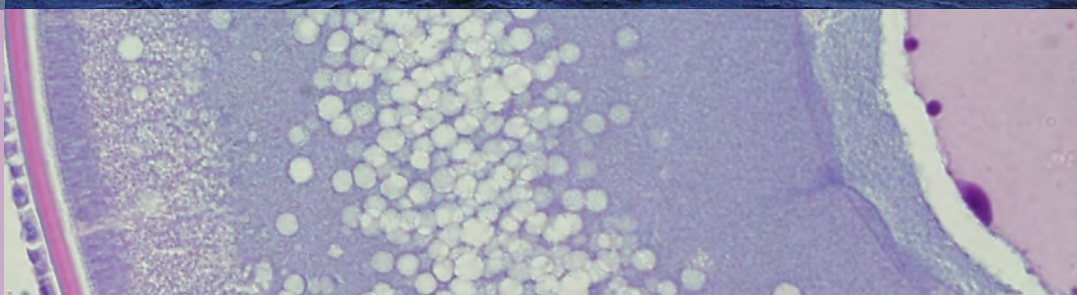
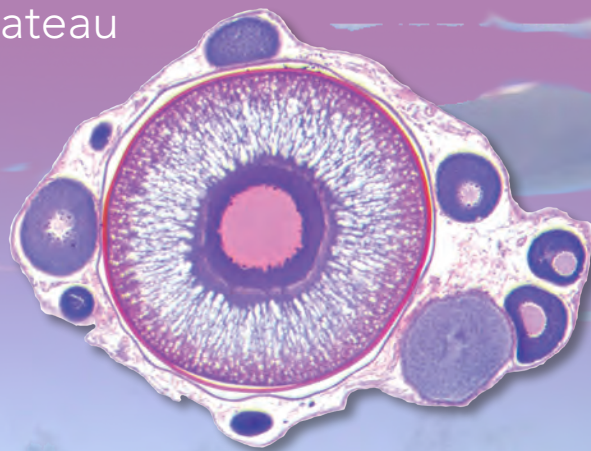


The spawning dynamics of patagonian toothfish in the Australian EEZ at Heard Island and the McDonald Islands and their importance to spawning activity across the Kerguelen Plateau



## FRDC project TRF 2010/064 FINAL REPORT

D. C. Welsford, J. McIvor, S.G. Candy, G.B. Nowara

July 2012



Australian Government  
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**Draft Final Report**

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## **Non-Technical Summary**

2010/064	The spawning dynamics of Patagonian toothfish in the Australian EEZ at Heard Island and the McDonald Islands and their importance to spawning activity across the Kerguelen Plateau
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### **Objectives**

1. Describe the size distribution, spatial extent and dynamics of toothfish in spawning aggregations in the Australian EEZ at Heard Island and McDonald Islands (HIMI) based on data collected from commercial fishing and survey data.
2. Produce refined estimates of age and size at maturity for male and female toothfish in the HIMI fishery.
3. Test the sensitivity of current toothfish harvest strategies to revised estimates of toothfish age and size at maturity, and the contribution of HIMI spawning aggregations to overall spawning activity across the Kerguelen Plateau.

## Outcomes Achieved to Date

The outputs of this project have contributed to the following outcomes:

**1. Improved stakeholder (including the Australian fishing industry, AFMA and CCAMLR) confidence in the results of HIMI stock assessments and Kerguelen Plateau-wide toothfish population models to provide advice on long term sustainable catch limits.**

As a result of this project, data is now available for the first time to develop stock assessment models that incorporate population specific estimates of size and age at maturity. This resolves a significant source of uncertainty in previous assessments that included maturity estimates derived from data collected outside of the Australian EEZ, providing increased confidence to resource managers and other stakeholders that these models will ensure harvest levels are ecologically sustainable.

This has benefitted stakeholders and Australia through:

**2. The establishment of the presence of spawning toothfish within the Australian EEZ.**

Prior to this project, the nearest substantial spawning location to the HIMI fishery was in the French EEZ west of Kerguelen. The knowledge that toothfish spawning activity occurs at several locations along the Kerguelen Plateau, including in the HIMI EEZ will improve the assessment of the impact that past commercial and illegal fishing has had in the region. It is also an important step in developing strategies for long term sustainable harvest that take into account the spatial complexity of the populations of toothfish that interact across the Kerguelen Plateau.

**3. A higher probability of maintenance of toothfish spawning biomass at or above target levels in the long term as required by the Commonwealth Harvest Strategy Policy (HSP) and CCAMLR.**

The results of sensitivity tests and 35 year projections in this project indicate that the current harvest rate within the Australian EEZ at HIMI has less than a 10% chance of resulting in the toothfish spawning stock being depleted below 20% of the estimated median pre-exploitation biomass (Bo), and has a greater than 50% chance to remain at or above 50% (Bo). Therefore the current harvest rate satisfies the CCAMLR decision rules, and the current target and limit reference points as defined by the HSP for the HIMI fishery.

The results of this project have been communicated to AFMA and industry stakeholders in the form of a draft report, and will be discussed at the forthcoming Subantarctic Resource Assessment Group and industry meetings in August 2012.

This report will also be presented at CCAMLR in October 2012.

For most of its 15 year history, very few spawning Patagonian toothfish had been recorded in the fishery in the Australian EEZ at Heard Island and the McDonald Islands (HIMI), despite comprehensive observer coverage and data collection. The fact that toothfish were known to move large distances during their lives suggested that most spawning activity may be occurring to the northwest, in the adjacent French EEZ around the Kerguelen Islands. However, in 2009 a number of spawning toothfish were captured during prospective longline fishing on the deep slope to the west of HIMI. FRDC and industry funding to collect data and samples to enable an investigation of spawning activity was approved in 2010, and over 11 000 fish were sampled during a winter 2011 longlining voyage that included sampling across a large area of the deep slope to the northwest, west and south of HIMI. For the first time, male and female gonads at all stages of reproductive development were collected, enabling the histological analysis of gonads of both sexes, the calibration of macroscopic staging criteria and the development of the HIMI specific estimates of size and age at maturity.

The data shows that toothfish at HIMI increase gonad size and spawn throughout the late autumn/ winter months (May-August), and appear to concentrate spawning activity on slopes to the northwest, west and south of HIMI at 1700-1900m depth. Strong biases in sex ratios of the catch at length were found, with size classes above 100 cm dominated by females.

Comparison of histological analysis with macroscopic staging indicated that many fish that had spawned, as confirmed by the presence of post-ovulatory follicles, return to a resting stage which is macroscopically indistinguishable from first maturing fish. A large number of females of all size classes had low gonad weights as a proportion of body weight and low macroscopic stages even during the spawning season. Therefore it seems likely that a substantial proportion of the mature female population do not spawn every year, possibly due to the relatively high cost of provisioning large quantities of large yolky eggs.

To investigate the impact of misclassification of maturity based on macroscopic staging, sex-specific and pooled size-at-maturity ogives were fitted assuming either: i) stage 2 and above were mature, accounting for fish that were mature but resting when sampled, or: ii) stage 3 and above were mature, including only those fish with clear macroscopic characteristics indicating that they were likely to spawn or had recently spawning in the season sampled. In the latter case, the resulting ogives gave an unrealistically high estimate of size at maturity for females, with less than 50% of fish mature in the largest size class. In all cases strong sexual dimorphism in maturity ogives were found, with males maturing at significantly smaller sizes than females, which seems to be common to all toothfish stocks in the Southern Ocean.

Sensitivity testing of the current HIMI stock assessment model to the new ogives revealed a strong effect on the estimate of median pre-exploitation spawning stock biomass ( $B_0$ ), but a less pronounced effect on the estimate of status (current spawning stock biomass as a proportion of  $B_0$ ).

This study has identified that several areas on the deep slope to the west and south of HIMI supports spawning activity, and this data, as well as the fact that large areas of apparently suitable habitat remain to be sampled, indicates that it is likely that spawning within the Australian EEZ makes an important contribution to the overall spawning output of the populations on the Kerguelen Plateau. The development of spatially explicit models of the population dynamics of toothfish at the scale of the Kerguelen Plateau is a priority to quantify the importance of the known French and Australian spawning locations to the populations across the region. Furthermore it is recommended that sex-specific estimates of catch at age, growth and size at maturity be incorporated in future stock assessments.

### **Keywords**

Patagonian toothfish, *Dissostichus eleginoides*, size-at-maturity, age-at-maturity, Heard Island and McDonald Islands, integrated assessment



## **Acknowledgements**

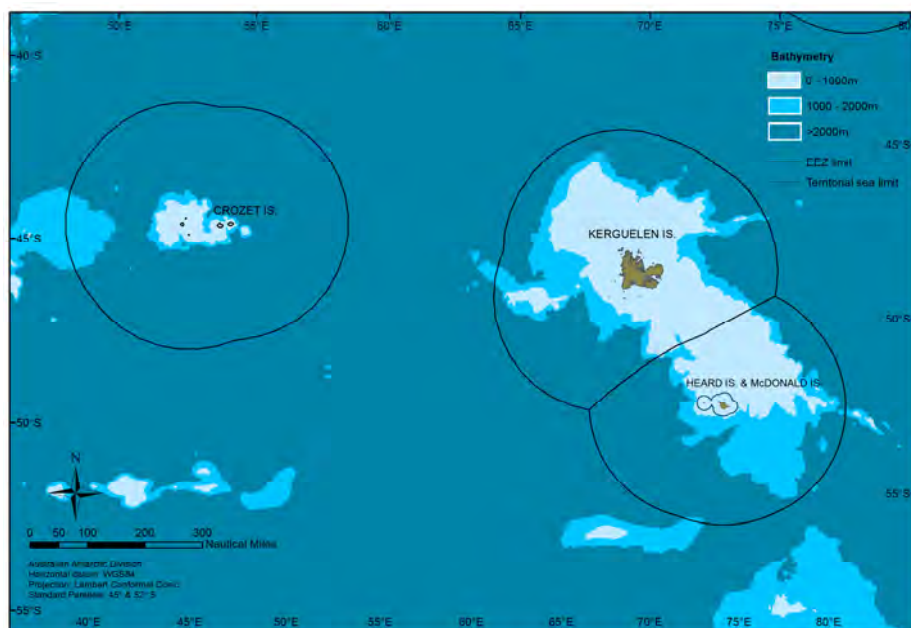
We gratefully acknowledge the invaluable assistance of the crew and skippers aboard Austral Fisheries P/L and Australian Longline P/L vessels in collecting data for this project. In particular we thank the crew of the FV *Antarctic Chieftain* and skipper Tangi Kitson for conducting the fishing program during winter 2011 that lead to the collection of many of the samples and data used in this project. We also thank the crew aboard the FV *Austral Leader II* and skipper Yuri Guskov for agreeing to not fish in the sampling area for three months while the 2011 sampling program took place.

Our sincere thanks also, to the Australian Fisheries Management Authority scientific observers and Data Collection Officers from Fisheries Audit Services and Capfish P/L for assisting with the collection of data on maturity stages since the fishery commenced. Many thanks also to Dick Williams for establishing the original scientific data collection program, Tim Lamb, Troy Robertson and Steve Cameron for database management, and Graeme Ewing for assistance with preparing histological sections.

This study was funded through the Fisheries Research and Development Corporation Tactical Research Fund Project 2010/064, Austral Fisheries P/L and Australian Longline P/L, with significant in-kind support from the Australian Antarctic Division.

## Background

The nototheniid genus *Dissostichus* contains two species, the Antarctic toothfish, *D. mawsoni* and the Patagonian toothfish *D. eleginoides*. The Patagonian toothfish has a broad geographic distribution, having been recorded from around all subantarctic islands, and extending as far north as 20°S along the west coast of South America (Arkhipkin *et al.*, 2003; Eastman, 1993; Gon and Heemstra, 1990). It is one of the most common demersal fishes in shelf and slope waters around the subantarctic Islands in the Indian Ocean Sector of the Southern Ocean, including the Australian EEZ around Heard Island and the McDonald Islands (HIMI) and the French EEZ around Kerguelen Islands (Duhamel and Hautecoeur, 2009; Williams and de la Mare, 1995) (Figure 1). Toothfish had been reported as a bycatch in Soviet demersal trawling in the region in the 1970s and became a target species in the French EEZ in the mid 1980s (Duhamel and Williams, 2011). Fishing for toothfish at HIMI commenced in 1997. Initially trawlers targeted fish in waters less than 1000m in depth and mainly caught juveniles. Since 2003, longlining has grown to become an increasingly important capture method at HIMI, leading to an expansion in the range of depths fished and size of toothfish captured (Welsford *et al.*, 2011; Welsford *et al.*, 2009). Further details on the development of the toothfish fishery at HIMI can be found in Patterson and Skirtun (2011), Welsford *et al.* (2011) and Constable and Welsford (2011).



**Figure 1.** Map of the main subantarctic island groups adjacent to the Australian Territory of Heard Island and McDonald Islands (HIMI) in the Indian Sector of the Southern Ocean. The Kerguelen and Crozet Islands to the north and northwest of HIMI form part of the Terres australes et antarctiques françaises (French Southern and Antarctic Lands). The Kerguelen Plateau refers to the large area of elevated seafloor around HIMI and the Kerguelen Islands. Bathymetry data courtesy of the Australian Antarctic Data Centre.

Patagonian toothfish are unusual in that they occupy a very broad depth range, with juvenile toothfish tending to be restricted to waters less than 1000m while larger adult fish are encountered in deep slope waters down to at least 2700m (Agnew *et al.*, 1999; Arkhipkin *et al.*, 2003; Arkhipkin and Laptikhovsky, 2010; Duhamel, 1987; Duhamel, 1993; López Abellán, 2005; Welsford *et al.*, 2011). As a result the Kerguelen Plateau represents a very large expanse of contiguous habitat for toothfish with over 640,000 km<sup>2</sup> of seafloor area between 0m and 2700m deep (AAD unpublished data).

