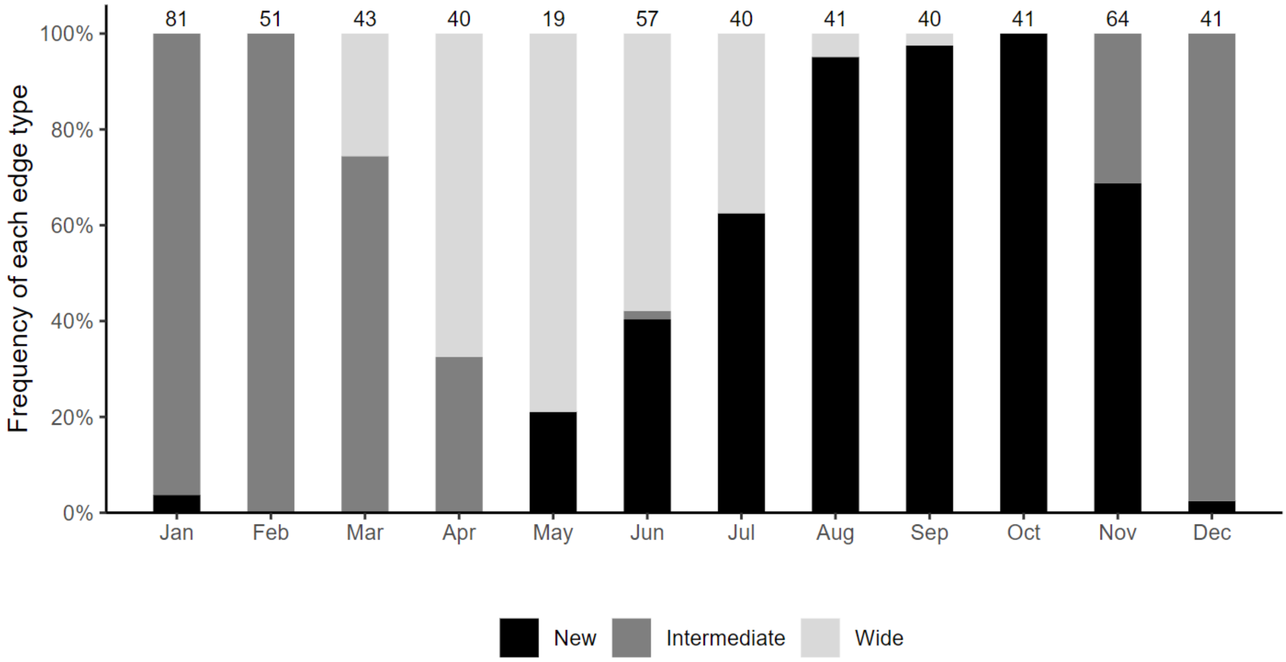

Figure 17. Frequency of otolith edge types for *Protonibea diacanthus* in each month, pooled by sex and region.

Counts of opaque zones were made for all sectioned otoliths and ranged from 1 to 15. Due to the formation of new bands during winter, and with spawning known to occur for *P. diacanthus* over summer, the first increments were interpreted as representing ~6-12 months of growth. The oldest sampled specimen was a 121 cm female estimated to be 15 years of age, while the largest fish was a 140 cm female estimated to be 14 years old. The oldest male fish were estimated to be 13 years, with total lengths of 121.5 cm and 122 cm. There was 85% total agreement in edge type and annuli count when a sub-sample of otoliths was aged for validation (*n = 150*), with 100% agreement in annuli count (± 1 year). Transitions in year classes, marked by proportionally similar class strength through time, were observable across all fully recruited year classes (> 6 years and 110 cm). The two clearest examples of this were the transition of the strong seven year old and 10 year old classes collected in 2020 into strong eight year old and 11 year old classes in 2021 samples (Figure 18).

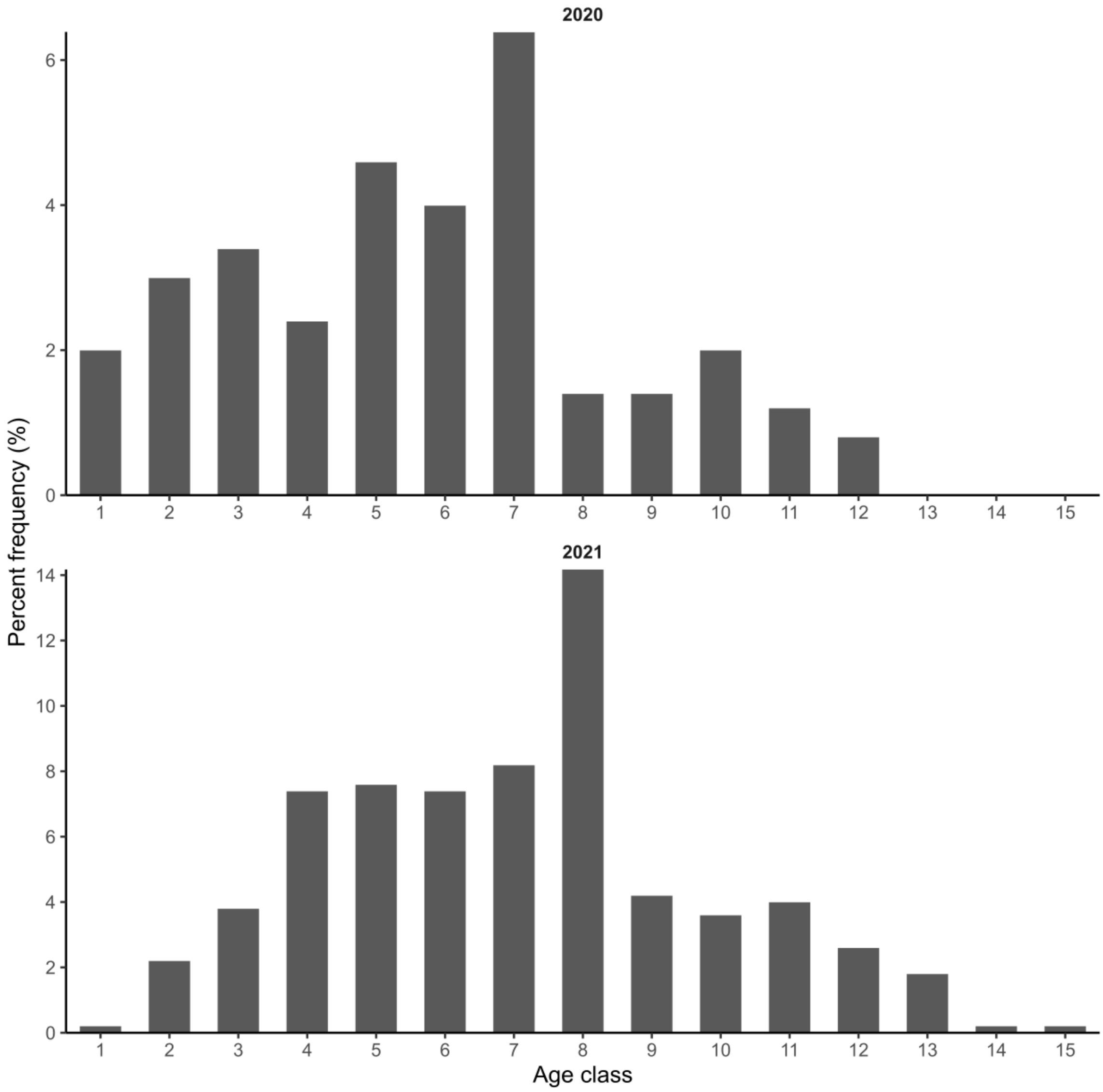

Figure 18: The age-frequency of *Protonibea diacanthus* sampled in central Queensland waters during the 2020 and 2021 with visible transition of age cohorts.

Comparison of growth rates between males and females indicated mostly no significant differences, with the only difference observed being a 2.3 cm larger L_{inf} value for females ($p < 0.05$). *Protonibea diacanthus* reached an average of 60 cm TL after 1+ year (indicative of 13-23 months), 84 cm TL after 2+ years, and 98 cm TL after 3+ years. Von Bertalanffy Growth Function parameters for females were L_{inf} = 119cm TL, k= 0.62 and t_0 =-0.10 year, and for males was L_{inf} = 117.2 cm TL, k = 0.57 and t_0 = -0.18. The VBGF parameters best describing the growth of *P. diacanthus* as a whole population were L_{inf} = 118 cm TL, k = 0.60, and t_0 = -0.16 year (Figure 19).

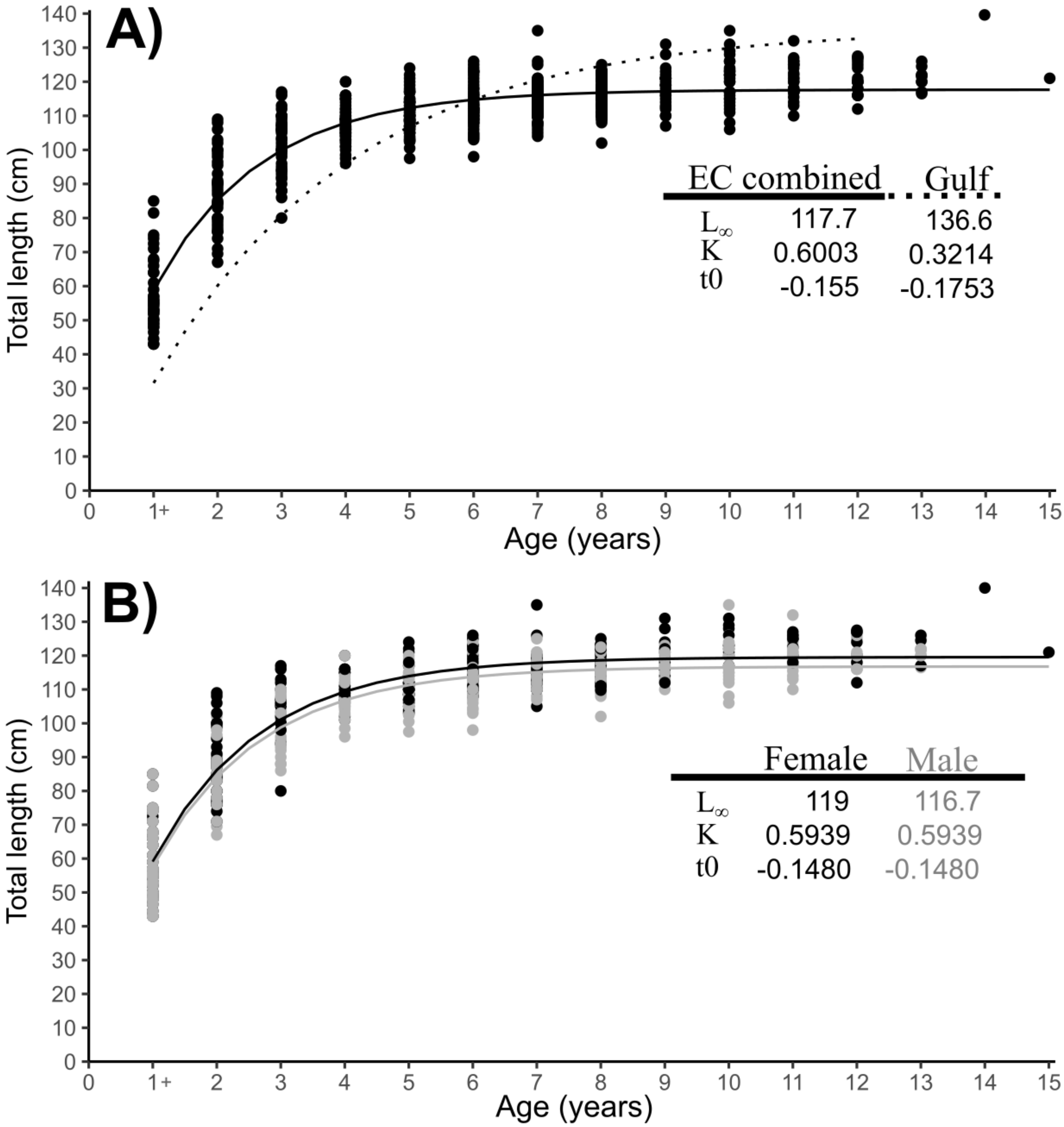

Figure 19. von Bertalanffy growth function (VBGF) fitted to (A) pooled East Coast data by sexes (black) and data from the Gulf of Carpentaria collected by Mcpherson (1997) (dotted line); and (B) males (grey) and females (black) for *Protonibea diacanthus* on the Queensland east coast. Growth parameters generated from VBGF shown on graph.