There is increasing evidence that there is regular mixing between the populations of Patagonian toothfish across the Kerguelen Plateau and the Crozet Islands to the west. Direct evidence of mixing exists in the form of recaptures of tagged toothfish that have travelled over 1000 km from the Australian Exclusive Economic Zone (EEZ) around HIMI to the French EEZ around Kerguelen, and occasionally as far as the Crozet Islands (Welsford *et al.*, 2011; Williams *et al.*, 2002). Recently tagged fish released in the French EEZ have also been captured in Australian waters (AAD, unpublished data).

Genetic data also indicate little genetic differentiation between individuals collected in French EEZ around the Crozet Islands and Kerguelen Island and those from HIMI, suggesting that at least small number of individuals are regularly exchanged between these populations (Appleyard *et al.*, 2002; Appleyard *et al.*, 2004). Similar studies in the southwest Atlantic indicate that males may be more mobile than females (Shaw *et al.*, 2004), and this also appears to be the case for tagged individuals at HIMI, with males making up a higher proportion of individuals travelling more than 500 km from their release site (Welsford *et al.*, 2011).

However, development of more quantitative predictions regarding the distribution of Patagonian toothfish across the Kerguelen Plateau, such as what proportion of the stock resides in the French EEZ or the Australian EEZ and where the major sources and sinks of recruits are, have been hampered by the lack of data on key life stages, in particular early life history stages and spawning individuals.

Early life history stages such as eggs and larvae are very rarely encountered. The small amount of material that has been collected, primarily around South Georgia indicates the eggs of toothfish are very large (4.3-4.7 mm in diameter) and yolky (Kock and Kellermann, 1991). The eggs are pelagic, floating up into the top 700 m of the water column after fertilization, and have mostly been encountered over deep (2200+m ) oceanic waters (Evseenko *et al.*, 1995; Kellermann, 1989). Eggs hatch several months after spawning, with the yolk-sac larvae over 14.5 mm SL at hatching (Evseenko *et al.*, 1995; North, 2002). The pelagic larval phase is apparently prolonged, with relatively large (60-70 mm) individuals still retaining larval characters, up to 8 months after their likely spawning date (Evseenko *et al.*, 1995;

North, 2002). The majority of early larvae described to date have been captured in the upper 200m of the water column, and larger larvae tend to be found closer inshore than eggs, indicating that they may be capable of actively swimming inshore to settle, and/or exploiting oceanographic features to enhance onshore transport (Evseenko *et al.*, 1995; North, 2002). Despite extensive research trawling and collection of biological data from all commercial fishing operations, only a small number of recently settled (<20 cm) juvenile toothfish have been captured in the Australian EEZ (Welsford *et al.*, 2011). By contrast, recent surveys around Kerguelen Islands captured such juveniles in substantial numbers (Duhamel and Hautecoeur, 2009; Duhamel *et al.*, 2011), suggesting that the majority of small toothfish in the region settle on the plateau to the north and east of the Kerguelen island and then a proportion move south into the Australian EEZ by the time they are 2-3 years old where they are observed in research trawls (Welsford *et al.*, 2006; Welsford *et al.*, 2009).

Fish in spawning condition were also largely absent in samples from the Australian EEZ, whereas mature male and female toothfish were encountered in deep water (1000m or more) by demersal longliners at Crozet and in particular on the western margin of the Kerguelen plateau and on Skif Bank, with running ripe fish captured on Skif Bank during July and August (Duhamel *et al.*, 2011; Lord *et al.*, 2006). In the study by Lord *et al.* (2006), the smallest mature males were around 50 cm in length, and the estimated length at 50% maturity was 63 cm in males and 85 cm in females. This information, in combination with the genetic, tagging and size distribution data noted above, led to a hypothesis that all recruits on the Kerguelen plateau may derive from spawning activity in the French EEZ, with significant implications for Australian and French management of these toothfish stocks (Williams *et al.*, 2003).

In 2009, during commercial longline fishing in winter in deep slope waters west of HIMI, the first substantial numbers of toothfish with gonads in advanced reproductive stages were recorded by AFMA and industry observers. Recognising the importance of locating spawning activity, the AAD worked with representatives of the fishing industry and AFMA to collect data which confirmed the presence of spawning fish in winter of 2010. Subsequently this project was developed and supported by the FRDC Tactical Research Fund, and commenced in May 2011.

## Need

Stock assessment of the Heard Island and McDonald Islands (HIMI) toothfish fishery currently uses an integrated population model. This model relies on estimates of parameters that predict the productivity of the population e.g. growth rates, mortality rates, recruitment rates and spawning stock biomass (Candy and Constable, 2008).

Advice on sustainable catch limits for toothfish use decision rules developed by CCAMLR, which seek to ensure that there is sufficient escapement of spawning biomass to ensure the productivity of the stock is not reduced by fishing (Constable *et al.*, 2000). Hence stock assessments are very sensitive to estimates of size-at-maturity, as this determines the proportion of the population that is mature and contributing to the spawning stock. When this project was developed the size and age at which toothfish matured at HIMI was unknown. The estimate used in the model was derived from spawning fish sampled in the French EEZ around Kerguelen Island. There are clear linkages between the French and Australian stocks; however the differing fishing history and environment in the French EEZ means using the French data for HIMI toothfish may create bias in the assessment.

With the commencement of demersal longlining in 2003 and fishing of deeper slope waters in recent years, spawning toothfish were captured by demersal longlines for the first time in 2009 and again in July 2010, and hence an opportunity existed to collect data on spawning activity in the Australian EEZ for the first time. The urgency of this project was high due to the need for understanding of the spawning dynamics for assessments of the HIMI stock, as well as contributing to joint efforts by France and Australia to model the toothfish populations across the Kerguelen Plateau.

## Objectives

1. Describe the size distribution, spatial extent and dynamics of toothfish in spawning aggregations in the Australian EEZ at Heard Island and McDonald Islands (HIMI) based on data collected from commercial fishing and survey data.
2. Produce refined estimates of age and size at maturity for male and female toothfish in the HIMI fishery.
3. Test the sensitivity of current toothfish harvest strategies to revised estimates of toothfish age and size at maturity, and the contribution of HIMI spawning aggregations to overall spawning activity across the Kerguelen Plateau.

## Methods

### Biological sampling

As the Australian Longlining owned longliner *Antarctic Chieftain* was the vessel that had caught spawning fish in the 2009 and 2010 seasons, all sampling by the AAD Fisheries Research Officer employed under the project was conducted from aboard this vessel. Prior to sampling during the 2011 winter, agreement was reached between the two license holders in the HIMI fishery, Austral Fisheries P/L and Australian Longlining P/L allowing exclusive access by the *Antarctic Chieftain* to the sampling area to avoid interference from other commercial fishing activities.

Standard biological data was collected (when possible) from at least 50 randomly sampled fish from the catch on each line hauled during the fishing trip. Standard biological data included: total weight (g); total and standard length (cm); sex; macroscopic gonad stage; and gonad weight. Fish lengths were taken using an Electronic Fish Measuring Board (EFMB) designed and built at the AAD. Fish and gonad weights were taken using electronic motion compensated scales. Sex and macroscopic gonad staging was determined according to the scale proposed by Kock and Kellerman (1991) for Antarctic fishes (Table 1). The geographic coordinates at the start and finish of each line, setting and hauling times, depth and topography were also recorded. All data was entered directly into a Microsoft Access database using the Fishlog interface developed at AAD for routine data collection by observers aboard commercial fishing vessels in the subantarctic.

**Table 1. Criteria and stages used for macroscopic (naked eye) assessment of gonad development of toothfish, based on Kock and Kellerman (1991).**

Female Maturity Staging		Male Maturity Staging	
<b>1. Immature</b>	Small, firm, pink/translucent, no eggs visible to naked eye.	<b>1. Immature</b>	Small, whitish, narrow strips lying close to the vertebral column
<b>2. Maturing virgin or resting</b>	More extended, firm, small oocytes visible, giving ovary a grainy appearance	<b>2. Developing or resting</b>	White, flat, convoluted easily visible to the naked eye, about ¼ length of the body cavity
<b>3. Developing</b>	Large, starting to swell the body cavity, contains yellow-orange oocytes of two sizes	<b>3. Developed</b>	Large, white and convoluted, no milt produced when pressed or cut
<b>4. Gravid</b>	Large, filling or swelling the body cavity, when opened large ova spill out	<b>4. Ripe</b>	Large, opalescent white, drops of milt produced when pressed or cut
<b>5. Spent</b>	Shrunken, flaccid, contains a few residual eggs and small ova	<b>5. Spent</b>	Shrunken, flabby, dirty white in colour

The HIMI toothfish fishery has had comprehensive observer coverage since it began in 1997, with biological data collected from random sampling of toothfish from nearly all fishing events. A proportion of these fish, as well as all those

individuals sampled during the 2011 sampling aboard Antarctic Chieftain, also had gonad weights recorded, enabling the calculation of a gonosomatic index (gonad as a proportion or percentage of total body weight) for these individuals. These data were also included in the analyses of length at maturity and spatial and temporal patterns of spawning activity described below.

### **Gonad sampling**

Post-ovulatory follicles (POFs) are the remaining follicular structure that had surrounded the ovum prior to release (Saidapur, 1982). In teleost fishes, POFs are visible in the ovary after spawning. As a result, presence of POFs constitutes clear evidence that an individual is mature and has spawned recently. In temperate and tropical water fishes POF degeneration can be rapid, and POFs may breakdown within a few days (Hunter and Goldberg, 1980). However in some studies of cold-water fishes they have been identified many months after spawning, and thus have been used as an indicator of spawning activity that might have occurred many months, or even the season before (Parker and Grimes, 2010; Saborido-Rey and Junquera, 1998). Previous studies of toothfish have indicated that macroscopic staging alone cannot reliably detect the POFs that indicate a female is mature and has released eggs some time prior to sampling (Parker and Grimes, 2010), and these structures can only be reliably identified through histological analysis. However as POFs begin to deteriorate, it can become difficult to differentiate between a POFs and atretic oocytes, and this may lead to incorrect identification of fish that have spawned as opposed to fish that have arrested egg development prior to spawning (Ganias, 2011). For the purposes of this study POFs were expected to be readily identifiable and an important indicator of recent spawning activity, as the majority of samples derived from fish sampled within a maximum of a few months of the predicted spawning season, and because POFs in the closely related Antarctic toothfish are shown to persist for several months post-spawning.

To provide samples for histological analysis, and taking account of restrictions on processing and curation, a target of up to 50 ovaries and 10 testes per maturity stage was set for the 2011 sampling voyage. Gonads samples were excised whole, weighed to the nearest gram, cut into sections of 3-7 grams and then preserved in 10% FAACC (Formaldehyde 4%, acetic acid 5%, calcium chloride 1.3%) in seawater. Samples were monitored for a few hours after first being placed in preservative, and were transferred into fresh FAACC if there was evidence that they were not fixing rapidly (i.e. if tissue consistency had not firmed slightly). On return to Australia, samples were transferred to 10% ethanol prior to histological processing.

### **Histological analysis**

A representative subsample from each gonad was enclosed in a tissue cassette, mounted in paraffin and sliced using a microtome into ~6 µm sections, and then



stained using a standard Haematoxylin and Eosin procedure. Sections were then viewed under a microscope (10×-400×) with a digital camera attached, to identify and categorise the developmental stages of gametocytes seen.

Histological maturity staging was determined by using characteristics derived from descriptions by West (1990), Ewing and Lyle (2009) and Murua *et al.* (2003). For each histology sample, all stages of oocyte development were identified and recorded. Microscopic maturity was classified by the most advanced type of oocyte (West 1990) or cell type in sperm development (Parker and Grimes, 2010) present. Presence of postovulatory follicles, oocyte atresia, and residual eggs were also recorded. For male gonad histology, maturity was determined by the development stage of spermatocytes and the general microscopic morphology of the gonad.

### **Estimation of size- and age-at-maturity**

For all individuals with total length and gonad maturity stage data, total length measurements were binned into 50 mm length bins, and individuals assigned as either immature or mature based on their macroscopic gonad stage. Size at maturity ogives were estimated by fitting a logistic regression to the maturity at length data using the *glm* (generalised linear modelling) function in R (R Development Core Team, 2010). Age at maturity ogives were estimated the same way, after lengths were converted into age using the age length key used for the most recent HIMI stock assessment (Candy and Welsford, 2011) (Appendix 3).

### **Sensitivity testing of the assessment models to revised maturity estimates**

To investigate the effect of the revised age-at-maturity ogive on the interpretation of spawning stock biomass, current stock status and the recommended long-term sustainable catch, a comparison was made between the results of the CASAL assessment model presented to CCAMLR in 2011, denoted *a2-2011-alkall-PE* in Candy and Welsford (2011), and a models including the new ogives. Details of the development and structure of the CASAL models used for the HIMI stock assessment are available in Candy and Constable (2008), Candy and Welsford (2009, 2011) and SC-CAMLR (2011).

The harvest strategy within CCAMLR fisheries (Constable *et al.*, 2000) seeks to only allow harvest levels that ensure the escapement of a sufficient amount of the spawning stock biomass so that recruitment levels and ecological relationships are maintained. The decision rules for setting catch limits in toothfish fisheries are described as follows:

1. choose constant annual catch  $\gamma_1$  so that the probability of the toothfish spawning biomass being depleted below 20% of its pre-exploitation median level over a 35-year harvesting period is 10%; and

2. choose constant catch  $\gamma_2$  so that the median escapement in the toothfish spawning biomass over a 35-year period is 50% of the pre-exploitation median level; then
3. select the lower of  $\gamma_1$  and  $\gamma_2$  as the total allowable catch.

Therefore the key statistics used to compare these models were the estimates of  $B_0$  (median spawning biomass prior to exploitation), current status (estimated spawning stock biomass as a proportion of  $B_0$ ) and status after 35 years at the current annual catch limit of 2 730 tonnes for the 2011/12 and 2012/13 seasons<sup>1</sup>.

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<sup>1</sup> The season at HIMI runs from 1 December to 30 November of the following year. Since 2007, CCAMLR has reviewed and set catch limits at HIMI for two consecutive seasons, with the next review due in 2013.

## Results

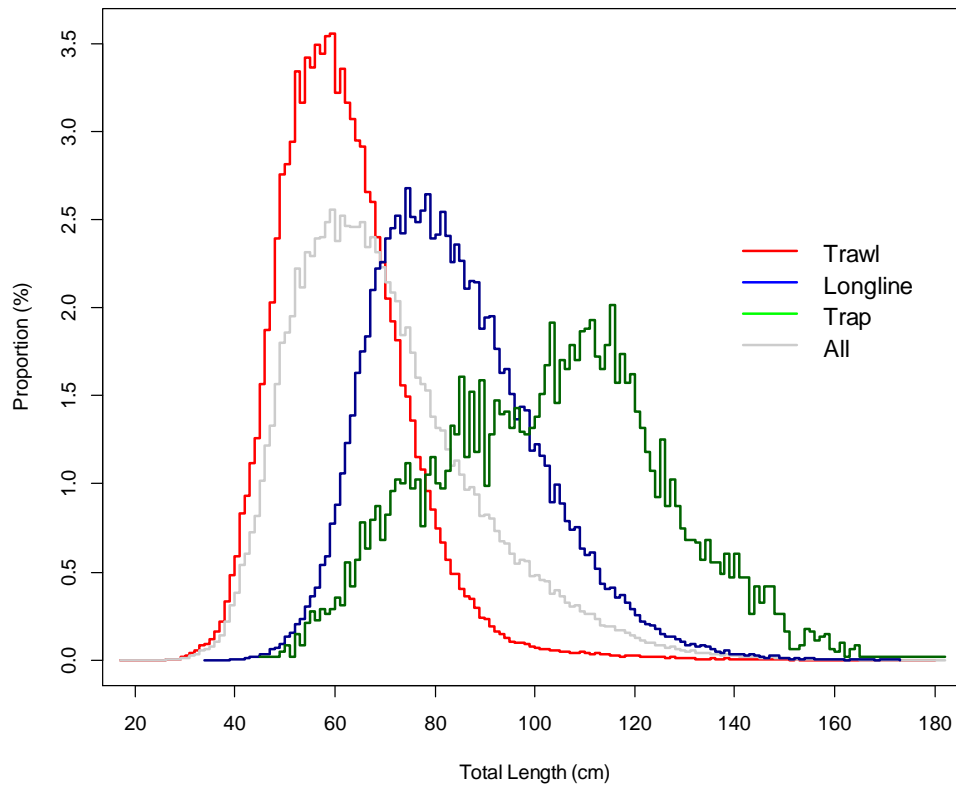
### Biological sampling

Since the commencement of the fishery, a total of 337,199 individual records of toothfish had the full complement of biological data including length, sex and maturity stage (Table 2). Trawl samples dominated as it was the primary capture method prior to 2003.

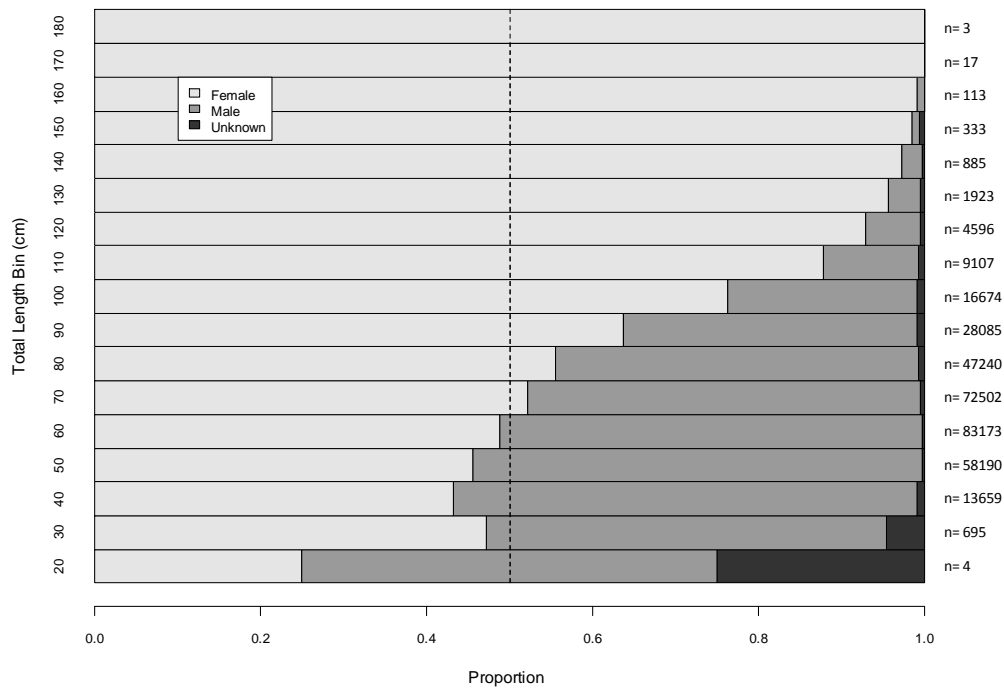
**Table 2. Numbers of toothfish for which standard biological measurements, sex and gonad stage data were available, by method and calendar year. Note: 2011 includes data collected specifically for this project aboard Antarctic Chieftain.**

Year	Capture Method			Total
	Trawl	Longline	Trap	
1997	5997			5997
1998	10670			10670
1999	9424			9424
2000	17674			17674
2001	23941			23941
2002	14026			14026
2003	19645	3971		23616
2004	16724	8449		25173
2005	16338	7792		24130
2006	14932	8770	1788	25490
2007	13756	8156		21912
2008	15147	15024	8	30179
2009	13407	24559	761	38727
2010	14176	15709	2431	32316
2011	11272	21466	1175	33913
<b>Total</b>	217129	113896	6163	337188

The different fishing gears are known to select a different size and age range of toothfish (Candy and Welsford, 2011). This is clearly evident in the proportions at length for the biological samples, with fish from trawl samples generally less than 80 cm total length, from longline samples mostly between 60 and 120 cm, and from trap between 60 and 140 cm. The overall length distribution of fish is also dominated by fish less than 100 cm due to the large numbers of trawl caught fish in the samples (Figure 2). It is also clear that toothfish sex ratios vary with size; a sex ratio of approximately 1:1 is apparent in smaller size classes, however the proportion of males rapidly declines above 100cm with no males observed in the (albeit small) samples over 170 cm (Figure 3). Individuals of unknown sex are more common in the smallest size classes due to the difficulty in reliable identification and sexing of juvenile gonad tissue.



**Figure 2. Proportion by length of toothfish for which standard biological measurements, sex and gonad stage data were available, by capture method and pooled across all methods.**



**Figure 3. Sex composition by length of toothfish for which standard biological measurements, sex and gonad stage data were available. Vertical dotted line indicates a 1:1 sex ratio.**

### Variation in gonosomatic index

A total of 12,563 individuals that had associated biological data also had gonad weight measured. The majority of these samples (11,735) were derived from samples collected for this project in 2011. As might be expected, the gonosomatic index (GSI) varied substantially across macroscopic gonad stages with stages 3 and 4, and to a lesser extent stage 5 having substantially higher median GSI than earlier developmental stages in both sexes, although males do not reach as high GSI values as females (Figure 4 and Table 3).

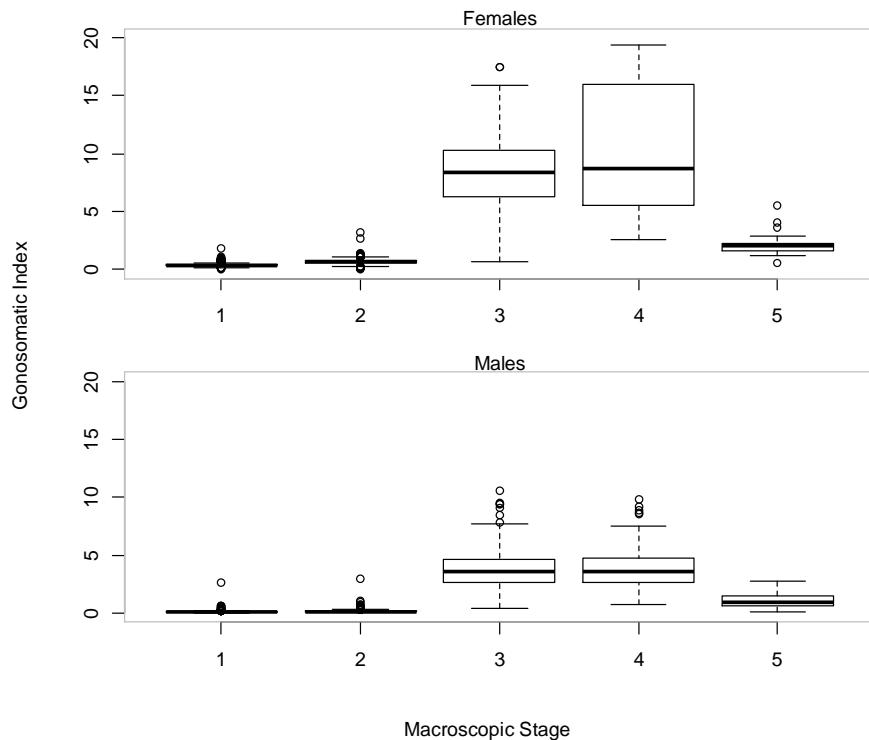


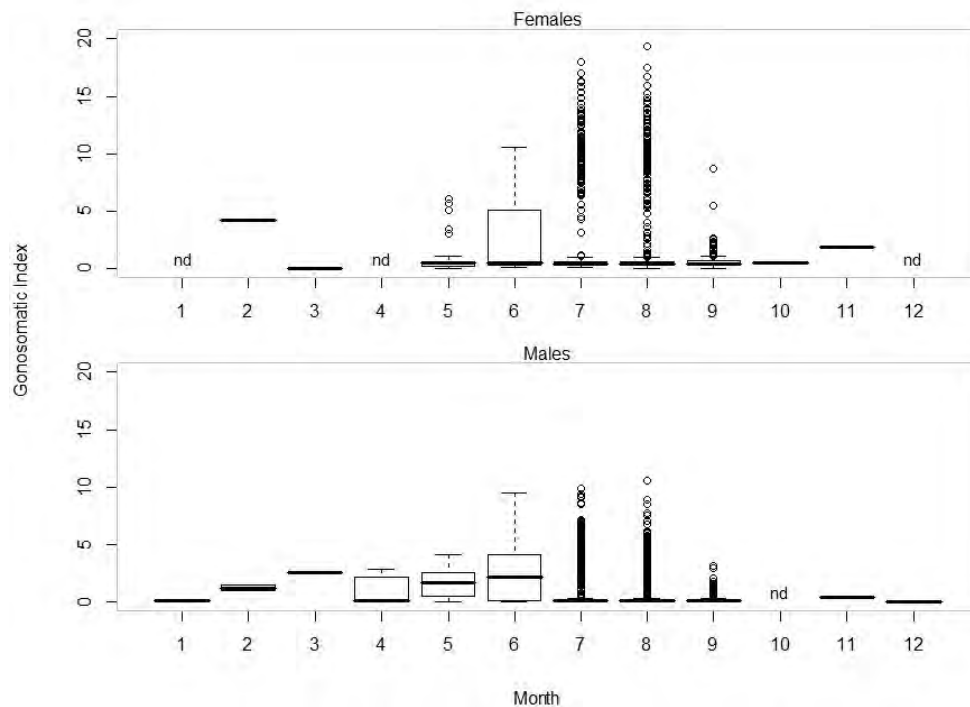
Figure 4. Box plots of gonosomatic index by sex and macroscopic gonad stage. Circles indicate values outside of 1.5 times the 25% to 75% interquartile range.

Table 3. Median and 95% quantile range of gonosomatic index by sex and macroscopic gonad stage.

Stage	Gonosomatic Index			
	Females		Males	
	Median	Range	Median	Range
1	0.35	0.21-0.64	0.08	0.04-0.16
2	0.63	0.35-1.01	0.13	0.06-0.26
3	8.36	3.35-13.92	3.61	0.85-7.06
4	8.70	2.97-18.30	3.60	1.03-6.78
5	2.01	1.07-4.24	0.88	0.11-2.27

The monthly trend in GSI showed that gonad growth is greatest between May and June for females and April and June for males, suggesting that June is the start of the spawning season (Figure 5). Between July and September the median GSI value

declines rapidly in both sexes, but there are a substantial number of outliers throughout these months indicating that there is likely to be some spawning activity throughout these months.



**Figure 5. Box plots of gonosomatic index by sex and month. Circles indicate values outside of 1.5 times the 25% to 75% interquartile range. nd=No data.**

Plots of GSI during the months of the spawning season (June – August) by length indicates that many sampled females show a weak trend of increasing GSI with length, while a second group (most likely participating in spawning due to GSI overlapping with the values for stages 3 and 4 as shown in Table 3) show a rapid increase in GSI above approximately 70 cm total length (Figure 6). A similar pattern is seen in males, with the minimum size of transition to high GSI values at around 60 cm.

Above 120 cm, there is some evidence that females with low GSI values are less common, however some of the largest individuals still show GSI values indistinguishable from smaller immature fish, which may indicate that females may rapidly revert to a resting stage after spawning, or not participate in spawning every season. Males with low GSI values are less common above 110 cm, indicating that above this size males are able to maintain gonads at stage 3 or 4 throughout the spawning season.