Length, weight, bladder weight and age composition

There was no variability in the length-weight relationship between sexes, with data from both sexes forming an exponential relationship. There was no noticeable change in the weight at length of *P.*

diacanthus after reaching sexual maturity (Fig. 20a). The ratio of swim bladder weight to fish length was shown to have an exponential relationship (Fig 20b), with some variability between sexes at larger size classes. Specifically, an inflection point occurred at ~120 cm, where the weight of swim bladders in male fish above this size continued to increase exponentially, while the weight of bladders from female fish followed a more linear trajectory.

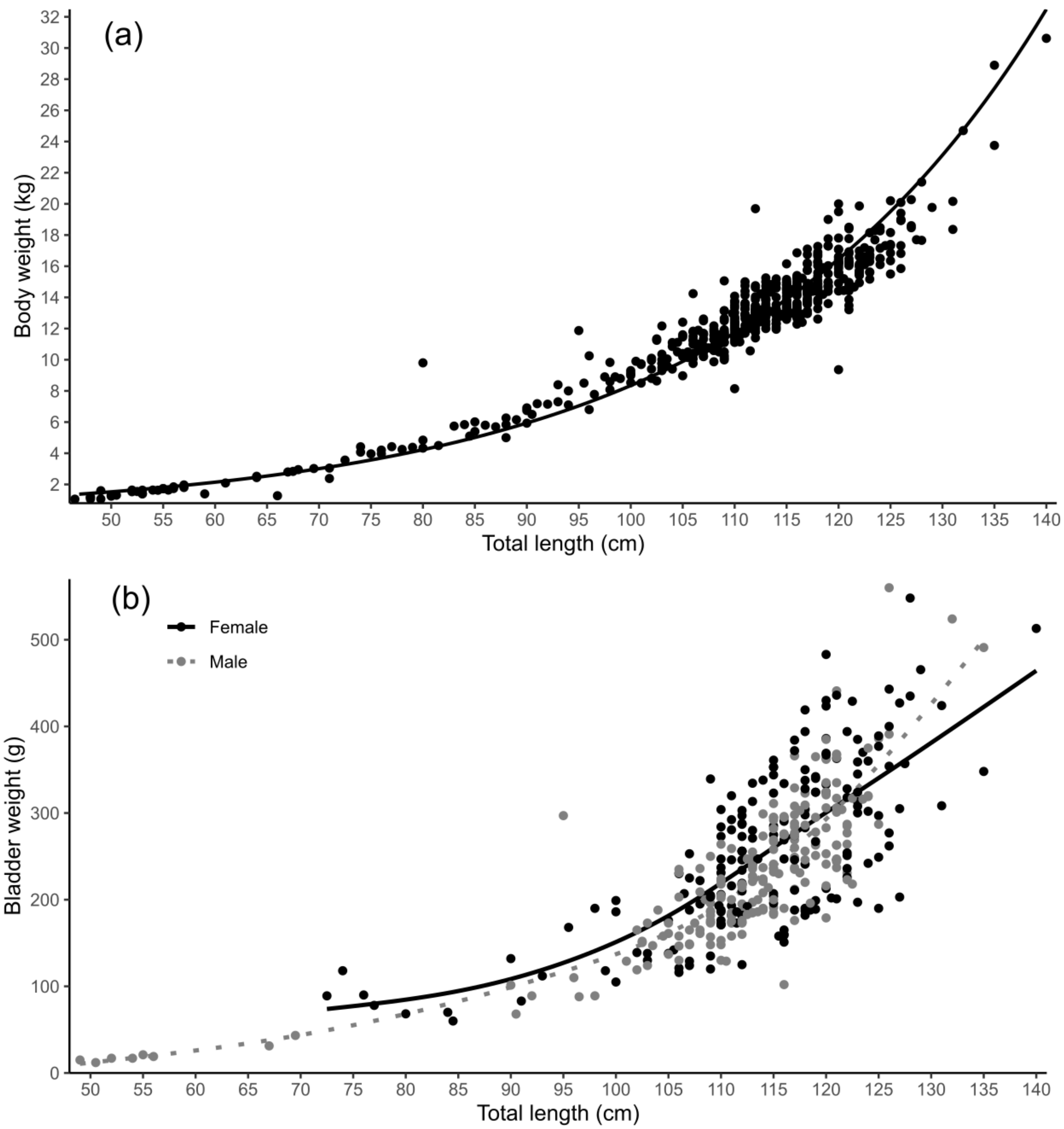

Figure 20. Total weight fitted to (a) pooled data for bodyweight, and (b) bladder weight by sex, for *Protonibea diacanthus* in Queensland.

Discussion

The results of this study yielded important insights into the reproductive biology of *P. diacanthus* from the Queensland East Coast. GSI values for *P. diacanthus* and histological staging of ovaries indicated a single major spawning period from October to February, which coincided with the peak of the wet season. Based on counts of hydrated oocytes, mature females preparing to spawn were capable of releasing 489,738 to 27,893,709 eggs during each season, with very large females (>140cm) potentially capable of producing exponentially greater numbers of eggs. Histological and macroscopic staging data indicated that size at 50% maturity was 83 cm and L100 was 105 cm, with no significant differences between sexes. Annuli were clearly identifiable in sectioned sagittal otoliths, and we reported fish older than any previously aged *P. diacanthus,* as well as age-based demographics with a higher percentage of larger and older fish than in other fisheries (McPherson 1997; Phelan 2008; Phelan et al. 2008). These results are important not only for informing life-history parameters required to assess *P. diacanthus* populations within Queensland waters, but also for establishing a new baseline for age-based demographics of a population that is not age-truncated.

Reproduction

Macroscopic and microscopic examination of *P. diacanthus* gonads showed clear seasonality in spawning periodicity. The presence of both stage 4 and 5 fish from November to February indicate that spawning activity was occurring throughout this period. This information was supported by GSI values which peaked in October indicating the commencement of spawning activity, before reducing throughout the subsequent months as the reproductive condition of fish deteriorated following the release of eggs. The timing of the spawning season coincided with the wet season in Central Queensland. The occurrence of spawning in periods of monsoonal activity is consistent with the timing of peak reproductive activity in NT (October to April), and Indian (June to August) populations (Rao 1963; Phelan 2002; Phelan 2008). Improving the understanding of whether environmental cues are linked to spawning behaviour would be valuable for informing any regulatory measures associated with spawning behaviour (e.g. seasonal closures) and understanding variability in recruitment such as that observed in the NT (Saunders et al. 2020) .

As with many other Sciaenids, *P. diacanthus* are known to form large aggregations around oceanographic features and structures (e.g. rocky reefs and jetties) with high current differentials (Domeier and Colin 1997; Semmens et al. 2010). The purpose of these aggregations has long been speculated to be linked to spawning behaviours, as seen in many other commercially important species (Phelan et al. 2008). In the current study, aggregations appear to occur year-round at the majority of sites and spawning condition did not seem to vary among sampling locations or fishery management regions, with similar proportions of spawning and spent fish observed across all surveyed sites. This finding suggests that the presence of aggregations may simply be a result of schooling behaviour at favourable habitats, with spawning occurring at specific times of the year, rather than aggregations forming for the sole purposes of spawning (Domeier and Colin 1997). Additional information around the residency of fish at, and movement of fish between, prominent aggregation sites would help to further resolve the importance of this behaviour (Semmens et al. 2010). An acoustic telemetry study is currently underway to collect more information on the movements, residency and habitat use of *P. diacanthus* in Central Queensland (Barnett et al., unpublished data).

The gravimetric fecundity estimates identified herein represent the first quantitative evaluation of fecundity from *P. diacanthus* populations in the southern hemisphere. The mean fecundity estimate of 4,495,172 oocytes from Queensland and 6,291,035 from the Northern Territory were within the range of previous work from populations in the northern hemisphere. However, the range of estimates observed in the current study (489,738 - 27,893,709 oocytes) greatly expands on known information regarding intraspecific variability and maximum spawning potential of the species. The maximum estimated fecundity for the species from previous studies was ~7 million (Rao 1963), which is around four times smaller than the maximum estimate identified here. Interestingly, we found a very weak relationship between fecundity and fish length when examining fish ranging from ~105 to ~130 cm. The inclusion of a single very large specimen (142 cm) that was estimated to have ~28 million eggs was the main driver of the observed exponential relationship between fish size and fecundity. We recommend examination of more specimens with a TL greater than 130 cm to help verify the strength of this relationship.

The length at maturity reported in the current study indicates an early maturation which is typical of many Sciaenids (Silberschneider et al. 2009; Hegarty et al. 2021). Our estimate of 50% maturity at ~83 cm was found to be within the length range (80-84 cm) observed in Cape York (Phelan et al. 2008) and India (Rao 1963), but considerably lower than estimates in both the Northern Territory (89 cm) (Phelan 2008), and the Gulf of Carpentaria (98 cm) (McPherson 1997). our estimate is also higher than the current minimum legal size (MLS) for *P. diacanthus* within the Queensland East Coast fishery, which is set at 75 cm. However, as fish smaller than 83 cm are uncommon within the commercial fishery, it is not likely that increases to the MLS would provide significant sustainability benefits to the stock.

The presence of large urinary bladder stones and degenerate ovaries have not previously been reported in *P. diacanthus*. Documentation of urinary stones (urolithiasis) in marine fish remains rare, and form due to the excretion of excessive amount of oxalate into the urine which gets incorporated into a stone (Samal et al. 2015; Applegate Jr et al. 2016). It has been hypothesised that these stones may be linked to rapid acclimation to low salinity environments, however, this remains largely unvalidated (Applegate Jr et al. 2016). Although the majority of urinary bladder stones observed in *P. diacanthus* were small and did not appear to have negative effects on the reproductive biology, urinary bladder stones in several fish were significant in size and had visually impacted the fishes' gonads. The presence of large objects within the peritoneal cavity has been shown to contribute additional stress and have negative effects on the spawning of fish (Samal et al. 2015). The stones identified in this study were located at the beginning of the oviduct and appeared to obstruct the development of oocytes. Despite the noted effects on gonadal development, the fish were large in size and the stones did not appear to impact their body condition. Further reporting of urinary bladder stones in wild marine populations along with targeted work to determine how urinary bladder stones may link to fish condition factors such as diet or infection would be of benefit.

Age and growth

Annual periodicity of opaque zones was validated by edge type analysis indicating that opaque zones were generally laid down during the austral winter-spring period (May to October). This periodicity is similar to that which has been observed in other species on the East Coast of Queensland such as barramundi (*Lates calcarifer)* (Stuart and McKillup 2002; Staunton-Smith et al. 2004), but is in contrast to the late spring to early summer annuli formation found in *P. diacanthus* from the Gulf of Carpentaria

(McPherson 1997). This regional difference in the timing of increment formation may be driven by differences in the timing of the seasonal transition in environmental conditions and food availability between the Gulf of Carpentaria, which is part of the Wet Tropics, and the Central Queensland Coast which is characterised as the Southern dry tropics (Staunton-Smith et al. 2004; Leahy and Robins 2021). These slight differences in seasonality may offset the peaks in feeding rate and changes in water temperature which are known to be influential to the formation of the opaque zone (Rao 1966; Phelan 2008).

The age and growth characteristics of *P. diacanthus* from Eastern Australia indicate that this species is similar to other Australian Sciaenid species in being moderately long-lived (>15 years) and fast growing (VBGF growth parameter k > 0.3 year^{-1}). They are most similar to mulloway (*Argyrosomus japonicus*) in terms of asymptotic maximum sizes (L_{inf} = 131.7 cm TL and 24 years), but have a maximum recorded age which is more similar to teraglin (*Atractoscion atelodus*) (L_{inf} = 73.8 cm FL and 14 years) (Silberschneider et al. 2009; Hegarty et al. 2022). However, when compared to age and growth parameters from other *P. diacanthus* populations we also found variation in the growth characteristics, as well as the overall age structure (McPherson 1997; Phelan 2008; Phelan et al. 2008).

The oldest *P. diacanthus* aged herein (15 years old) was older than has previously been reported from elsewhere in their range. The maximum reported age of *P. diacanthus* from the Northern Territory and Gulf of Carpentaria is 13 and 12 years old, respectively (McPherson 1997; Phelan 2008). We also observed a greater proportion of older fish than have been described in other *P. diacanthus* populations, with a modal age class of eight years compared to four years in the NT and three years in Cape York (Phelan 2008; Phelan et al. 2008). As these latter two populations have been subjected to higher rates of exploitation over a longer time period, it is likely that this may have contributed to the depletion of older animals. While the truncation of age-classes has been well described within fish from Cape York, it remains largely unrecognised within the NT which hosts Australia's largest Black Jewfish fishery (Phelan 2008). The fact that we sampled multiple fish over the age of 13 years of age and that the age composition of the Queensland catch had strong numbers of fish >8 years suggests that age-class truncation within the NT fishery may have occurred prior to the sampling carried out by Phelan et al (2008).

One other notable difference between our study and those from other *P. diacanthus* populations in Australia was in observed growth rates. We estimated a combined sex growth rate of K = 0.6, which is halfway between the estimate of K = 0.9845 from the NT (Phelan 2008), and the K = 0.32 value from the Gulf of Carpentaria and India (Rao 1966; McPherson 1997). Although it is well recognised that factors such as the water temperature and food availability affect growth, we consider it unlikely to have resulted in variability in the rate of up to ~0.6. Differences among the estimates of K for *P. diacanthus* are therefore likely to be more reflective of sampling biases, such as where population sub-samples are characterised by a fewer number of samples at or above the asymptotic length, therefore leading to a reduced overall growth rate, and higher estimate of L_{inf} (e.g. McPherson 1997). Alternatively, the noted difficulty in reading *P. diacanthus* otoliths from tropical regions may also have resulted in differences in the detecting increments which may have impacted the allocation of individuals to age classes.

The value that commercial fishers receive for *P. diacanthus* is determined primarily by the price of the swim bladder, which is based on the bladder weight, and secondly by the volume of meat. Swim bladders are valued in price bands which correspond to bladders weighing less than 150 g, between 150-200 g, 200-250 g, 250-300 g and more than 300 g. Those falling into the larger weight band receiving exponentially greater value. For instance, a 295 g bladder may fetch approximately half of the value of a 305 g bladder. As a result, it is in the best interest of commercial fishers to ensure that harvest levels do not reduce the proportion of larger fish (> 120 cm) in the population, which are more likely to retain a mean bladder weight of 300 g or greater. The body weight of larger fish also results in a secondary economic benefit of having a larger volume of meat to sell. Based on both these economic considerations, we highlight it is important for *P. diacanthus* stocks to be managed at a level that achieves Maximum Economic Yield (MEY) by ensuring harvest levels do not result in size truncated populations (which represent a lower market value per fish and reduced recreational satisfaction).

Implications

The presence of a single population across the Gulf of Carpentaria, spanning multiple fisheries and multiple jurisdictions (Northern Territory and Queensland), means that considerable cooperation will be required between management agencies and stakeholders to effectively manage this stock. Within the Gulf of Carpentaria, commercial, recreational and indigenous fishers all have a strong interest in *P. diacanthus* and meeting the needs of each through management should be a foremost consideration (i.e. recognising their importance to traditional owners while still supporting commercial and recreational fisheries). Despite the widespread spatial footprint of the populations, there remains multiple pressures on different life history stages of the population (e.g. fin fish trawl on adults as well as Indigenous and recreational harvest on juveniles and adults) which should be monitored to understand their impact on the stock. This is particularly important given anecdotal reports from Traditional Owners, which were verified during our on-water sampling, that the size and abundance of *P. diacanthus* in the Cape York Peninsula has yet to fully recover from historical fishing pressure.

The identification of a single population and characterisation of key biological parameters spanning the main East Coast inshore fishery management regions (Management Regions 3 and 4) resulted in a clearly definable stock and biological inputs, which were able to be directly integrated into the *P. diacanthus* stock assessment for the East Coast of Queensland. The stock assessment estimated the population to be at or above a spawning biomass level of 56% and recommended an increase in the total harvest for the fishery, which has now been implemented in the fishery. The result of this was an increase in the TACC from 20 tonnes to 54 tonnes, and the ability for recreational anglers to fish year-round for the species. It is estimated that these management changes provide access to an additional ~$1.36m worth of harvestable product to the commercial fishery each year, as well as commensurate social and economic benefits to the recreational sector. The full assessment can be found at: https://era.daf.qld.gov.au/id/eprint/8870/.

The age composition and observation of strong recruitment of *P. diacanthus* on the East Coast of Queensland suggest that the brief period of increased fishing pressure from 2016 to 2020 is unlikely to have significantly impacted the health of the stock. This contrasts with many other Sciaenid fisheries which are characterised by truncated age frequencies. The present study provides robust age and growth information to improve the accuracy of the *P. diacanthus* stock assessment and provides a baseline on what the age composition of a healthy stock may resemble. As a result, the age-structure observed in the East Coast population raises some concerns around the status of other *P. diacanthus* stocks throughout Australian waters. We also reaffirm that managing *P. diacanthus* stocks to a level that achieves MEY should reflect harvest levels which retain a proportion of larger, more economically valuable fish in the population.

The observed timing of spawning provides evidence to support the implementation of a seasonal spawning closure for the fishery. Currently the fishing season opens on 1 January each year under a competitive total allowable commercial catch limit, which means that most of the catch is occurring during the peak period of reproductive activity. Implementing a spawning closure between October and February would provide extra protection by offsetting the time where fishers target and catch *P. diacanthus* until spawning is complete.

Currently the minimum legal size (MLS) is set ~8 cm below the size at 50% maturity. While it is common practice to set the MLS at L50, for this species it is unlikely to have a considerable influence on the sustainability of the fishery. This is because very few Black Jewfish below 100 cm are landed by the commercial fishery, which predominantly targets aggregations of larger fish in order to maximise profitability (due to the larger size of their swim bladders). The recreational sector does interact with fish in the 75-83 cm range, however, a large proportion of these fish are released and preliminary evidence indicates that small Black Jewfish, which are generally caught in shallow waters (< 10 m), have good post-release survival (Barnett et al., unpublished data). As a result, we do not recommend a change in the MLS as we expect it to have very little effect on overall fishing mortality.

Recommendations

We recommend that management arrangements for the Queensland East Coast population of *P. diacanthus* be relaxed in line with recommendations from the recent stock assessment. To support the long-term sustainability of the East Coast population we recommend that peak harvest levels do not occur during the spawning period. Seasonal spawning closures should be sufficiently long to encompass interannual variability in peak spawning activity, but without compromising the ability for the fishery to fully utilise the TACC.

Routine biological monitoring of the East Coast *P. diacanthus* population through the collection of age and length information is of high priority. This monitoring should focus on Management Regions 3 and 4 which account for the majority of the current fishery catch. This recommendation is particularly important given the impacts of fishing on the age structures of *P. diacanthus* populations elsewhere in the world, and the aggregating behaviour of the species which suggests that commercial catch rates are likely to be affected by hyperstability.

Further work needs to be undertaken to understand the status of the Gulf of Carpentaria population. Components of this population such as in Cape York have experienced localised depletion and there is a lack of follow-up monitoring to understand whether recovery has occurred. Furthermore, with the re-commencement of the Gulf of Carpentaria Fin Fish Trawl Fishery in 2022, the population is now likely to be experiencing harvest pressure throughout its range. Therefore, we recommend that monitoring and assessment of *P. diacanthus* should be prioritised and jointly undertaken by Queensland and Northern Territory fisheries agencies. Where possible this should consider uniform data collection and ageing protocols, joint stock assessments to encompasses the extent of genetic stocks identified herein, and complimentary management among jurisdictions.

Additional research considerations include:

- Research into the movement and connectivity of *P. diacanthus*. Specifically understanding the connectivity of fish in the Lockhart River area and Inshore Fishery Management Region 2 (Cooktown to Hinchinbrook) with adjacent populations would help to further refine the assessment of this species in Queensland waters.
- The determination of post-release survival of *P. diacanthus* through direct observation or tagging would help to provide more conclusive information around best handling practices and estimates of mortality from fish released by the recreational sector.
- The estimation of fecundity in large specimens (> 130 cm) would help clarify the shape and strength of the fecundity at length relationship and as a result build further certainty around stock assessment inputs.
- Identifying essential fish habitats for all stages of the life cycle: Understanding the value of habitats that are most important to each stage of the life cycle of *P. diacanthus* will help inform any impact or drivers of recruitment.

Extension and Adoption

Information arising from this study has been well received by stakeholders and has been used to directly inform stock assessment and management of *P. diacanthus* in Queensland. As a result, the recommendations here have been adopted in a way that has re-shaped the management of the fishery, and includes recommended adoption of a seasonal spawning closure, increase in the commercial total allowable catch, and removing the link between the recreational fishing season and commercial TACC which were all supported at the most recent East Coast Inshore Working Group meeting. https://www.daf.qld.gov.au/business-priorities/fisheries/sustainable/fishery-working-groups/east-coast-inshore-working-group/communiques/communique-6-october-2022

The project was and continues to be extended and communicated to end users, including: fishery managers, stock assessment scientists, fishery working groups, the wider recreational fishing community, commercial fishing industry associations, and the wider fisheries science community. This includes through:

- the dissemination of information through fact sheets,
- presentations at scientific and industry conferences,
- articles in popular fishing magazines, and
- presentations to interested fishing groups and associations.

Media coverage

The project has received a range of coverage through formal communication channels such as official media releases and radio interviews, as well as through social media. A summary of consultation, extension and adoption associated with the development and delivery of this FRDC project is outlined below, and media clips are included in Appendix 4 – media coverage.

Radio

- The media release generated some interest from ABC Broadcasting and the PI was interviewed by Inga Stunzner from ABC Capricornia. The interview was aired on 3 June 2020 across the ABC network.