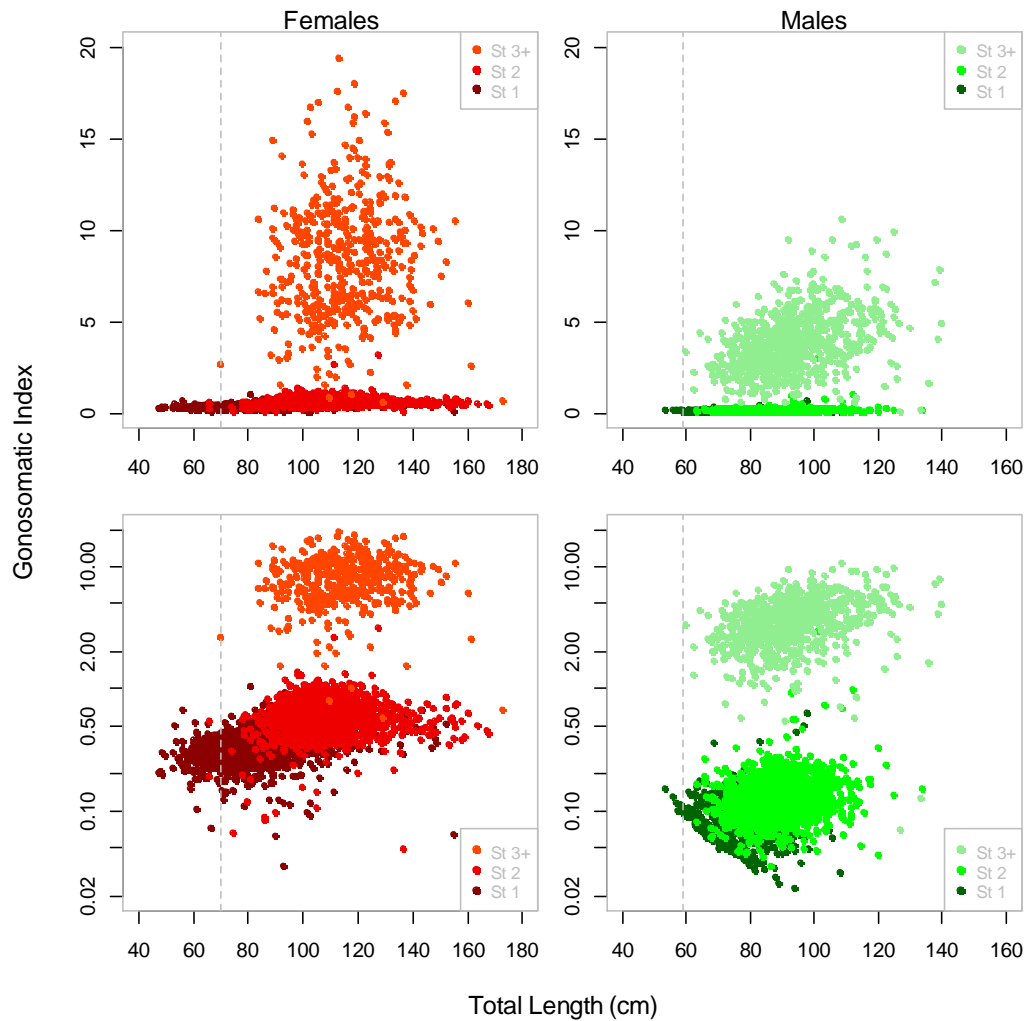


Figure 6. Gonosomatic index (GSI) by sex, stage and total length for toothfish measured during the spawning season (May-August). Vertical dotted lines indicate the minimum size at which toothfish transition to higher GSI values and macroscopic stages. Note that in the lower panels, GSI are plotted on a logarithmic scale to clarify the separation between the high and low GSI clusters.

### Histological analysis

Male and female toothfish from all macroscopic maturity stages were collected during the winter voyage to HIMI in 2011, with a total of 197 female and 75 male gonads being suitable for histological analysis (Table 4).

Table 4. Number of gonad samples prepared for histological analysis by sex and macroscopic maturity stage (see Table 1 for description of stages).

Sex	Macroscopic Stage					Total
	1	2	3	4	5	
Female	46	48	75	7	21	197
Male	17	13	16	16	13	75

Histological sections showed typical tissue cell structures as described for other teleost fishes (e.g. Ewing and Lyle, 2009; Murua *et al.*, 2003; West, 1990), enabling the characterisation of eleven distinct stages of oocyte development (Table 5 and Figure 7). Atretic oocytes and two age classes of post-ovulatory follicles were also identified (Table 6 and Figure 8).

**Table 5. Description of oocyte development in toothfish and its corresponding macroscopic (after Kock and Kellerman 1991, see Table 1) and microscopic (modified after West 1990) developmental stages. See Figure 7 for images of oocyte developmental stages.**

Macroscopic Stage	Microscopic stage	Most advanced oocytes present	Appearance of oocytes in Patagonian toothfish
1. Immature	Chromatin nucleolus	Oogenesis (OOG)	<ul style="list-style-type: none"> <li>• smallest oocyte stage present</li> <li>• nucleus just visible</li> <li>• small and granulose in appearance</li> </ul>
	Perinucleolar	Early Perinucleolus (EPN)	<ul style="list-style-type: none"> <li>• cytoplasm stains uniformly</li> <li>• nucleus (stains pink) increases in size</li> <li>• nucleoli begin to form</li> </ul>
		Late Perinucleolus (LPN)	<ul style="list-style-type: none"> <li>• nucleus further increases</li> <li>• larger than EPN</li> </ul>
2. Maturing virgin	Yolk vesicle formation	Cortical Alveoli (CA)	<ul style="list-style-type: none"> <li>• yolk vesicles form around nucleus. These vesicles appear empty with HandE staining.</li> <li>• zona radiata (chorion) widens</li> <li>• no yolk granules yet</li> </ul>
		Early Vitellogenesis (EVG)	<ul style="list-style-type: none"> <li>• very small yolk granules (yolk proteins in fluid filled spheres) form in cytoplasm, mostly towards periphery of the cell</li> <li>• chorion widens slightly through each vitellogenic stage</li> </ul>
			<ul style="list-style-type: none"> <li>• yolk granules more prevalent and larger and now also located towards nucleus</li> </ul>
3. Developing	Ripe (mature) stage	Early Migratory Nucleus (EMN)	<ul style="list-style-type: none"> <li>• yolk granules very large but not forming one mass</li> <li>• nucleus begins to migrate towards cell periphery</li> <li>• chorion thickens and H&amp;E stains a deeper red</li> </ul>
		Late Migratory Nucleus (LMN)	<ul style="list-style-type: none"> <li>• nucleus on cell periphery</li> <li>• yolk granules look like larger droplets and stains purple with H&amp;E</li> </ul>
		Post Migratory Nucleus (PMN)	<ul style="list-style-type: none"> <li>• yolk granules fused</li> <li>• nucleus begins to disperse</li> </ul>
4. Gravid		Hydrated/Mature (HYD)	<ul style="list-style-type: none"> <li>• small lumen between oocyte and follicle</li> <li>• oocyte stains a uniform pink and appears smooth.</li> <li>• sometimes distorted in shape due to preparation</li> </ul>
5. Spent/2. Resting	Post-spawning	Varies, however EPN, LPN and CA are always present	<ul style="list-style-type: none"> <li>• Post ovulatory follicles present</li> <li>• Atretic oocytes sometimes present</li> <li>• Left over mature oocytes sometimes present slightly shrunken from follicle</li> <li>• May contains oocytes up to LVG stage but more frequently oocytes only at the CA and EVG stage</li> </ul>



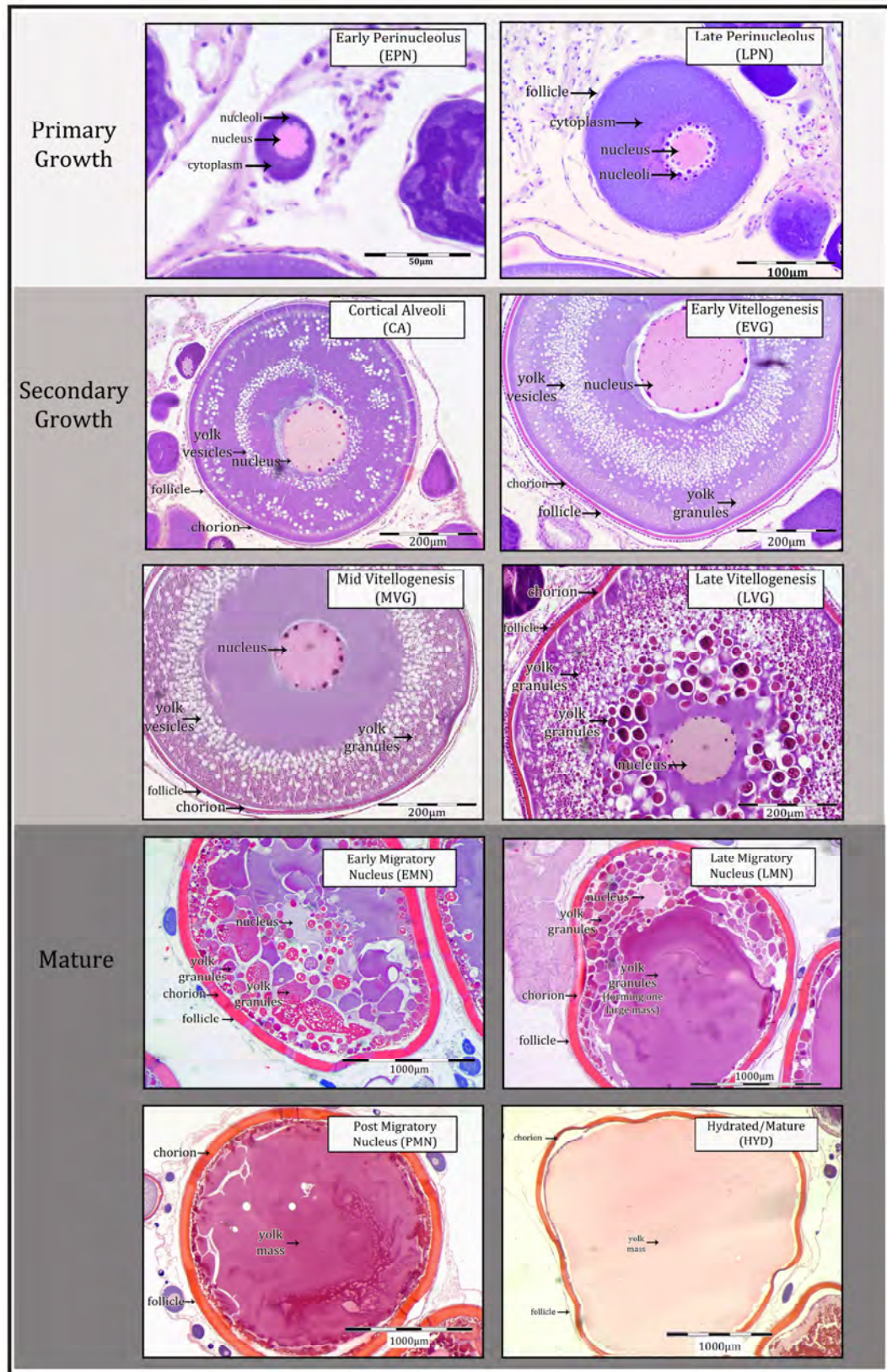
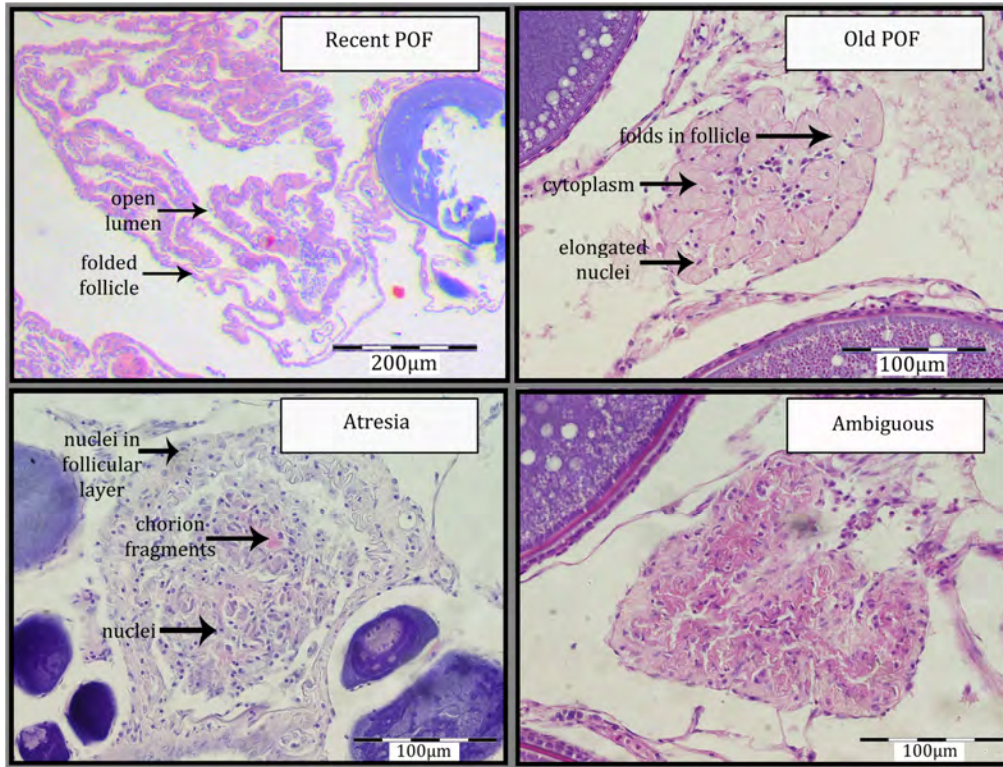


Figure 7. Appearance of oocytes developmental stages in Patagonian toothfish ovaries stained with haematoxylin and eosin.