Print and online

- The project media release was distributed to news outlets on 29 May 2020 (see Appendix 4).
- A public information notice to fishery stakeholders was delivered on 8 March 2021 and included a message of appreciation to fishers assisting with the research.
- Project updates were posted on the Fisheries Queensland and Agriculture Queensland Facebook pages (see Appendix 4).
  - Project-related post that appeared on the Fisheries Queensland Facebook page on 25 June 2020.
  - Project-related post that appeared on the Agriculture Queensland Facebook page on 27 August 2020.
  - Project-related post that appeared on the Agriculture Queensland Facebook page on 9 November 2021.

Presentations

- Regular updates were provided at the inshore fishery working group meetings by the PI (scientific member on the working group). These included:
    - An overview of the project aims, scope and progress to-date including information related to the reproductive biology and spawning periodicity, delivered on 1 July 2021.
    - A general update on the project progress, delivered on 7 December 2021.
    - An overview of the results related to genetic population structure and ageing information, delivered on 23 July 2022.
- Presentation on '*The population biology of Protonibea diacanthus from Eastern Australia'* at the 2023 Australian Society for Fish Biology Conference, Gold Coast.

Scientific literature

- The learnings from this project have been published in the "Stock Assessment of Queensland East Coast Black Jewfish *Protonibea diacanthus*, Australia, with data to December 2021" which can be found here: Stock assessment program | Department of Agriculture and Fisheries, Queensland (daf.qld.gov.au)
- Two scientific manuscripts have been submitted to international peer-reviewed journals:
    - Williams et al., (in review) *Reproductive biology of Black Jewfish (*Protonibea diacanthus*) off the East coast of Australia*: The Journal of Fish Biology
    - Williams et al., (in review) *Age and growth of Black Jewfish (*Protonibea diacanthus*) off the East coast of Australia*: The Journal of Fish Biology

Appendix 1. Project Staff

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- Dr Adam Barnett, Research Fellow James Cook University & Principal Scientist Biopixel Oceans Foundation

Appendix 2. Intellectual property

No intellectual property has been generated by the project

Appendix 3. References

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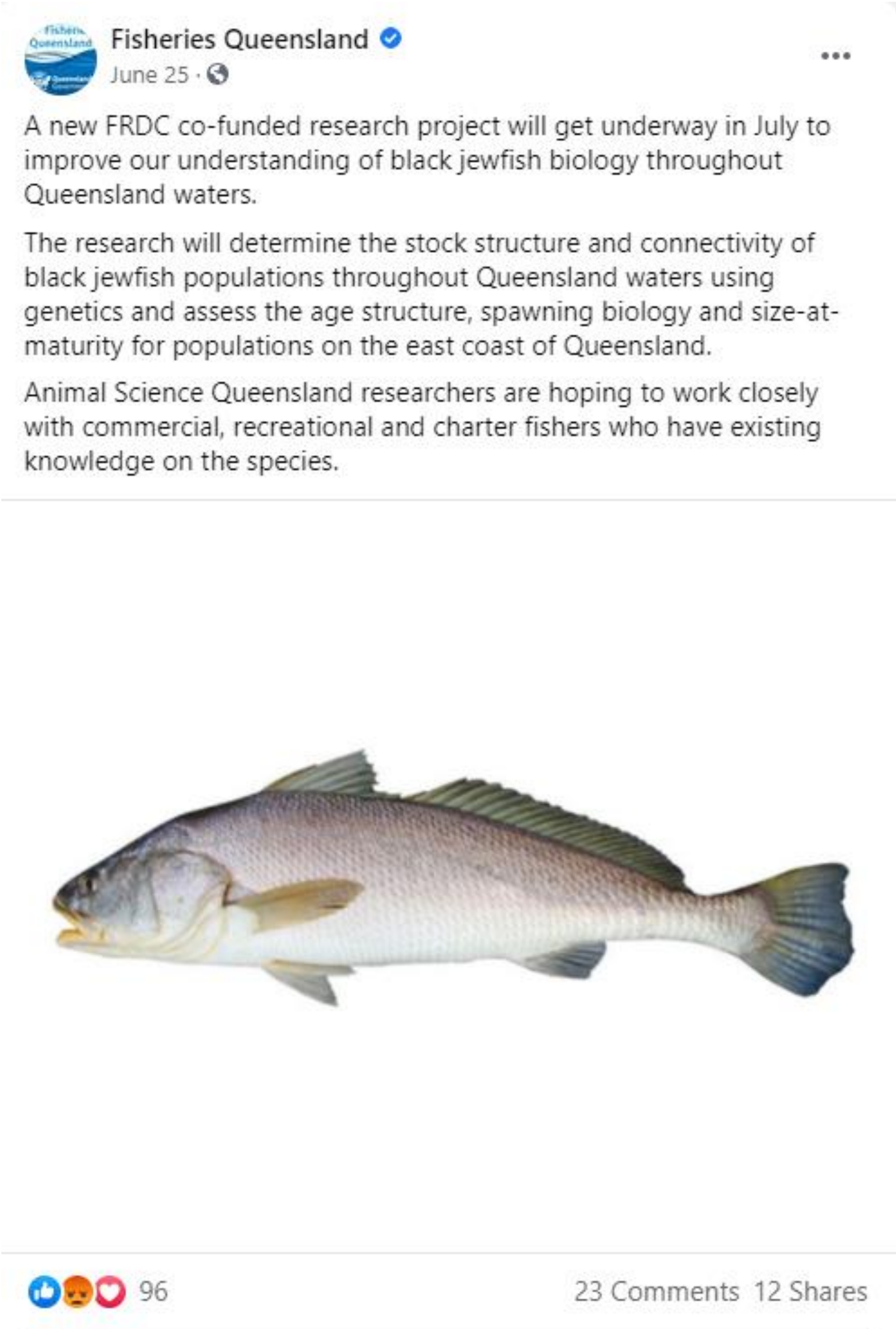
Appendix 4. Project media



Fisheries Queensland

June 25 ·

A new FRDC co-funded research project will get underway in July to improve our understanding of black jewfish biology throughout Queensland waters.

The research will determine the stock structure and connectivity of black jewfish populations throughout Queensland waters using genetics and assess the age structure, spawning biology and size-at-maturity for populations on the east coast of Queensland.

Animal Science Queensland researchers are hoping to work closely with commercial, recreational and charter fishers who have existing knowledge on the species.

96

23 Comments 12 Shares

Figure 1: Project-related post that appeared on the Fisheries Queensland Facebook page on 25 June 2020.

Figure 2: Project-related post that appeared on the Agriculture Queensland Facebook page on 27 August 2020.

Figure 3: Project-related post that appeared on the Agriculture Queensland Facebook page on 9 November 2021.

New Queensland study to shed light on Black Jewfish population

Published Friday, 29 May, 2020 at 01:30 PM

Minister for Agricultural Industry Development and Fisheries
The Honourable Mark Furner

Queensland Government scientists will assess the population biology of Black Jewfish in a new research project to better understand stocks and manage the species.

Minister for Agricultural Development and Fisheries Mark Furner said the three-year priority project would provide crucial information to help protect the sustainability of Black Jewfish.

“We need to know more about Black Jewfish on the east coast of Queensland to inform future assessments and management decisions that will lead to a long-term sustainable resource,” Mr Furner said.

“The outcomes of this research project should give fishery managers an improved understanding of the biology of Black Jewfish stocks to be able to set catch limits for the species.”

Mr Furner said scientists will work closely with commercial, recreational and charter fishers to collect samples and learn from their existing knowledge of Black Jewfish.

“While the research will be Queensland-wide, there will be a strong focus around the Black Jewfish hotspots such as Mackay and Rockhampton,” Mr Furner said.

The research project’s objectives are to:

- Determine the stock structure and connectivity of Black Jewfish throughout Queensland waters using genetics; and
- Assess the age structure, spawning biology and size-at-maturity for Black Jewfish populations on the east coast of Queensland.

Funded by the Fisheries Research and Development Corporation, scientists from the Queensland Department of Agriculture and Fisheries will conduct the research project which commences on 1 July 2020.

Stakeholders who would like to participate can contact the project team by emailing samuel.williams@daf.qld.gov.au.

Since 2017, there has been a rapid increase in targeted commercial fishing of Black Jewfish following a rise in market demand for their swim bladders, which are sold fresh or dried in South-East Asia.

In May 2019, the Queensland Government introduced a total allowable catch limit of 20 tonnes for Black Jewfish in response to escalating catches and concerns about sustainability.

https://statements.qld.gov.au/statements/89924

East coast black jewfish season closed after catch limit reached

News release | 08-Mar-2021

The commercial catch limit for black jewfish in Queensland's east coast waters has been reached, putting the species off limit to all fishers until next year.

Fisheries Queensland Director of Management and Reform Kimberly Foster said the 20-tonne limit for east coast black jewfish was fulfilled on 8 March 2021.

"Black jewfish is now a no-take species for commercial and recreational fishers in east coast waters until the fishery reopens on 1 January 2022," she said.

"I remind all fishers that the no-take applies to all east coast waters, while the key areas where black jewfish aggregate at Hay Point and the Dalrymple Bay coal terminal are closed to all fishing to protect the species".

"Fishing for black jewfish in the Gulf of Carpentaria remains open at this stage until a 6-tonne annual limit is reached."

Ms Foster said the Queensland Government took swift action in 2019 to prevent a collapse of black jewfish stocks.

"Black jewfish are vulnerable to overfishing and there is a risk of black-marketing due to the extremely high market prices for their swim bladders," she said.

"Any commercial or recreational fisher found in possession of black jewfish during the closure will be in breach of the *Fisheries Act* and could face a maximum fine of $133,450.

"Any fisher found in possession of commercial quantity of black jewfish, with the intention of black marketing the fish, may be subject to a maximum fine of $400,350 or three-years imprisonment."

Ms Foster also thanked fishers and processors for their involvement in a Queensland Government research project to assess the population biology of black jewfish.

"By collecting and donating fish samples during the season, they are making a significant contribution to science so we can better understand stocks and manage the species," she said.

The following rules apply to ensure the sustainability of black jewfish:

- a commercial catch limit of 20 tonnes per year on the east coast and 6 tonnes per year in the Gulf of Carpentaria;
- a reduction in the recreational in-possession limit from 2 to 1 for recreational fishers, with black jewfish becoming a no-take species when the commercial catch limit is reached;
- a requirement for black jewfish, scaly jewfish and mulloway to be kept whole while on board a vessel in order to prevent processing of the fish at sea to remove the valuable swim bladders; and
- closures to all fishing around key aggregation areas of Hay Point and Dalrymple Bay coal terminal.

For information about the management of black jewfish in Queensland visit www.daf.qld.gov.au (http://www.daf.qld.gov.au).

Follow Fisheries Queensland on Facebook, Instagram and Twitter (@DAFQld).

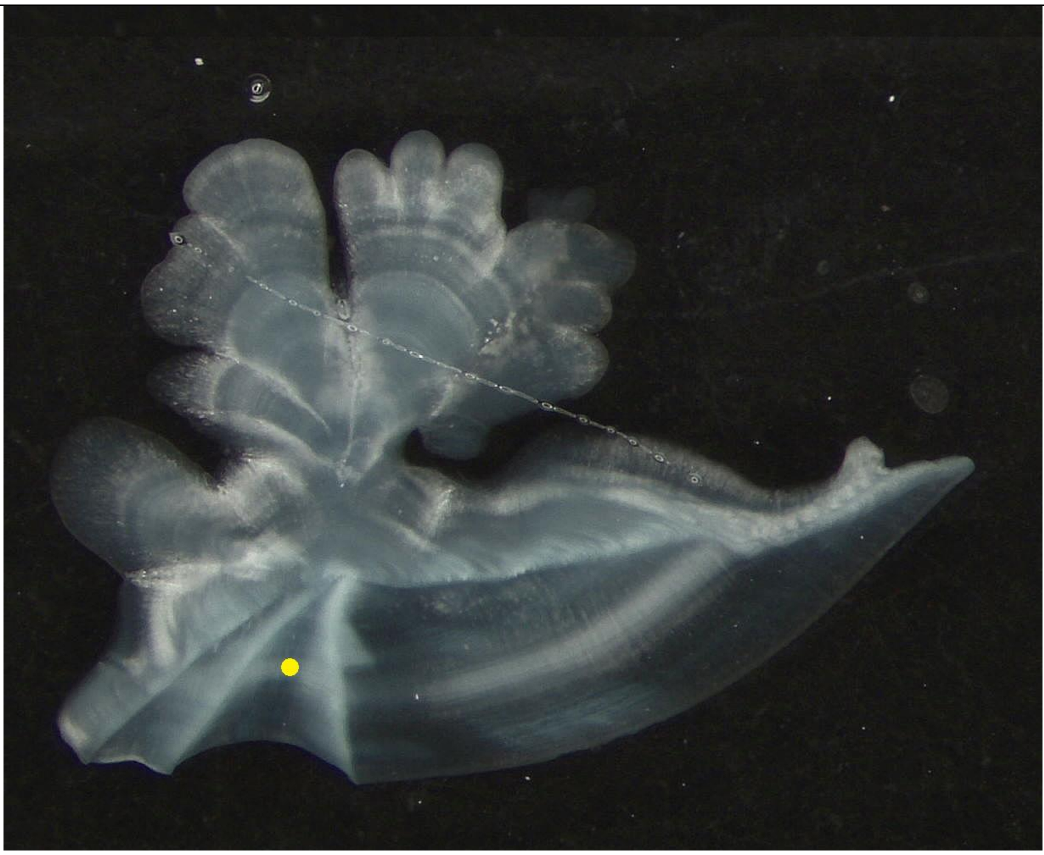
Media contact: DAF Media, media@daf.qld.gov.au

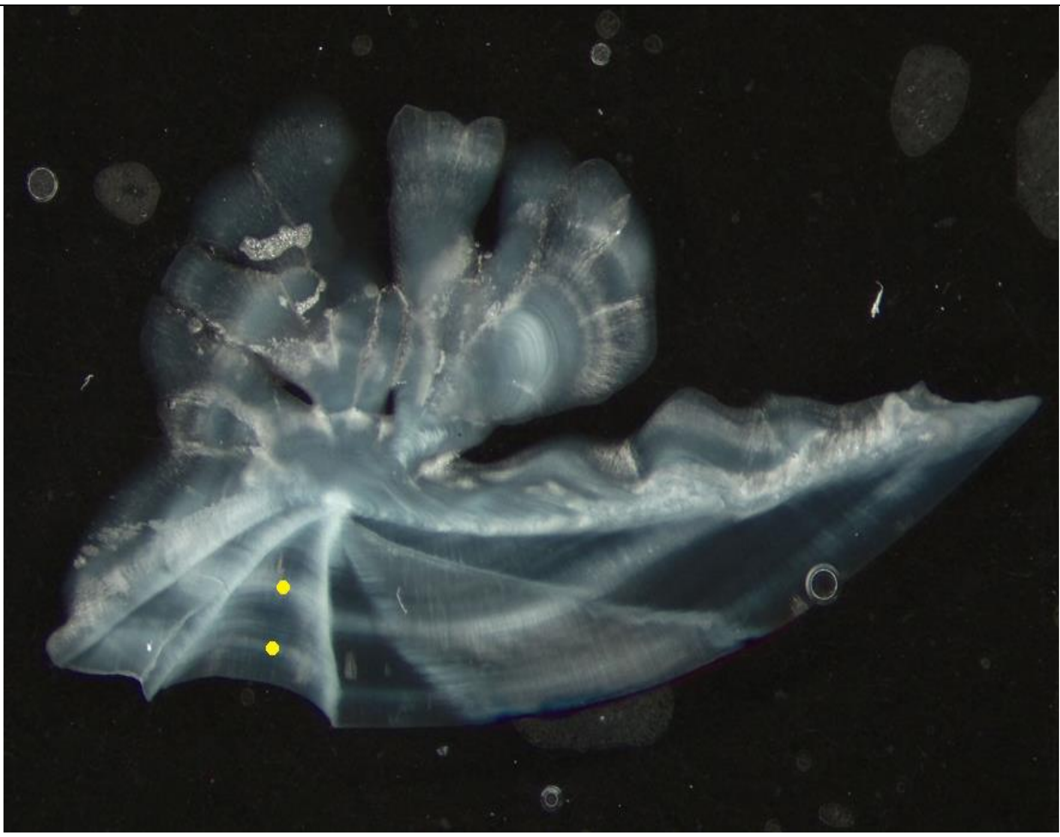
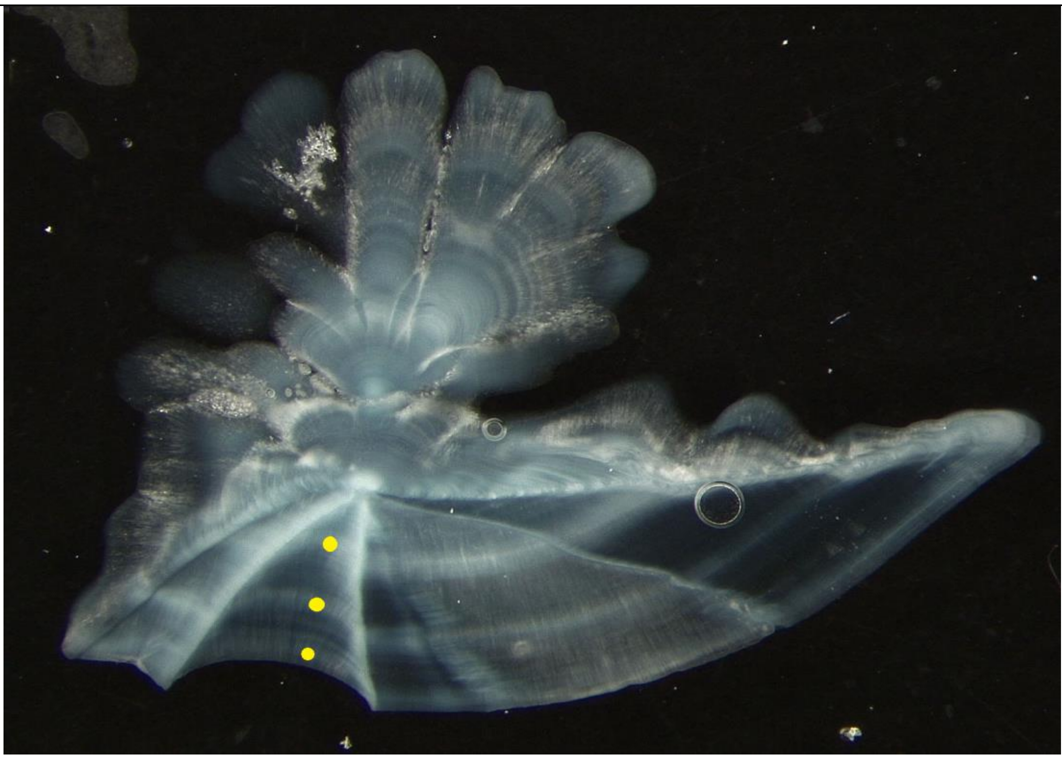
Appendix 5. East Coast Black Jewfish Stock Assessment

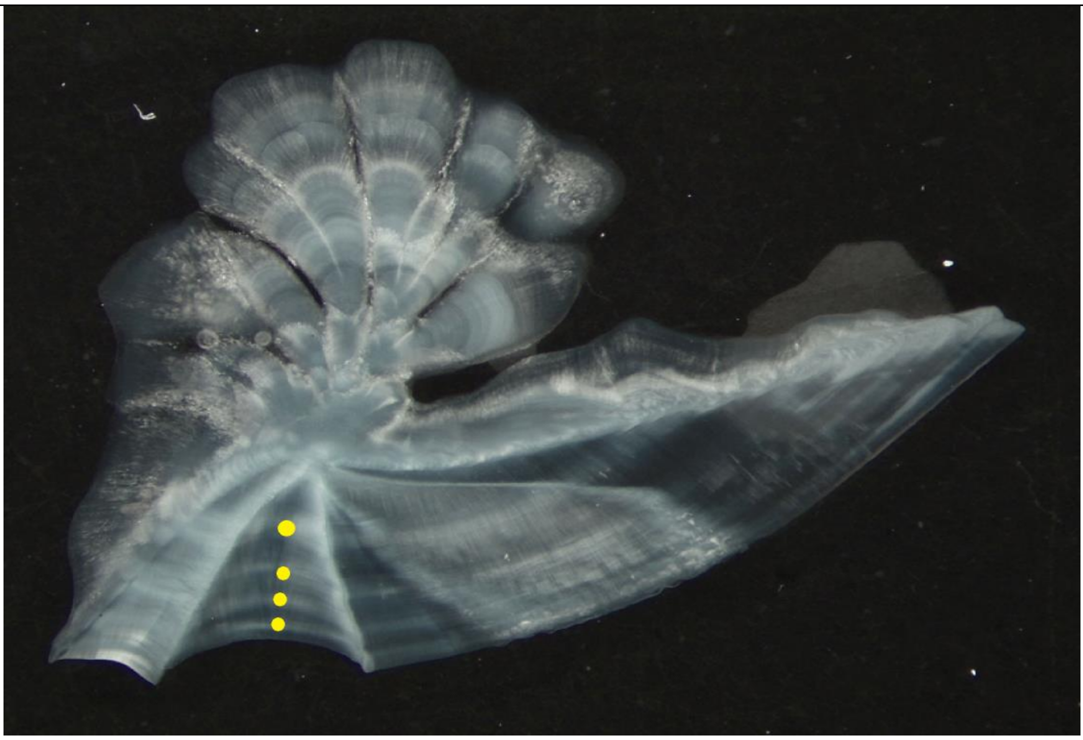
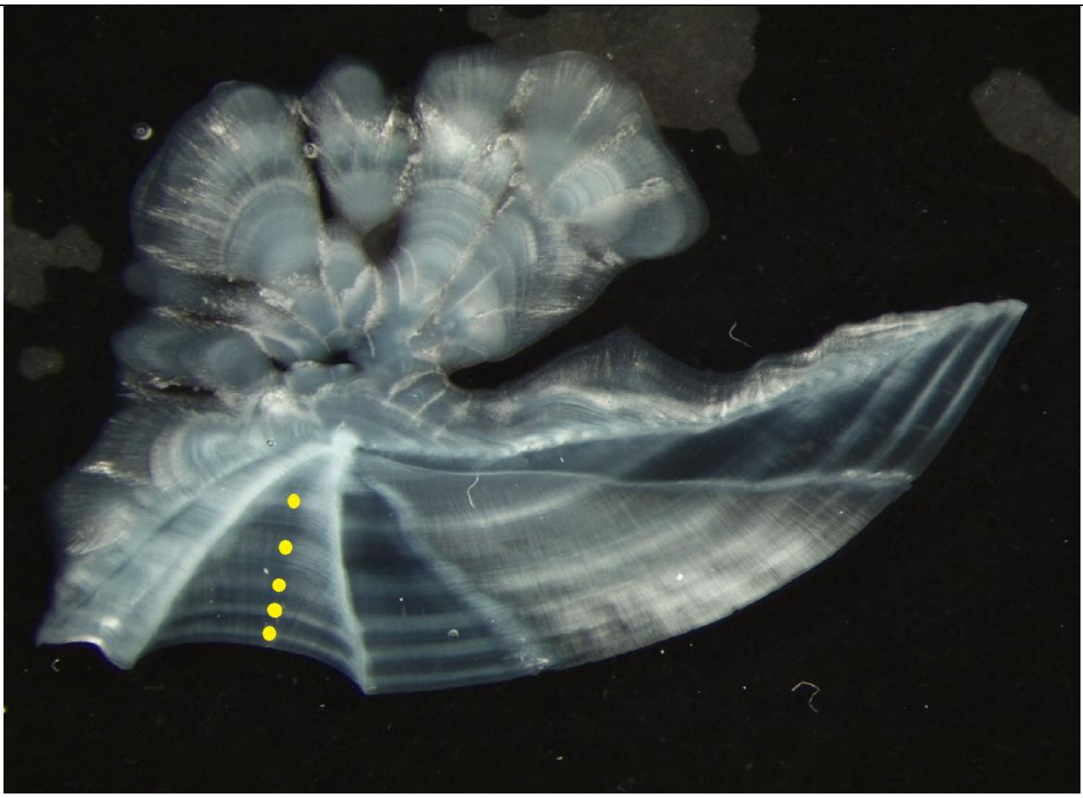
https://era.daf.qld.gov.au/id/eprint/8870/