**Figure 8. Appearance of post-ovulatory follicles (POFs), oocytes undergoing atresia and an ambiguous tissue structure with intermediate characteristics of both POFs and atretic oocytes.**

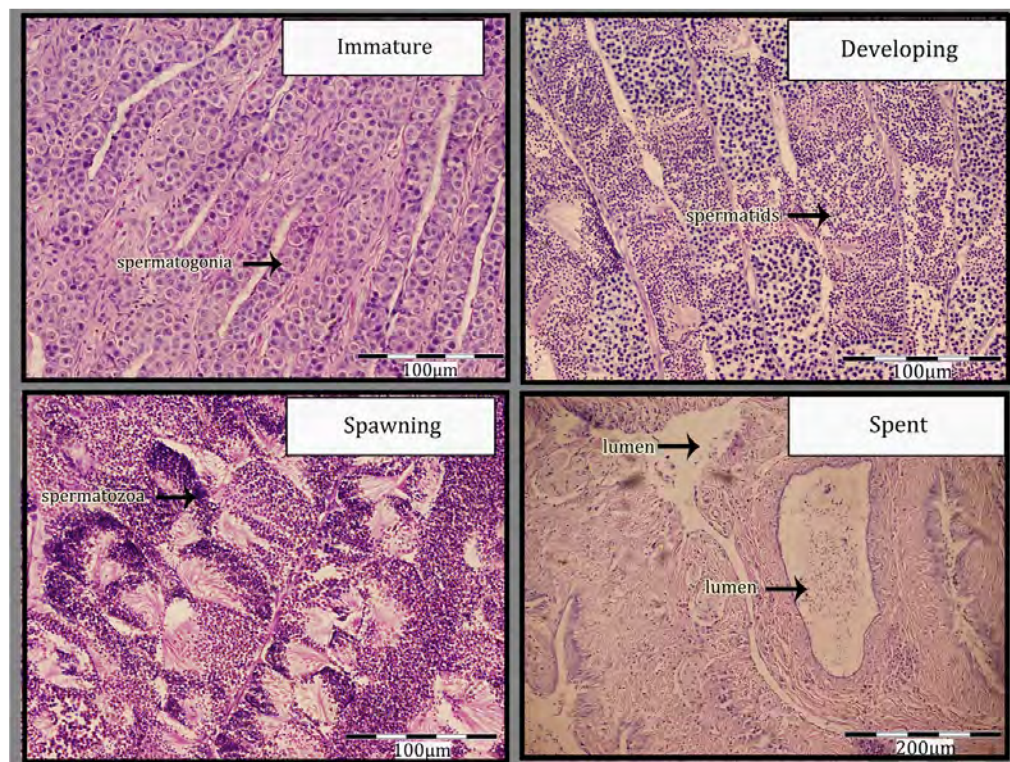
**Table 6. Characteristics of post-ovulatory follicles (POFs) and atretic structures identified in toothfish ovaries. See Figure 8 for images.**

Structure	Description
Recent POFs	The follicle is beginning to collapse and fold after ovulation and contains many elongated nuclei. A large follicular lumen is still visible.
Old POF	The follicle is compact and fully folded in itself, however a small lumen can still be evident between folds. Contains few elongated nuclei. Cytoplasm stains a uniform pink.
Atretic oocyte	Contains a denser band of nuclei in the follicular layer. In the centre nuclei are rounded and still numerous. Contain remnant degenerating chorion or vitellogenic bodies which stain pink.
Ambiguous	Classed as ambiguous as it appears to have traits of both a POF (including an apparent lumen) and an atretic oocyte (including abundant nuclei).

Histology of male gonads enabled classification into 4 distinct stages based on the appearance of cells dividing to produce spermatozoa development and testis morphology (Table 7, Figure 9). No clear cellular or tissue structures were identified that enabled a clear distinction to be drawn between developing fish in stages 2 and 3.

**Table 7. Description of spermatozoan development in toothfish and corresponding macroscopic (after Kock and Kellerman 1991, see Table 1) and microscopic developmental stages. See Figure 8 for images.**

Macroscopic Stage	Microscopic stage	Most advanced stage present	Appearance in Patagonian toothfish
1. Immature	Immature	spermatogonia	<ul style="list-style-type: none"> <li>Only spermatogonia-stage cells present</li> <li>Tissue uniform across section</li> </ul>
2./3. Maturing/ Developing	Developing	spermatids	<ul style="list-style-type: none"> <li>Patches of spermatocytes and spermatid cells present, smaller and darker staining than spermatogonia</li> <li>Small patches of spermatozoa with flagella may be evident</li> </ul>
4. Ripe	Spawning	spermatozoa	<ul style="list-style-type: none"> <li>Clumps of spermatozoa with flagella have accumulated in the lumen and dominate in sections</li> </ul>
5. Spent	Spent	spermatids	<ul style="list-style-type: none"> <li>Section has a 'collapsed' appearance, with a large lumen</li> <li>Some residual spermatozoa may be evident in the lumen</li> </ul>



**Figure 9. Appearance of developmental stages in Patagonian toothfish testes stained with haematoxylin and eosin. See Table 6 for detailed descriptions.**

When the macroscopic staging and histological analysis for females were compared, macroscopic staging successfully categorised individuals into the correct cellular developmental stage in the majority of cases (Table 8). However 26% of the individuals assigned as stage 1 macroscopically were revealed to have

characteristics of maturity, including POFs, indicating previous spawning activity. Some also contained atretic oocytes which may indicate that oocyte development had commenced in the past and been arrested. Others also showed oocytes progressing to the cortical alveoli and early vitellogenesis stages, however as the timeframe for these oocytes to progress further is not known for toothfish, it may be that these individuals may not have been likely to participate in spawning in the 2011 season.

**Table 8. Comparison of macroscopic staging of toothfish ovaries with oocyte stage present in histological sections e.g. 57% of individuals identified as stage 4 had hydrated oocytes present in histological sections. Shaded cells indicate the most advanced oocyte/post-ovulatory follicle (POF) stages expected given the assigned macroscopic stage is accurate i.e. individuals assigned to stage 1 should not contain oocytes further advanced than late perinucleolus stage.**

Oocytes Types/POFs Present		Assigned Macroscopic Maturity Stage (%)				
		Stage 1 (n=46)	Stage 2 (n=48)	Stage 3 (n=75)	Stage 4 (n=7)	Stage 5 (n=21)
Early Perinucleolus	(EPN)	100	100	100	100	100
Late Perinucleolus	(LPN)	72	98	100	100	100
Cortical Alveoli	(CA)	26	96	100	100	100
Early Vitellogenesis	(EVG)	4	17	48	57	52
Mid Vitellogenesis	(MVG)	-	27	39	29	67
Late Vitellogenesis	(LVG)	-	10	9	29	14
Early Migratory Nucleus	(EMN)	-	-	55	-	-
Late Migratory Nucleus	(LMN)	-	-	87	57	-
Post Migratory Nucleus	(PMN)	-	-	25	57	-
Hydrated	(HYD)	-	-	7	57	-
Residual Hydrated		-	-	-	-	52
Recent POFs		-	4	5	57	95
Old POFs		4	27	4	-	-
Atresia		-	19	23	14	24
Old Atresia		9	60	4	-	5
Unknown		2	35	1	-	5
<b>Individuals correctly staged</b>		74	94 (69 <sup>a</sup> )	92	57	95

<sup>a</sup> If only first maturing fish are included in stage 2, only 69% were correctly identified macroscopically.

Misclassifications of stage 2 individuals included a small number in which oocytes had not progressed beyond the late perinucleolus stage and one individual that was

likely to be stage 5 due to the presence of recent POFs. A large number also included old POFs, indicating they were likely to be individuals that had spawned at some time in the past. Misclassification of stage 3 included some stage 4s as determined by the presence of hydrated oocytes, and one stage 5 with recent POFs.

Only 7 of individuals assessed as macroscopic stage 4 were collected at sea, and while 57% were correctly staged, histological analysis indicated that one was a misclassified stage 3, with no hydrated oocytes evident but post-migratory nucleus stage oocytes present, and two were misclassified stage 5s with recent POFs but no hydrated oocytes present. Macroscopic classification of spent females had a very high classification success, with 95% of specimens including recent POFs. Only one individual could not be classified due to the absence of POFs and the presence of the ambiguous structures noted above and in Figure 8.

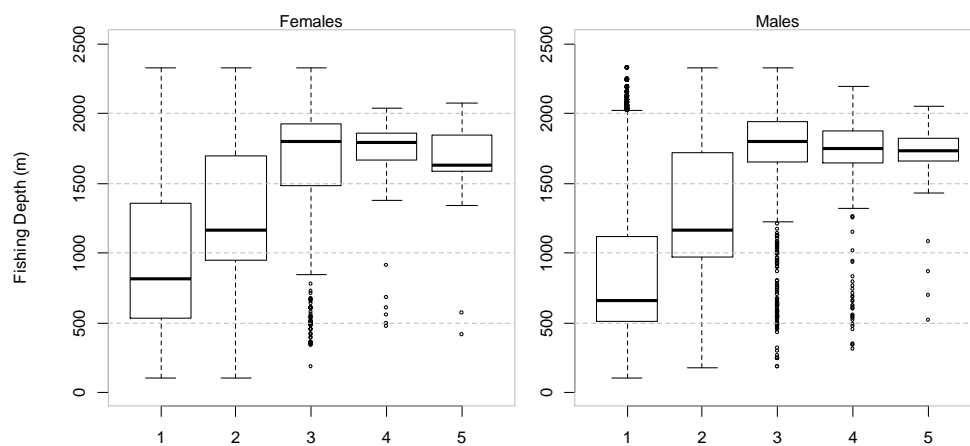
Macroscopic staging of males was generally successful at classifying stage 1, 4 and to a lesser extent stage 5 individuals, but was relatively poor at distinguishing between macroscopic stages 2 and 3 (Table 9), possibly indicating more of a continuum of spermatozoan development and turnover in males during the spawning season, relative to the more discrete stages identifiable in females. For example, a large proportion of spent individuals also included cells progressed to the spermatid stage, indicating the potential of continued production of sperm over a prolonged period.

**Table 9. Comparison of macroscopic staging of toothfish testes with spermatozoan development evident in histology sections. Shaded cells indicate cells that are expected to be present in a maturity stage. Shaded cells indicate the most advanced cell stages expected given the assigned macroscopic stage is accurate i.e. individuals assigned to stage 1 should not contain cells further advanced than the spermatogonium stage.**

Cell/Structures Present	Assigned Macroscopic Maturity Stage (%)				
	Stage 1 (n = 17)	Stage 2 (n = 13)	Stage 3 (n = 15)	Stage 4 (n = 16)	Stage 5 (n = 13)
Spermatogonia	94	77	27	19	77
Spermatids	6	23	100	94	69
Spermatozoa	-	-	60	81	62
Open lumen	-	-	13	13	77
Individuals correctly staged	94	23	40	81	77

### Spatial distribution of spawning activity

There was a strong trend for fish of both sexes with gonads in a more advanced state of development to be encountered at greater depths than earlier developmental stages (Figure 10). While stage 1 and 2 fish were encountered across the entire depth range fished (40-2300 m), the majority of stage 3-5 fish were encountered in depths of 1500-2000m, and stage 4 fish of both sexes in particular seem to have a relatively narrow depth preference of ~1700-1900m.



**Figure 10. Box plots of fishing depth by sex and macroscopic gonad stage. Samples collected outside of the putative gonad development and spawning season (before May or after August) are excluded. Circles indicate values outside of 1.5 times the 25% to 75% interquartile range.**

Maps of locations where fish of stage 3 or greater have been encountered reveal that spawning activity seems to primarily occur in locations on the deeper slopes northwest, west and southwest of HIMI, however there is some evidence of fish with advanced gonad states around much of the deeper slopes (Figure 11 and figures in Appendix 4). Stage 3 or higher males seem to be more wide spread, occurring at locations on the southern part of Shell Bank to the northeast of HIMI, where few females with advanced gonad development have been observed.

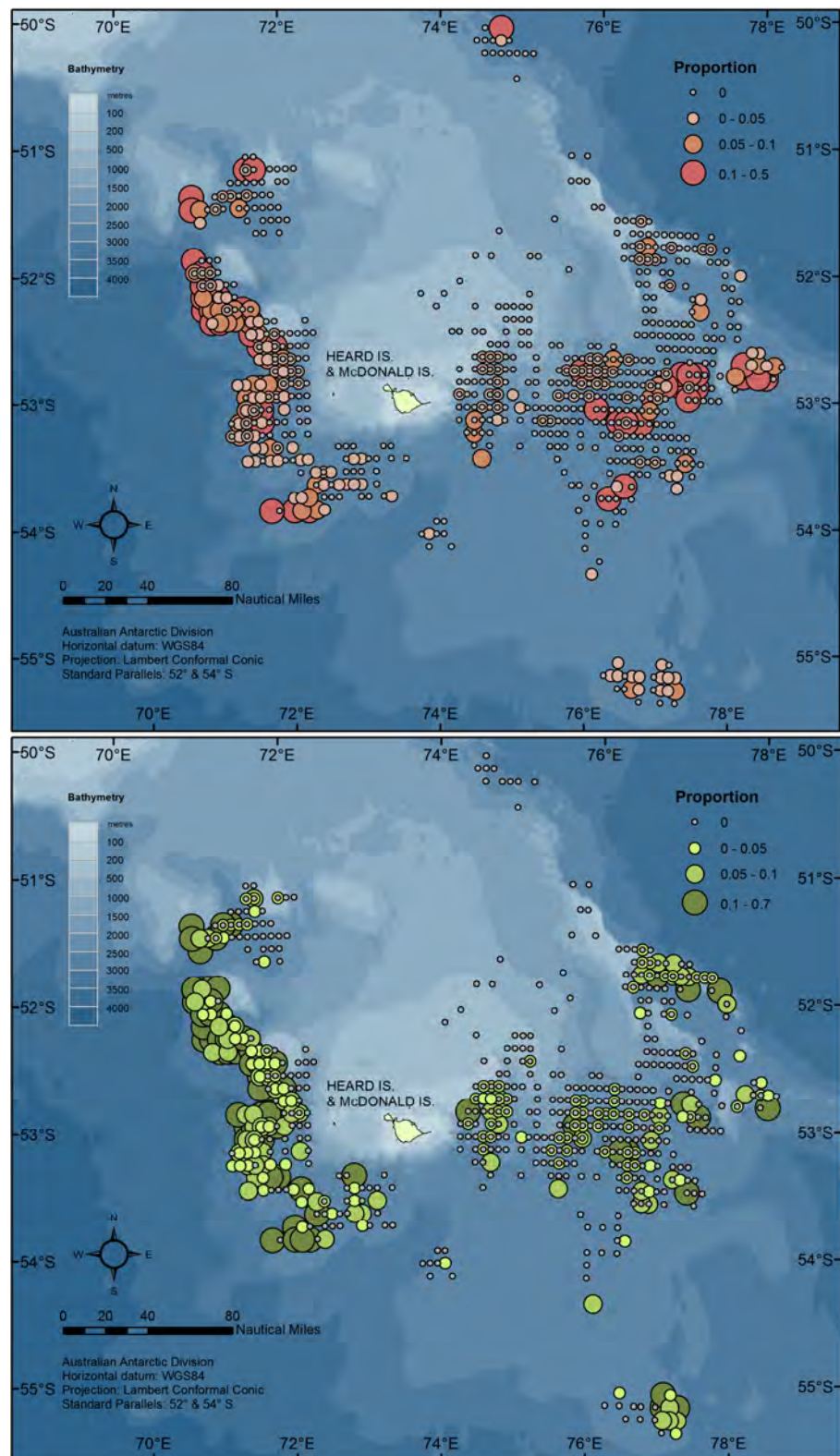


Figure 11. Proportion of toothfish with gonads assessed as macroscopic stage 3 or above. Females are shown in the upper panel, males in the lower panel. Proportions are calculated for all samples pooled across gears within 0.1 degree grids for data collected between May and August, 1997-2011. Grids where 10 or less individuals were measured were excluded. Bathymetry data courtesy of the Australian Antarctic Data Centre.

### **Estimation of size- and age-at-maturity**

For the purposes of stock assessment using the CASAL modelling framework and the CCAMLR decisions rules (see methods above), the critical quantity to estimate is the proportion of the population that can be considered 'mature' and therefore part of the spawning stock biomass. Different authors have used different threshold criteria to define maturity; for example Lord *et al.* (2006) used fish of stage 3 or higher, according to the Kock and Kellerman (1991) schema, to estimate size at first maturity and 50% maturity. Everson (1999) used the same criterion but fitted ogives to account for the fact that in some years a proportion of mature fish did not spawn. Parker and Grimes (2010) used a combination of forecasting from female Antarctic toothfish having oocytes progressed to at least the cortical alveoli stage, and hind casting from fish with post-ovulatory follicles, to estimate size and age at spawning ogives. Due to the difficulty noted above in accurately classifying toothfish as either mature or immature based on macroscopic staging, where the bulk of data was available in this study, size-at-maturity ogives were fitted assuming that stage 2 or greater was mature, as well as assuming stage 3 or greater was mature. In each case, sex specific models were fitted as well as with both sexes pooled.

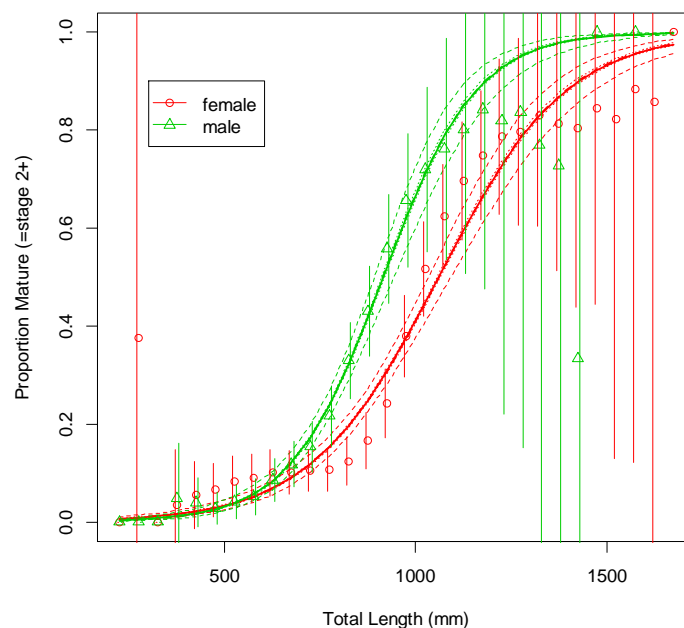
Initially generalised linear models (GLMs) were fitted to the length at maturity data assuming a binomial error distribution and a logit link function. Data was binned into 50 mm length bins, with the largest size class including all fish of 1600 mm or greater. Plots of the 95% confidence interval of proportion mature in a size class indicated that over dispersion was leading to poor estimation of the confidence intervals around the fitted ogives, as well as the standard errors of the parameters of the fitted logistic functions (Figures 12 and 13). As a result, GLMs were fitted using a quasibinomial error distribution. The resulting ogives were effectively identical, however the estimated standard errors and confidence intervals (estimated by scaling those for the binomial by the square root of the over dispersion parameter, which is in turn estimated by dividing the residual deviance by its degrees of freedom) were more plausible (Figures 12- 15).

The values of the parameters of the ogives and length at 50% maturity are shown in Table 10. The ogives based on the assumption that stage 2 or higher fish are mature produced estimates that indicate females and both sexes combined achieve 50% maturity at over 1000 mm, while males mature at around 915 mm. Assuming stage 3 or above is mature resulted in ogives for females and both sexes combined that estimated 50% maturity at over 1500 mm, with even the largest size classes in the population never reaching 100% maturity, and males reach 50% maturity at 1179 mm length.

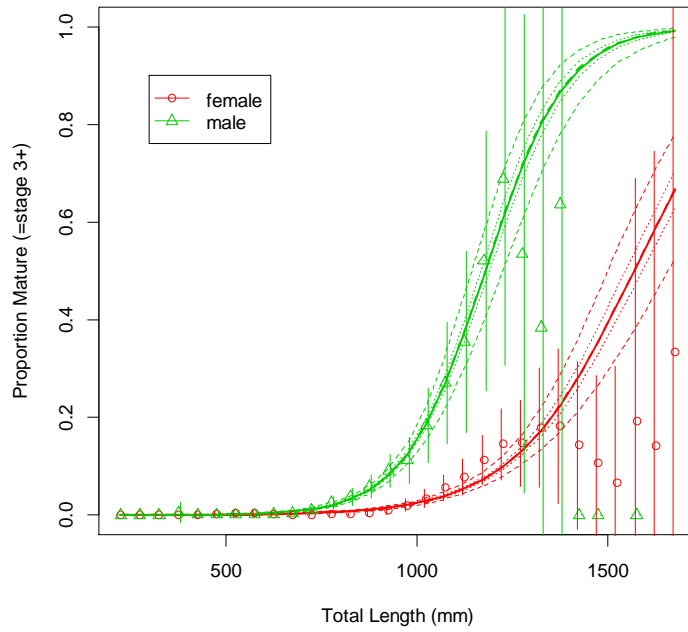


**Table 10. Model parameters for maturity ogives fitted to toothfish maturity at length data, by sex and combined and for maturity thresholds. Models are a logistic function fitted with a quasibinomial error distribution. Standard errors of the intercept and slope were estimated by scaling those for the equivalent model fitted with a binomial error by the square root of the over dispersion parameter, which is in turn estimated by dividing the residual deviance by its degrees of freedom.**

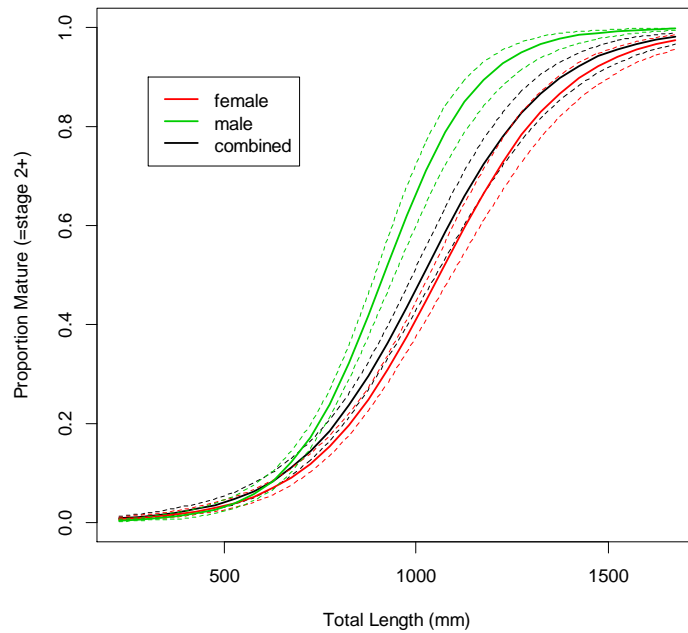
Model	Maturity threshold	Data	Residual deviance	Residual degrees of freedom	Intercept (SE)	Slope (SE)	Length at 50% maturity (mm)
1	Stage $\geq 2$	Females	1964	53	-6.348 (0.328)	0.0060 (0.0004)	1060
		Males			-1.211 (0.566)	0.0023 (0.0007)	915
2	Stage $\geq 2$	All	4243.3	55	-6.218 (0.328)	0.0061 (0.0004)	1017
3	Stage $\geq 3$	Females	549.1	53	-10.06 (0.500)	0.0064 (0.0005)	1568
		Males			-1.176 (0.753)	0.0031 (0.0008)	1179
4	Stage $\geq 3$	All	2596.9	55	-8.720 (0.645)	0.0057 (0.0006)	1526



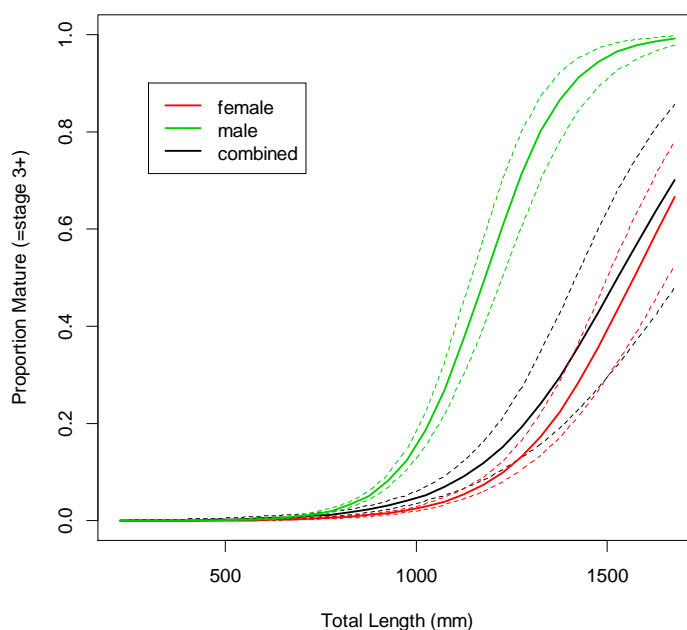
**Figure 12. Proportion mature (stage 2+) versus total length. Binned data proportions (points) with approximate 95% confidence limits (bars), fitted logistic curves (solid lines), approximate 95% confidence limits based on binned data and quasibinomial GLM (dashed lines), approximate 95% confidence limits based on binary data and binomial GLM (dotted lines).**



**Figure 13. Proportion mature (stage 3+) versus total length. Binned data proportions (points) with approximate 95% confidence limits (bars), fitted logistic curves (solid lines), approximate 95% confidence limits based on binned data and quasibinomial GLM (dashed lines), approximate 95% confidence limits based on binary data and binomial GLM (dotted lines).**



**Figure 14. Proportion mature (stage 2+) versus total length. Binned data fitted logistic curves (solid lines) for females, males, and combined data, approximate 95% confidence limits based on binned data and quasibinomial GLM (dashed lines).**



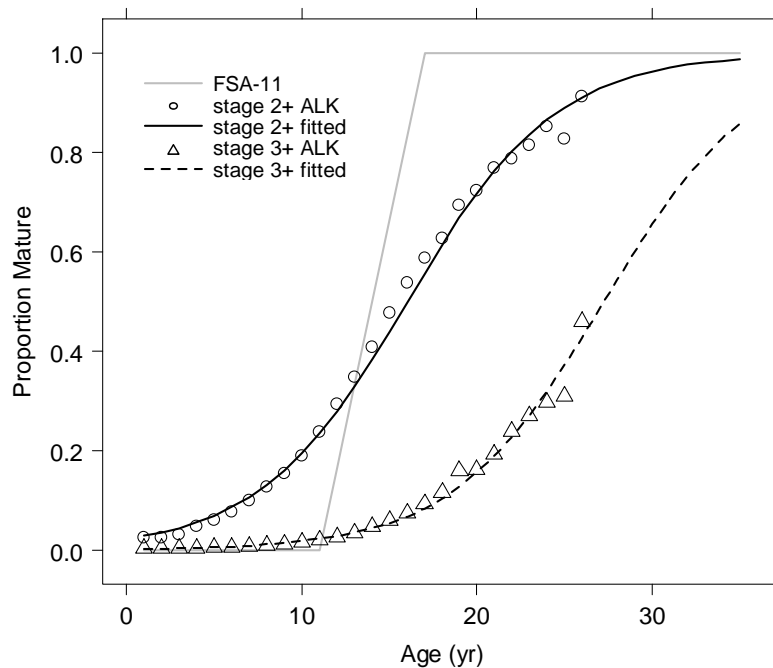
**Figure 15. Proportion mature (stage 3+) versus total length. Binned data fitted logistic curves (solid lines) for females, males, and combined data, approximate 95% confidence limits based on binned data and quasibinomial GLM (dashed lines).**

CASAL requires the maturity ogive to be defined as a function of age rather than total length. Therefore to convert the length-based ogive to an age-based ogive the age-length key for all commercial fisheries and all years of fishing combined (Appendix 3) were used to obtain the proportion in length bins as a function of age by simply multiplying the length-based ogive calculated at the midpoint of each 50 mm length bin and summing these products over all length bins for a given age. Figure 16 shows the proportions mature at each age obtained using the above method and smoothed ogives obtained by fitting a logistic curve to these raw proportions, as well as the function used in Candy and Welsford (2011) to estimate size at maturity.

### **Sensitivity testing of the assessment models to revised maturity estimates**

The current configuration of CASAL for stock assessment at HIMI does not include sex specific parameters. To evaluate the impact of the new estimates of maturity at age shown above, three scenarios were developed using the age at maturity ogives:

- 1) for both sexes combined, assuming fish of stage 2 or above are part of the spawning stock biomass;
- 2) for both sexes combined, assuming fish of stage 3 or above are part of the spawning stock biomass;
- 3) as for scenario 1, but with the age at maturity advanced by 2 years.