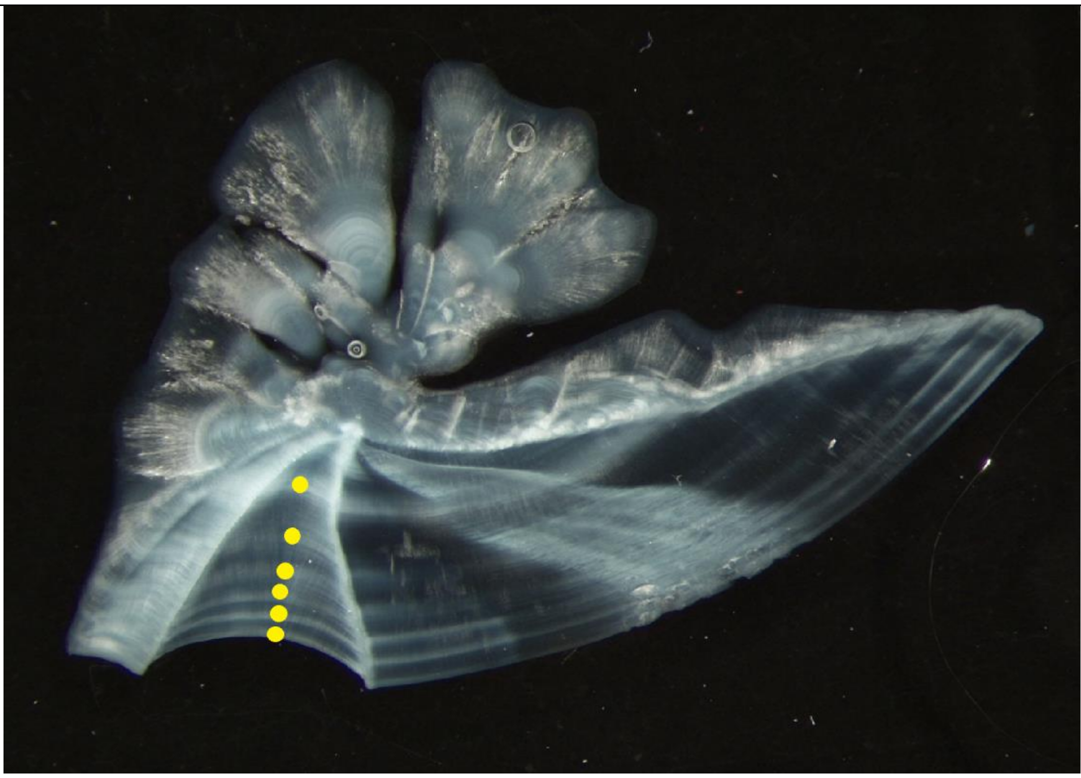
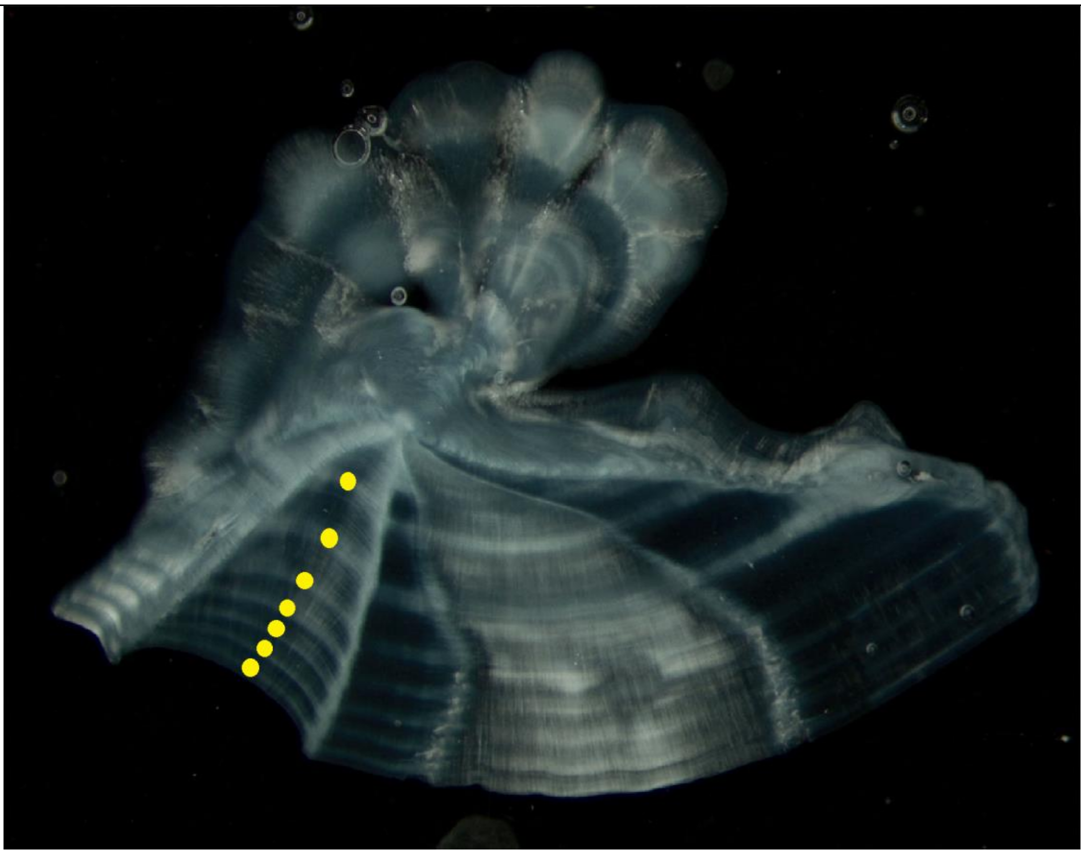
Appendix 6. Black Jewfish Otolith Ageing - Example Images for each increment count

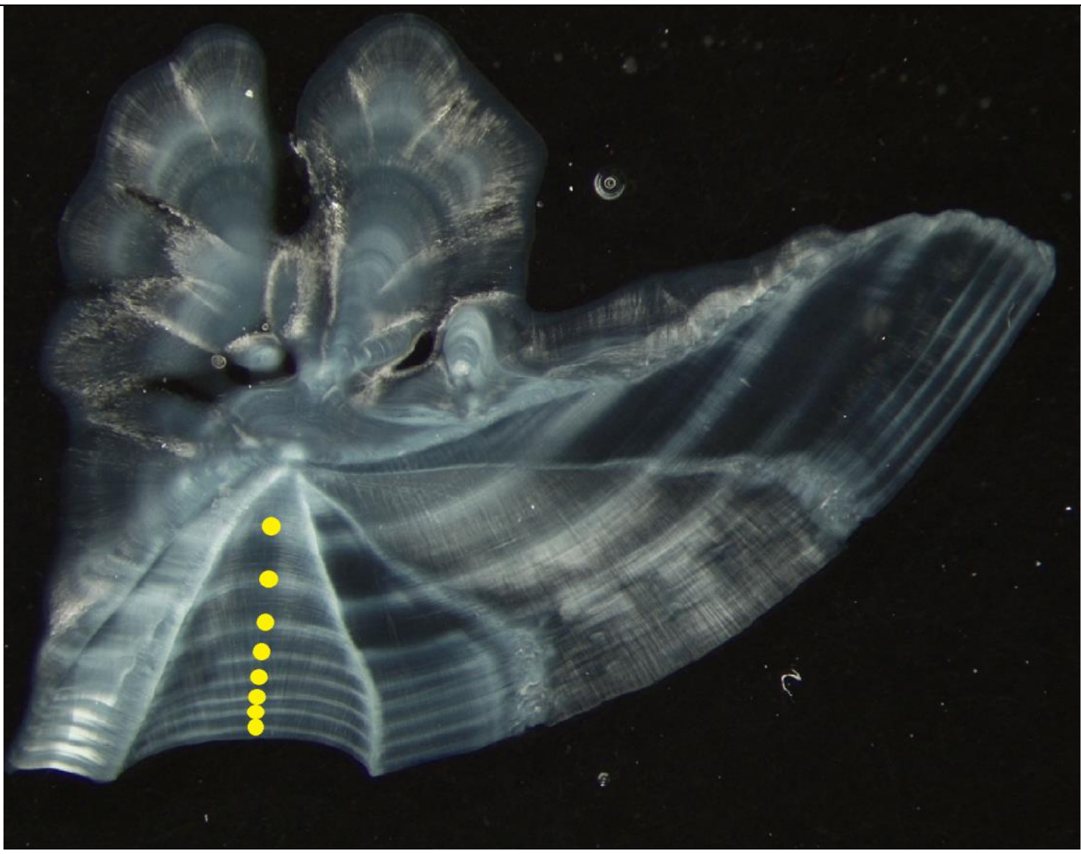
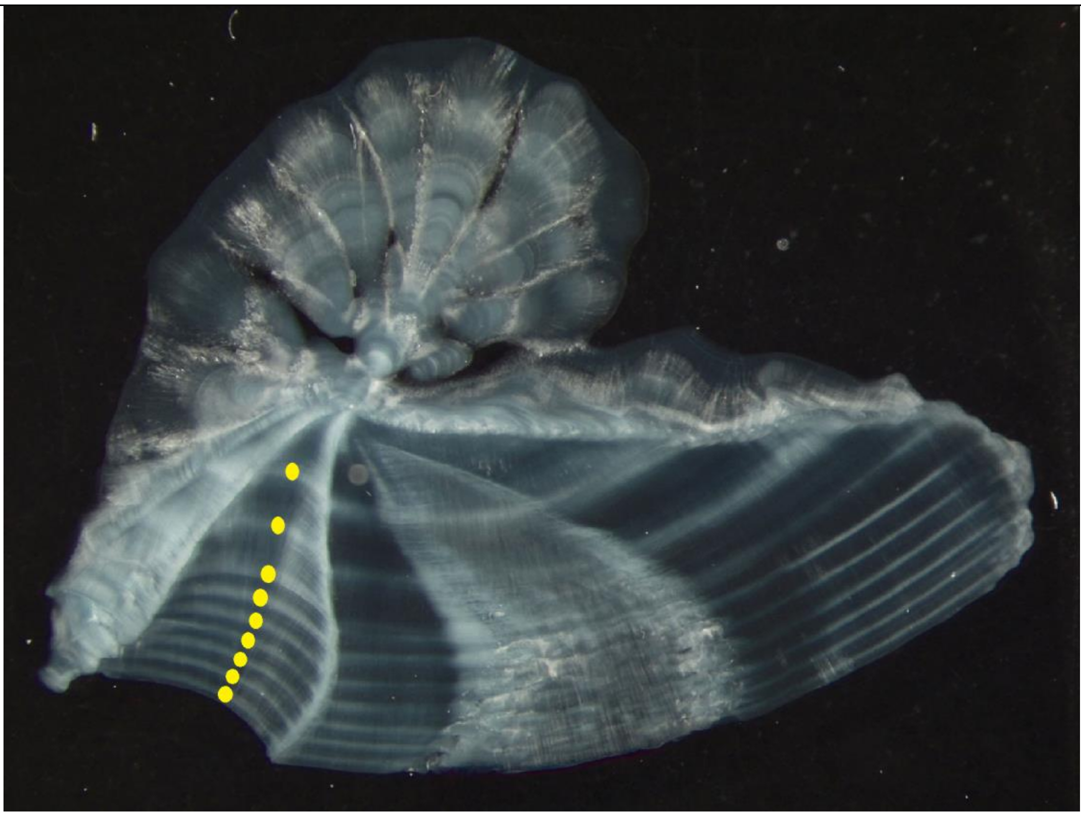
Please contact the principal investigator for a full otolith ageing protocol.