**Figure 16. Proportion mature (stage 3+) versus age. Raw proportions at age obtained by applying the ALK to proportions-at-length (points), smoothed proportions obtained from logistic curve fitted to points (lines), and maturity ogive used in the 2011 assessment (Candy and Welsford 2011).**

While many fish in stage 2 are likely to already have participated in spawning or had advanced to vitellogenesis and hence could be considered mature, the ogive in scenario 1 is still likely to underestimate size at maturity in the population.

Conversely, the ogive in scenario 2 is likely to substantially overestimate the size at maturity as it excludes fish with evidence of previous spawning or that are resting during the sampling period. Scenario 3 was therefore developed to reflect that although some fish in macroscopic stage 2 are likely to be mature, progressing from cortical alveoli stage to hydration in notothenioid fishes is estimated to take up to 2 years (Kock and Kellermann, 1991), and therefore an offset of 2 years represents a biologically plausible compromise between scenarios 1 and 2.

The smoothed ogives shown in Figure 16 were used to provide maturity-at-age proportions from each age classes (1-35) in the CASAL model, with the exceptions that for the ogive used in scenario 1 these proportions were fixed at 0 for ages less than 5 and fixed at 1.0 for ages above 29 yrs, and, for scenario 2 these proportions were fixed at 0 for ages less than 11 and reached a maximum of 0.859 at age 35 yrs. , Finally, for scenario 3 these proportions were fixed at 0 for ages less than 7 and fixed at 1.0 for ages above age 31. Other than these changes to the maturity ogive fixed input to CASAL all other data and input (fixed) parameters were identical to that used to fit CASAL in Candy and Welsford (2011). Results of the assessment scenarios are shown in Table 9. Projections of stock status for 35 years after 2011 were obtained for each scenario using future random lognormal recruitment from 2009 with an annual catch of 2 730 tonnes between 2012 and 2046 distributed

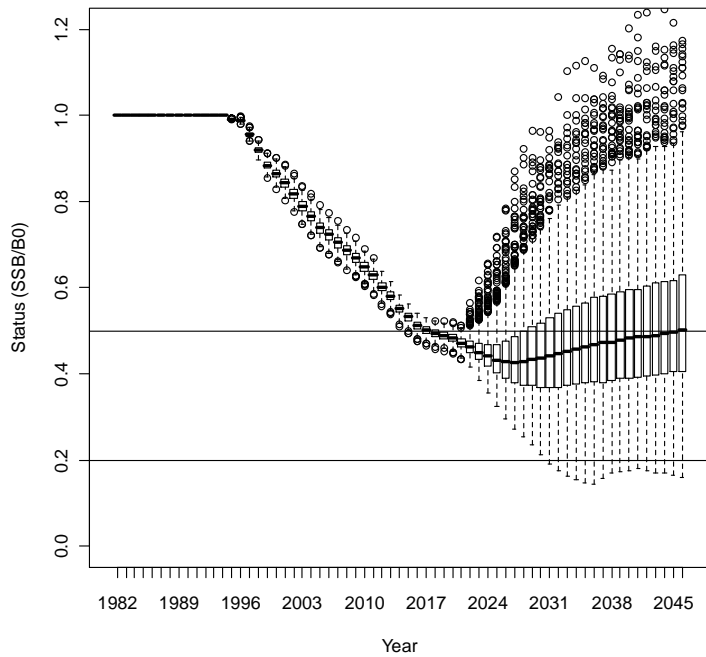
among sub-fisheries based on best estimates of future expected catches. The results of 1000 trajectories for each scenario are shown in Figures 17 to 20.

**Table 9. Results of assessments of stock status of *Dissostichus eleginoides* at Heard Island and the McDonald Islands, comparing the 2011 assessment model (Base) with models with estimated maturity ogives.  $B_0$  is the maximum posterior density (MPD) estimate of the pre-exploitation median spawning stock biomass (SSB), SSB status 2011 is the ratio of the model prediction of SSB in 2011 to  $B_0$ ,  $R_0$  is the MPD estimate of mean Age 1 recruitment prior to exploitation (1981), and  $CV_R$  is the coefficient of variation of the annual recruitment series (1996-2008). See Candy and Welsford (2011) for other details of the model input parameters and data.**

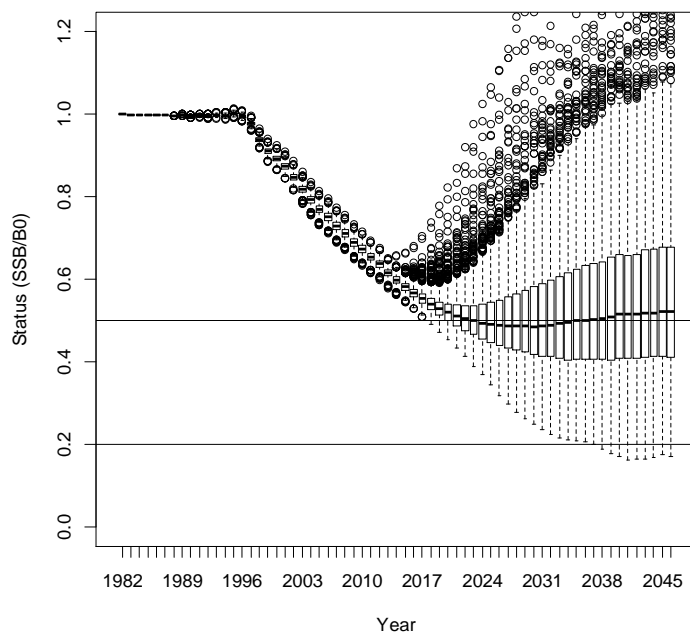
Scenario	Description	$B_0$ (tonnes) (SE)	SSB Status 2011	$R_0$ (mil.)	$CV_R$
Base case	Model in Candy and Welsford (2011)	86 400 (1 915)	0.629	5.765	0.78
1	Base case with maturity ogive based on stage $\geq 2$ as mature	74 406 (17 93)	0.654	5.8205	0.91
2	Base case with maturity ogive based on stage $\geq 3$ as mature	30 619 (776)	0.749	5.8206	0.95
3	Base case with maturity ogive based on stage $\geq 2$ as mature with 2 year shift	67 119 (776)	0.657	5.790	0.80

The absolute estimates of  $B_0$  were more sensitive to variation in the maturity ogives than the relative estimates of current status. The  $B_0$  estimates resulting from scenarios 1 were 14% and scenario 3 22% lower than the base case. However the current status only differed from the base by 3.8% and 4.4% from the base case. The forward projection of scenarios 1 and 3 were also similar, in both cases long term escapement was estimated to be above 0.5 of  $B_0$ , indicating that catch limits estimated by these model would be higher than the current limit of 2730 tonnes. This is likely to be due to the fact that the ogives used in these scenarios estimate many more ages classes are recruiting to the spawning stock before declines in biomass due to natural and fishing mortality.

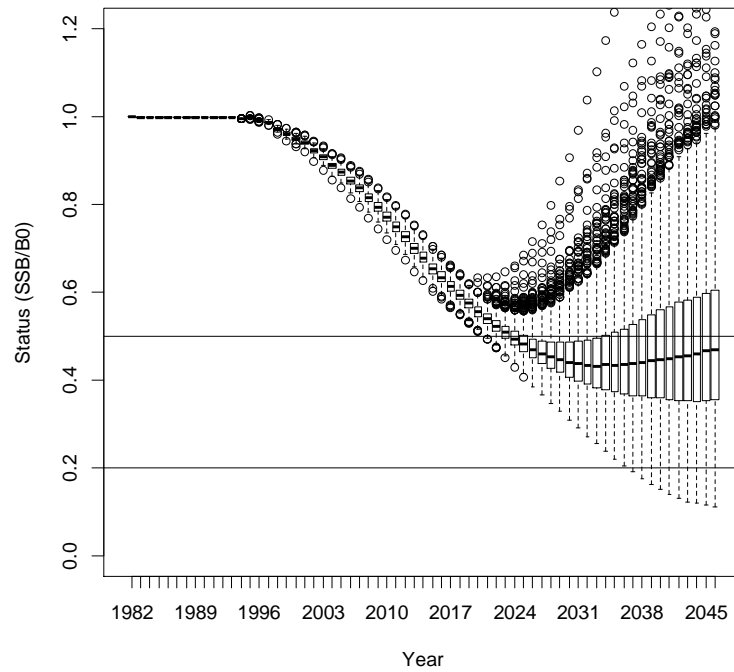
The most dramatic contrast with the base case was scenario 2, with a decline of 65% in  $B_0$  compared to the base case. This is attributable to a much smaller proportion of the population estimated to survive natural and fishing mortality to recruit to the spawning stock. As noted above, scenario 3 is based on an ogive that is likely to be substantially overestimating the size at maturity of toothfish at HIMI, and this is likely to also be driving the higher estimate of current status, as the stock assessment model that fewer of these large old fish are vulnerable to the fishery. However, over the 35 year projection period, the current catch level would violate the escapement rule, and therefore a catch limit set under this scenario would be lower than the current 2 730 limit (Figure 18).



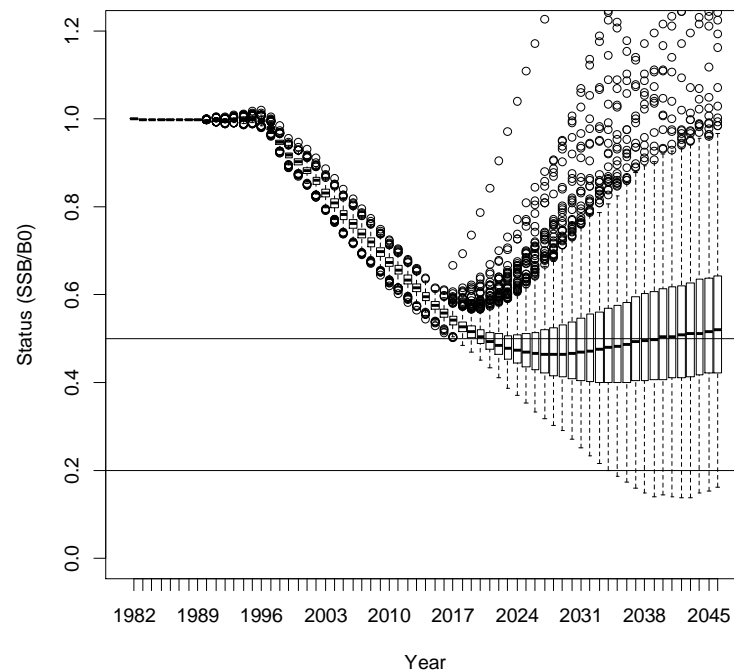
**Figure 16. Projections of stock status for the base case scenario as described in Table 10. Box plots represent the distribution of the status of spawning stock biomass in a trial relative to  $B_0$  in that projection trial across 1000 projection trials for that year. Horizontal lines show the 50% and 20% status levels for reference used in CCAMLR decision rules: in this scenario the escapement probability = 0.50 and the depletion probability = 0.009.**



**Figure 17. Projections of stock status for the scenario 1 as described in Table 10. Box plots represent the distribution of the status of spawning stock biomass in a trial relative to  $B_0$  in that projection trial across 1000 projection trials for that year. Horizontal lines show the 50% and 20% status levels for reference used in CCAMLR decision rules: in this scenario the escapement probability = 0.52 and the depletion probability = 0.006.**



**Figure 18.** Projections of stock status for the scenario 2 as described in Table 10. Box plots represent the distribution of the status of spawning stock biomass in a trial relative to  $B_0$  in that projection trial across 1000 projection trials for that year. Horizontal lines show the 50% and 20% status levels for reference used in CCAMLR decision rules: in this scenario the escapement probability = 0.47, depletion probability = 0.019.



**Figure 19.** Projections of stock status for the scenario 2 as described in Table 10. Box plots represent the distribution of the status of spawning stock biomass in a trial relative to  $B_0$  in that projection trial across 1000 projection trials for that year. Horizontal lines show the 50% and 20% status levels for reference used in CCAMLR decision rules: in this scenario the escapement probability = 0.52, depletion probability = 0.007.

## Discussion

The samples analysed in this study were strongly biased towards females in length classes over 100 cm. A common feature of toothfish populations in the South Atlantic (Collins *et al.*, 2010), Macquarie Ridge and the Ross Sea (Fay and Tuck, 2011; Horn, 2002) and at HIMI (Welsford *et al.*, 2011) is that females grow faster and reach larger maximum sizes than males. This is likely to contribute to the observed female bias, as males may not live long enough to grow into the larger size classes. Furthermore, there is evidence that males are more likely to move long distances than females, which may further bias sex ratios towards females as, over time, males move out of the HIMI EEZ (Welsford *et al.* 2011). Welsford *et al.* (2011) also found that larger males tend to be more common on the western side of the plateau and in deeper waters. This distribution is likely to be associated with spawning behaviour, as a male bias is seen in spawning populations to the north around Kerguelen as well as on the Patagonian shelf (Laptikhovsky *et al.*, 2006; Lord *et al.*, 2006). However, samples of large fish are still relatively uncommon in datasets from HIMI, and the deeper habitats to the south and west have not been as well sampled as the shallow slopes to the east and northeast, and so a more complete picture of the distribution of the larger size classes may reveal that this sex bias is less dramatic overall.

The seasonal pattern of GSI revealed in this study shows that gonad growth is most rapid in March and April, as oocytes undergo vitellogenesis and hydrate, and spermatozoa form, and then declines through May and August, as fish transition through running ripe into spent and resting stages. The spawning season is similar to that reported in other studies of toothfish, with a review by Collins *et al.* (2010) noting that the spawning seasons reported for toothfish throughout its range are generally between April and August. This timing is likely to have evolved to ensure that despite the relatively large (4-5 mm diameter) yolky eggs being energetically costly to produce (Evseenko *et al.*, 1995), and taking several weeks to hatch, larvae are likely to emerge coincident with the spring bloom and the eggs and larvae are less vulnerable to predation when in the plankton (Arkhipkin and Laptikhovsky, 2010). Spawning buoyant eggs at depth also allows a proportion of embryo development to occur away from surface layers where predators are more common.