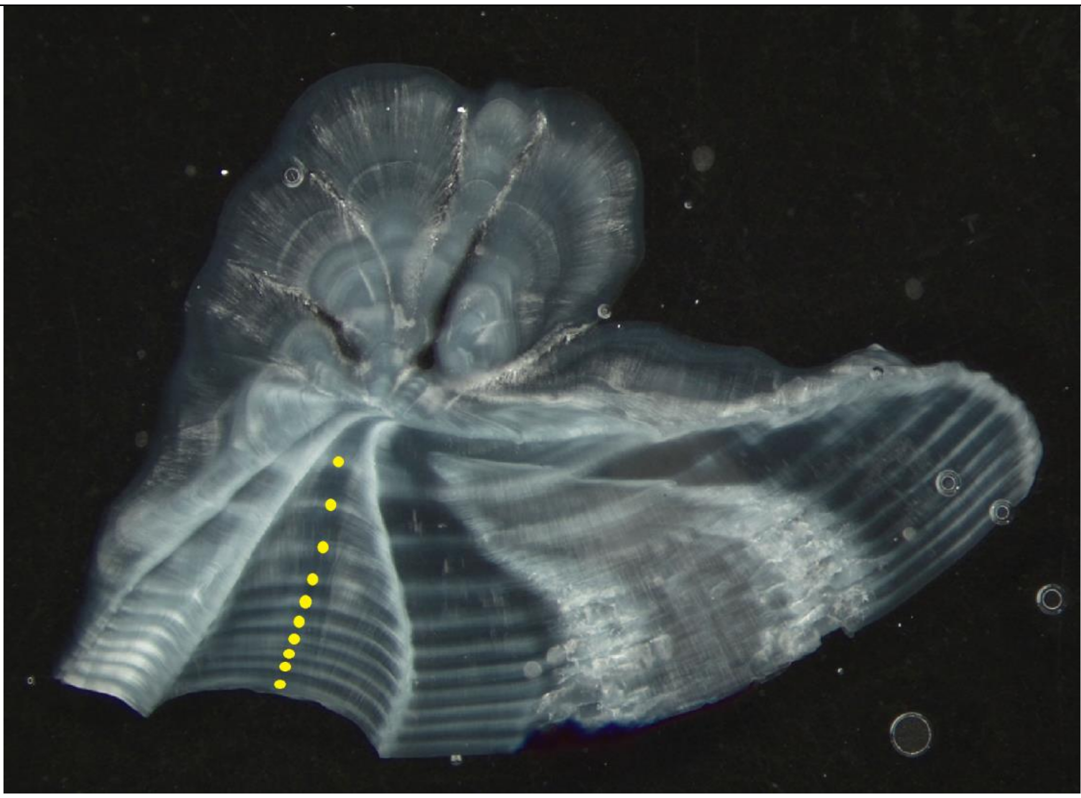
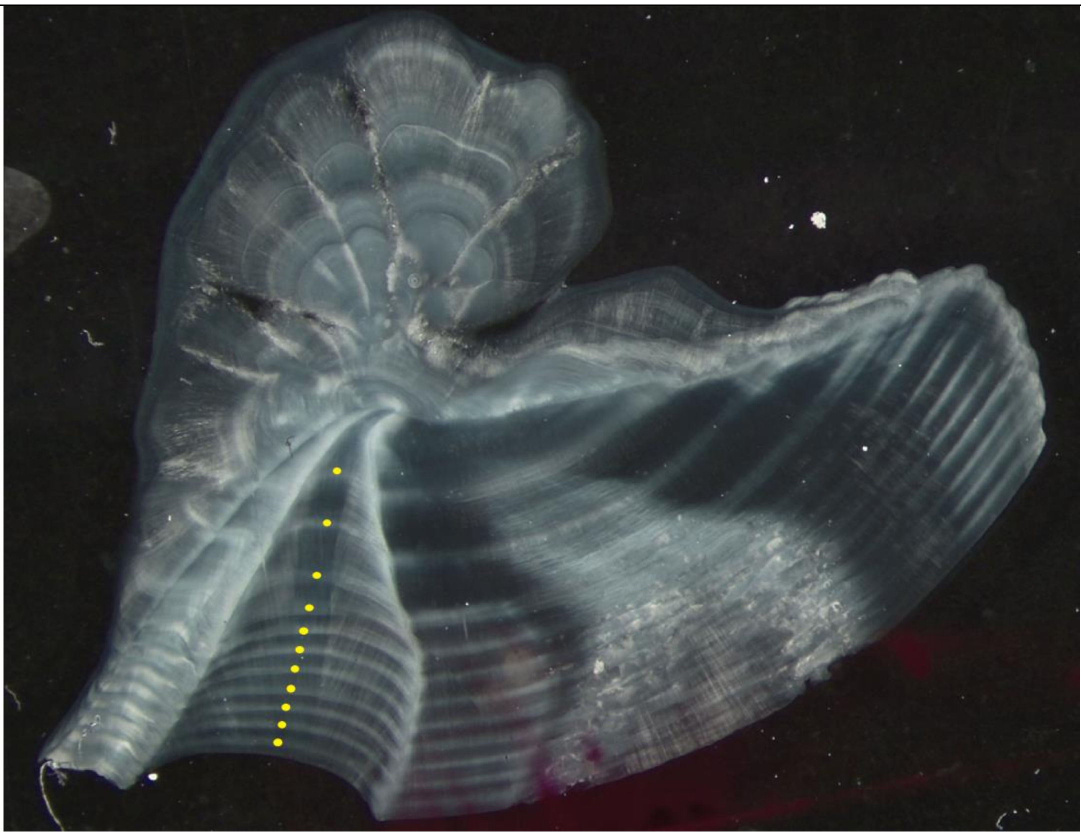
Increment count (edge type)	Annotated example annotated otolith images
1 (intermediate)	

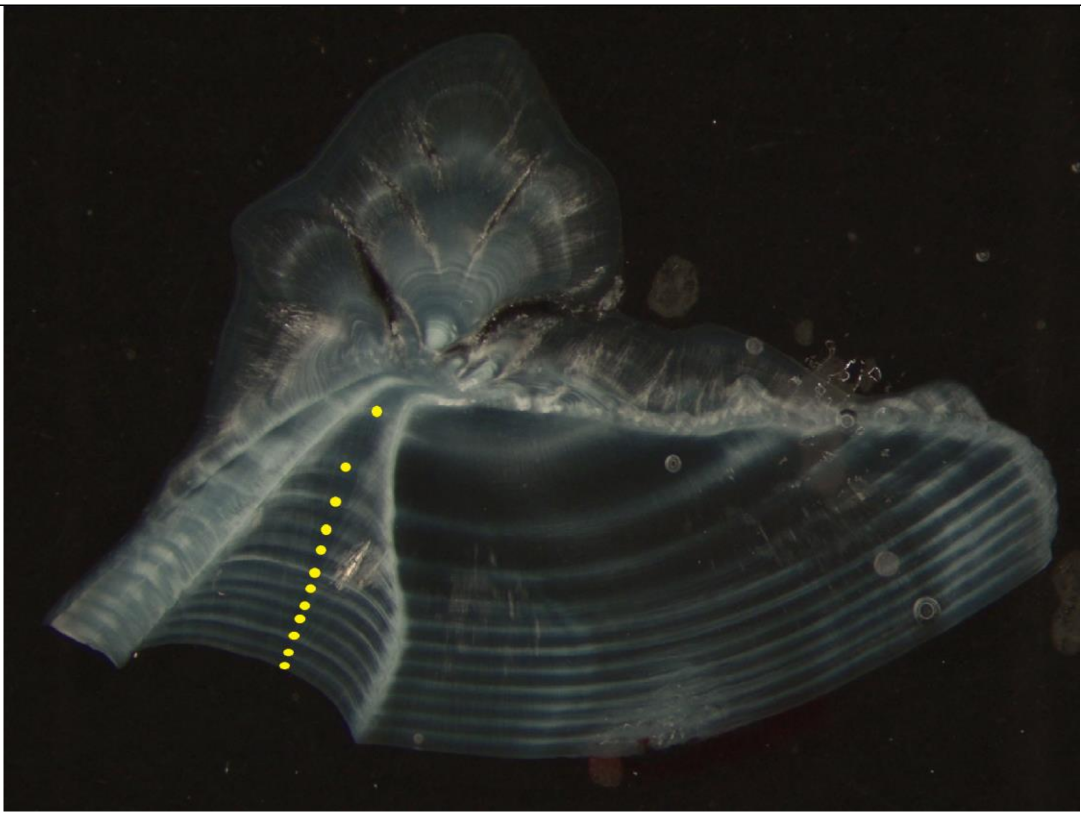
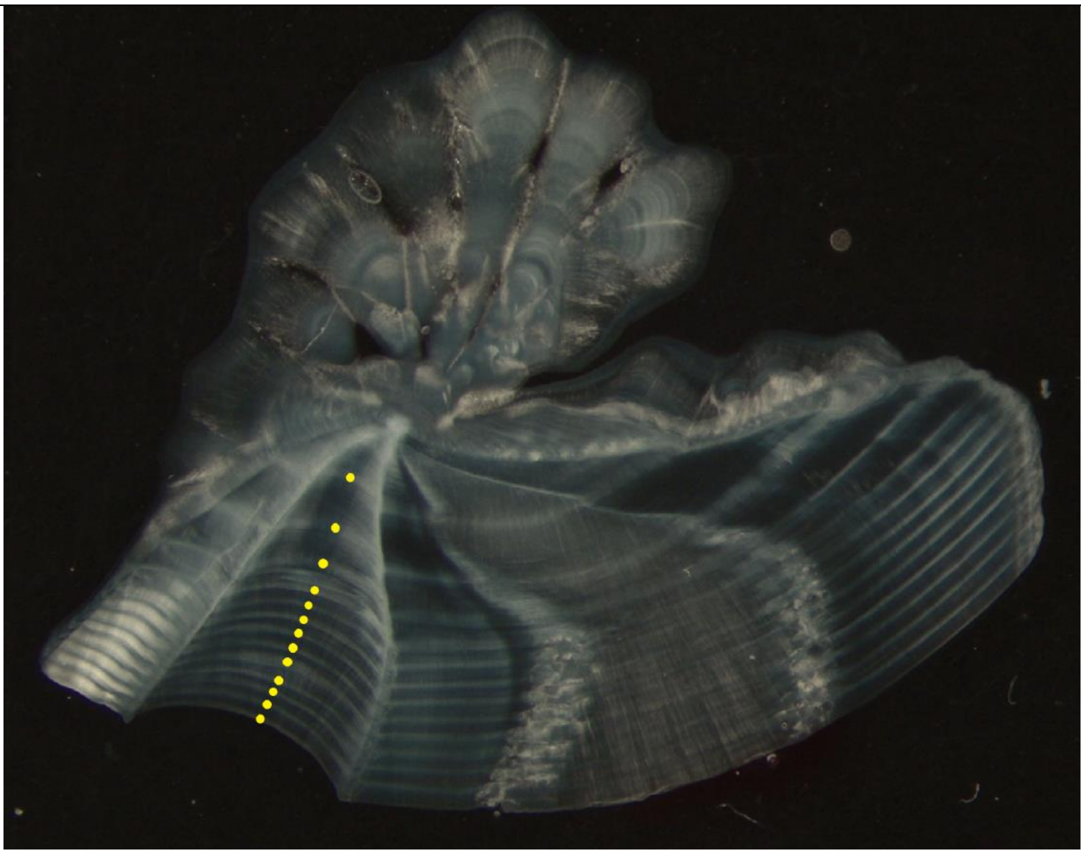
2 (intermediate)	
3 (New)	

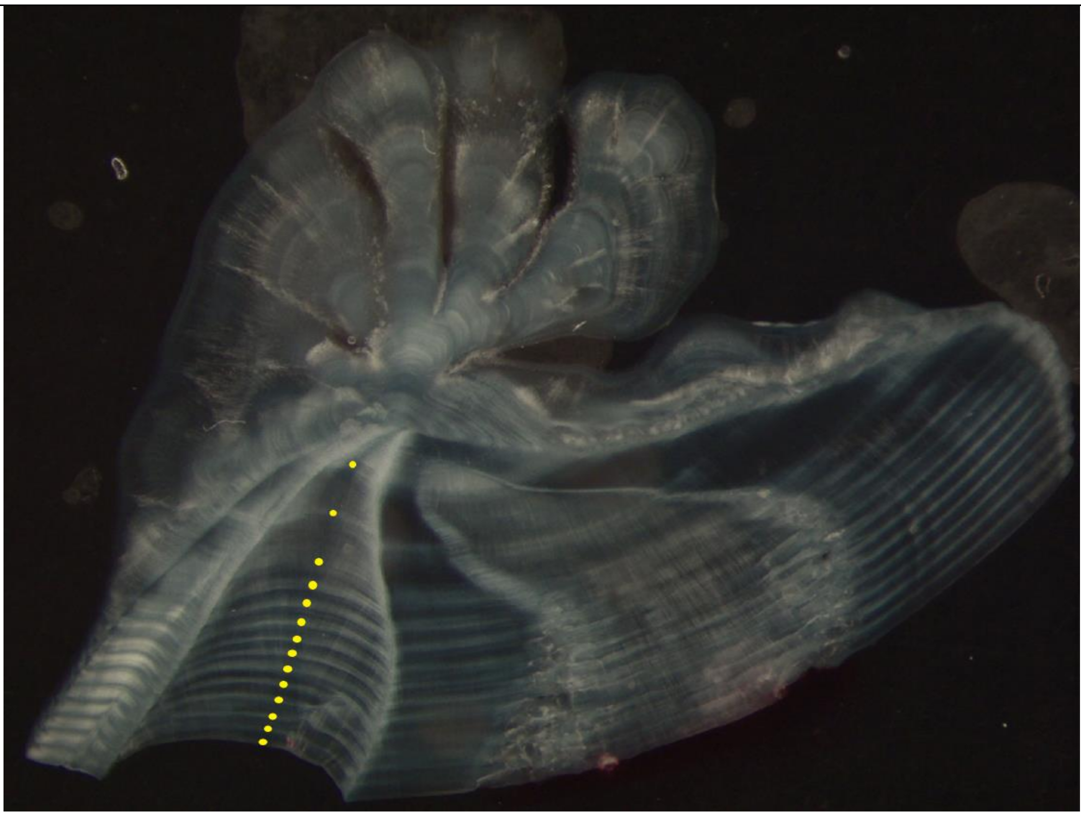
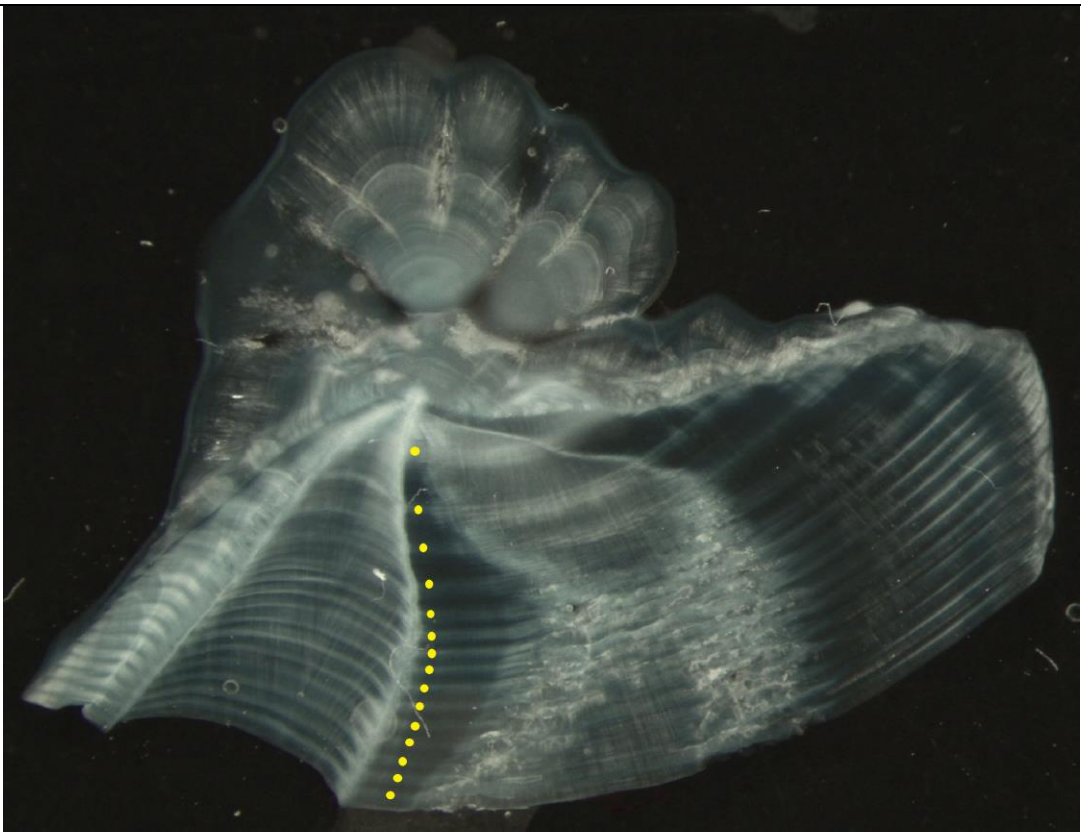
4 (intermediate)	
5 (intermediate)	

6 (new)	
7 (intermediate)	

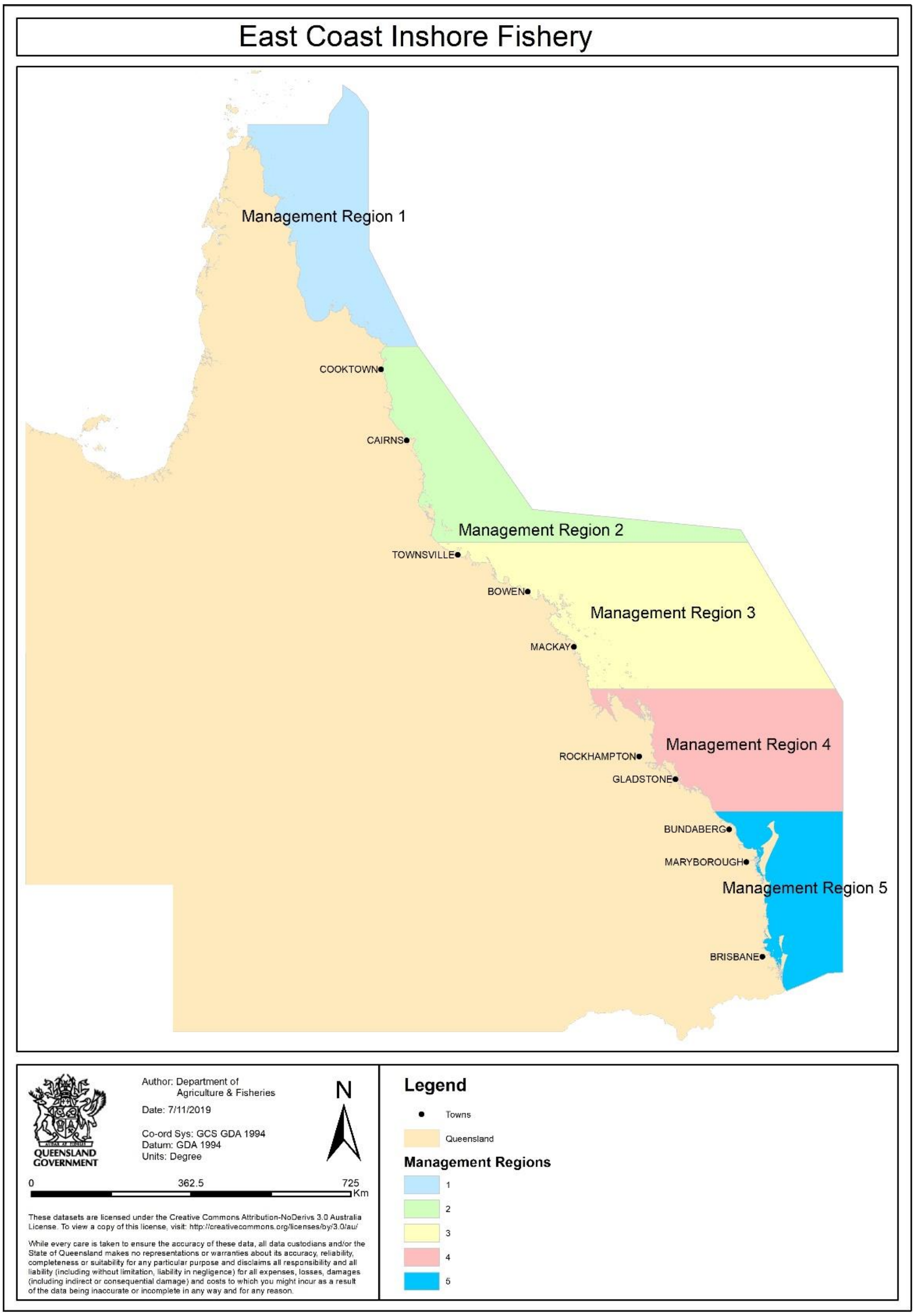
8 (intermediate)	
9 (intermediate)	

10 (intermediate)	
11 (wide)	

12 (new)	
13 (new)	

14 (intermediate)	
15 (wide)	

Appendix 7. Map of the East Coast Inshore Fishery Management Regions



FRDC FINAL REPORT CHECKLIST

The final report checklist can now be filled in when submitting your final report deliverable in FishNet.