The results of this study greatly extend the area where spawning activity maturing fish and spawning is likely to be occurring in the Kerguelen Plateau. The deep slopes along the entire western margin of the Kerguelen Plateau, in both the French and Australian EEZs seem to be used as spawning habitat by toothfish. There is evidence in this study that there are spawning locations on the southern slopes also, and large areas of seafloor in the 1500-1900m depth range were stage 4 and 5 fish concentrate that have not been sampled to date in the HIMI EEZ. Recent current models of the area indicate a retroflexion of the northern and southern



filaments of the Polar Front along the eastern margin of the Kerguelen Plateau (Park *et al.*, 2008; van Wijk *et al.*, 2010), which may be a feature that transports eggs and larvae from these 'upstream' locations to near the shallow northeastern end of the Plateau where the smallest juveniles have been recorded (Duhamel and Hautecoeur, 2009). Hydrodynamic modelling (e.g. Ashford *et al.*, 2012; Hanchet *et al.*, 2008) would be a useful extension of this work to determine the likely trajectories and destinations of propagules released from these locations.

The sexually dimorphic patterns seen in GSI by length and size at maturity is similar to that seen in other studies, with males maturing at a smaller size than females in both toothfish species (Arana, 2009; Collins *et al.*, 2010; Everson and Murray, 1999; Lord *et al.*, 2006; Parker and Grimes, 2010), most likely as a result of the lower energetic cost and smaller abdominal space required to produce quantities of sperm as opposed to large yolky eggs. The sizes reported for first maturity of 60 cm for males and 70 cm for females, as judged by the smallest size at which GSI increases in this study, is higher than seen in other studies. For example Lord *et al.* 2006 recorded macroscopic stage 3 and higher males of 50 cm total length and females of 54 cm from samples collected in the Kerguelen EEZ in 2002 and 2003. It is possible that there are genuine differences between these populations; however a more likely explanation for the large difference seen, particularly in the females, is interannual variability in the portion of the population that participates in spawning in any one year. For example Everson and Murray (1999) showed that at South Georgia more than 30% of the size classes of fish that were mature and in spawning condition in 1996 did not spawn in 1997. The majority of samples of GSI in this study derived from a single season, and evidence from histological analysis estimated that 31% of stage two fish may have been resting after spawning in the past, suggesting that 2011 at HIMI may have been analogous to 1997 at South Georgia. Consequently, no firm conclusions can be drawn regarding the differences between toothfish populations across the Kerguelen Plateau based these data alone. Histological analysis of samples collected over several years, collected both prior to, during and after the spawning season would allow a better characterisation of interannual variability in spawning fraction and analysis of rates of vitellogenesis and POF breakdown. Such data would also allow the exploration of relationship between spawning fraction, environmental conditions and subsequent recruitment.

Studies estimating size at maturity of toothfish generally to use stage 3 as the threshold for toothfish when estimating the proportion of the population mature (e.g. Arana, 2009; Everson and Murray, 1999; Lord *et al.*, 2006). The levels of mature resting fish that are indistinguishable macroscopically from first maturing fish at HIMI made the size at maturity estimated on this basis much higher than reported by most other studies. The review by Collins *et al.* (2010) found the size at 50% maturity to range between 80 and 129 cm for females and 63 and 105 cm in studies

across the species range, while the estimates in this study were higher than the upper end of these ranges by 28 cm for females and 15 cm for males. As a result, using these estimates is not recommended for use in future assessments. The results in this study using stage 2 as the threshold produced results that were within the range of estimates in the Collins *et al.* 2011 review, however they are also likely to be biased as not all stage 2 fish had microscopic characteristics indicating they were likely to mature during the season they were sampled. Therefore, we recommend that future assessments at HIMI use the ogive based on the stage 2 threshold, offset by two years as a compromise between more and less conservative assumptions about maturation rates.

Few studies have attempted to validate whether the Kock and Kellerman (1991) macroscopic stages reliably discriminates the transition to maturity in toothfish, and no study is available to our knowledge that assesses whether there is consistent interpretation of macroscopic stages across datasets. This study reveals that it may not reliably discriminate between stage 2 and 3 gonads. It is possible that the interpretation used on Australian vessels tends to underestimate levels of development. It is unlikely that unequivocal characteristics such as freely releasing eggs or milt in running ripe fish could be mistaken, so this effect is likely to be confined to interpretation of the transition between stage 2 and 3. Sampling in the lead up to the spawning season when the contrast between stage 2 and stage 3 fish may be higher, and the numbers of post-spawning stage 2 fish are likely to be lower would assist with understanding this phenomenon.

The lack of sampling across all potential spawning locations is also likely to have also influenced on the maturity ogives estimated in this study. The issue of spatial and temporal segregation of mature spawning fish away from the juvenile and resting population, and the potential for sampling biases influencing estimates of size at maturity was also noted for Antarctic toothfish by Parker and Grimes (2010). Modelling of scenarios of movement and distribution of mature and immature fish across the Plateau also has the potential to evaluate the extent of any sampling bias brought about by historical patterns of sampling, as well as being critical to developing assessments that can capture the spatial complexity of the behaviour and biology of toothfish.

## **Benefits and adoption**

The outcomes for this study have immediate benefits for AFMA and the industry stakeholders in the HIMI toothfish fishery:

- This study provides the first estimates of size and age at maturity for the HIMI stock that are based on data drawn from the HIMI stock, and is shown to have a significant impact on the estimates of stock status. This ensures that the catch limits set in future assessments achieve the objectives of sufficient escapement of spawning stock biomass to ensure the toothfish is sustained.
- This study confirms that toothfish spawn in both the Australian and French EEZs on the Kerguelen Plateau. Unequivocally mature or spawning toothfish have been encountered along the deep slope to the northwest, west and southwest of HIMI, and there remain large areas of suitable habitat that are unfished to the south and south east of the islands. As spawning activity is widespread and the longline fleet at HIMI is relatively small and effort is widespread, the likelihood that the current fleet at HIMI could substantially disrupt reproductive activity is less likely than if toothfish spawned at a few discrete locations where effort has focussed. Furthermore toothfish eggs and larvae being released over a large area may provide a hedge against recruitment failure due to changing current patterns from year to year and in the long term as climate change drives changes in local oceanography transporting propagules away from suitable habitat.
- This study reinforces the standing of the HIMI toothfish fishery as one of very few fisheries with stock specific empirical estimates of the majority of key population parameters (recruitment, size-at-age, catch-at-age, natural mortality and now age-at-maturity). This standing is critical for ensuring organizations such as CCAMLR and the Marine Stewardship Council continue to recognise Australia's sustainable management of this fishery.
- This study reinforces the value of ongoing collection of sex specific biological data by observers across the HIMI fishery. The analysis of spatial and temporal patterns in reproductive biology presented here would have been impossible based on a snap shot taken in a single voyage, and large areas of potential spawning habitat remain to be sampled.

## **Further development**

Sustainable management of the Patagonian toothfish stock at HIMI would benefit from further development on several themes that are beyond the scope or timeframe of this project. These include;

### **Standardised sampling across potential spawning habitat**

In this study, toothfish prefer to spawn on the deep slopes between ~1500 and 1900m. There are large areas of seafloor in this depth range that have not been sampled within the HIMI EEZ. As this is too deep to use absolute measures of abundance such as results from a trawl survey, analysing standardised catch rates from longlines as these areas are explored has the potential to enable the estimation of relative abundance of mature fish and spawners across this area.

### **Analysis of temporal dynamics in gonad development**

This study shows that the dynamics of maturation and oocyte development within Patagonian toothfish is complex, with a large proportion of the population that may be expected to be mature either resting or spent when sampling was conducted in 2011. Resampling spawning locations in the lead up to the spawning season (April-May), before they have had an opportunity to release eggs has the potential to allow macroscopic discrimination of these fish enabling more precise estimation of what proportion of the population is mature. Histological analysis would also provide data on how long post-ovulatory follicles persist, and how rapid oocytes transition from the cortical alveoli stage through to advanced vitellogenesis, which would provide a direct test of the validity of stock assessment scenario 3 above.

### **Development of two-sex assessment methods**

The results of this study reinforce the hypothesis that toothfish are sexually dimorphic, with males and females apparently having different growth and maturation rates. As the HIMI fishery moves to target larger fish, the vulnerability of the different sexes may also change. Developing assessment models that incorporate these data, as well as sex-specific catch at age and catch at length is a priority.

### **Studies of early life history processes and dispersal**

The dispersal and behaviour of the early life history stages of toothfish are poorly understood. Combining data from three-dimensional circulation models with data on important locations for spawning, egg buoyancy and larval behaviour would assist with identifying hydrographic conditions that result in dispersal or retention of eggs and larvae over the Plateau and the possible effects of variability in local oceanography on recruitment.

**Development of spatially explicit stock assessment methods**

Toothfish may take a decade to reach maturity and several decades to mix across the Plateau. The populations in the French and Australian EEZs have also had distinct histories in the magnitude of commercial and illegal fishing over the last few decades. Current stock assessment methods are unable to adequately represent the spatial complexities of fishing and toothfish distribution in the region and therefore may be biased. Therefore development of assessment methods that can estimate the distribution of age classes, by sex across the region and sustainable harvest strategies for the French and Australian EEZs is a priority.

**Data management after the project**

The datasets generated from this project including model inputs and outputs are stored on a secure server as part of the comprehensive holdings of Australian Antarctic and Subantarctic fisheries operations and biological data maintained by the Fish and Fisheries section at AAD. This server is backed up daily. Due to the commercial in confidence nature of all of the data on fisheries operations used during this project, data releases require agreement from AFMA, AAD and the industry stakeholders in the HIMI fishery.

## **Planned outcomes**

The outputs of this project have contributed substantially to all of the primary planned outcomes:

### **1. Improved stakeholder (including the Australian fishing industry, AFMA and CCAMLR) confidence in the results of HIMI stock assessments and Kerguelen Plateau-wide toothfish population models to provide advice on long term sustainable catch limits.**

Prior to this project, the estimates of size and age at maturity used in stock assessment models at HIMI were derived from other toothfish populations (Constable and Welsford, 2011). As a result of this project, maturity ogives are now available for the first time to develop stock assessment models that incorporate population specific estimates of size and age at maturity. The sensitivity tests ran as part of this project show that these estimates are very influential in estimating  $B_0$  and current stock status that in turn are influential in setting sustainable catch limits under the CCAMLR decision rules. Hence, producing these estimates resolves a significant source of uncertainty in previous assessments, and should providing increased confidence to resource managers and other stakeholders that these models will ensure harvest levels meet international and domestic obligations for responsible management of the HIMI stock and catch limits are ecologically sustainable. The results of this project have been communicated to AFMA and industry stakeholders in the form of a draft report, and will be presented at the forthcoming Subantarctic Resource Assessment Group (SARAG) and industry meetings in August 2012. This report will also be presented at to the CCAMLR Working Group for Fish Stock Assessment (WG-FSA) in October 2012, and the results will be used to develop a revised stock assessment for presentation to SARAG and CCAMLR WG-FSA in 2013.

### **2. The establishment of the presence of spawning toothfish within the Australian EEZ.**

Prior to this project, the nearest known substantial spawning location to the HIMI fishery was in the French EEZ west of Kerguelen. The knowledge that toothfish spawning activity occurs at several locations along the Kerguelen Plateau, including in the HIMI EEZ, combined with catch and effort from the legal fleet and the data on likely locations of IUU removals has the potential to greater improve the assessment of the impact that past fishing has had in the across the region. It is also an important step in developing strategies for long term sustainable harvest that take into account the spatial complexity of the populations of toothfish that interact across the Kerguelen Plateau.

**3. A higher probability of maintenance of toothfish spawning biomass at or above target levels in the long term as required by the Commonwealth Harvest Strategy Policy (HSP) and CCAMLR.**

The results of sensitivity tests and 35 year projections in this project indicate that the current harvest rate within the Australian EEZ at HIMI has less than a 10% chance of resulting in the toothfish spawning stock being depleted below 20% of the estimated median pre-exploitation biomass ( $B_0$ ), and has a greater than 50% chance to remain at or above 50% ( $B_0$ ). Therefore the current harvest rate satisfies the CCAMLR decision rules, and the current target and limit reference points as defined by the HSP for the HIMI fishery.

## Conclusion

The combination of consistent biological sampling by observers since the HIMI fishery commenced and the targeted samples collected during the 2011 spawning season has enabled the first detailed spatial and temporal patterns of Patagonian toothfish reproductive biology at HIMI, thereby achieving objective 1. The first detailed histological analysis of male and female toothfish gonads from HIMI enabled the estimation of sex specific size and age at maturity ogives, as outlined in objective 2, has also been achieved. Finally, objective 3 has been achieved through the sensitivity testing of the 2011 assessment to the inclusion of the new maturity ogives, and the establishment that large areas of the deep slope at HIMI are used by toothfish for spawning and are likely to make a substantial contribution to sustaining the toothfish populations across the Kerguelen Plateau. Therefore this project has successfully achieved all of its objectives and has made substantial progress towards achieving the planned outcomes noted above.

This study indicates that the current assessment framework is likely to provide improved estimates of sustainable catch levels when it include the maturity ogives and it is important this is taken into account when the toothfish stock assessment is revised in 2013. Furthermore this study highlights the need for the development of assessment models that can better capture the spatial structure of the toothfish populations that result from their movements from juvenile habitats to spawning areas, and the differences in growth and maturation rates between males and females.



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## **Appendix 1. Intellectual Property**

No intellectual property is identified as arising from this project.

The dataset generated from this project is maintained in a secure database at the AAD. A metadata record describing the datasets and terms of use has been lodged with Australian Antarctic Data Centre (<http://data.aad.gov.au/>).

**Appendix 2.                      Staff**

Dr Steve Candy

Mr Tim Lamb

Mr Jesse McIvor

Ms Gabrielle Nowara

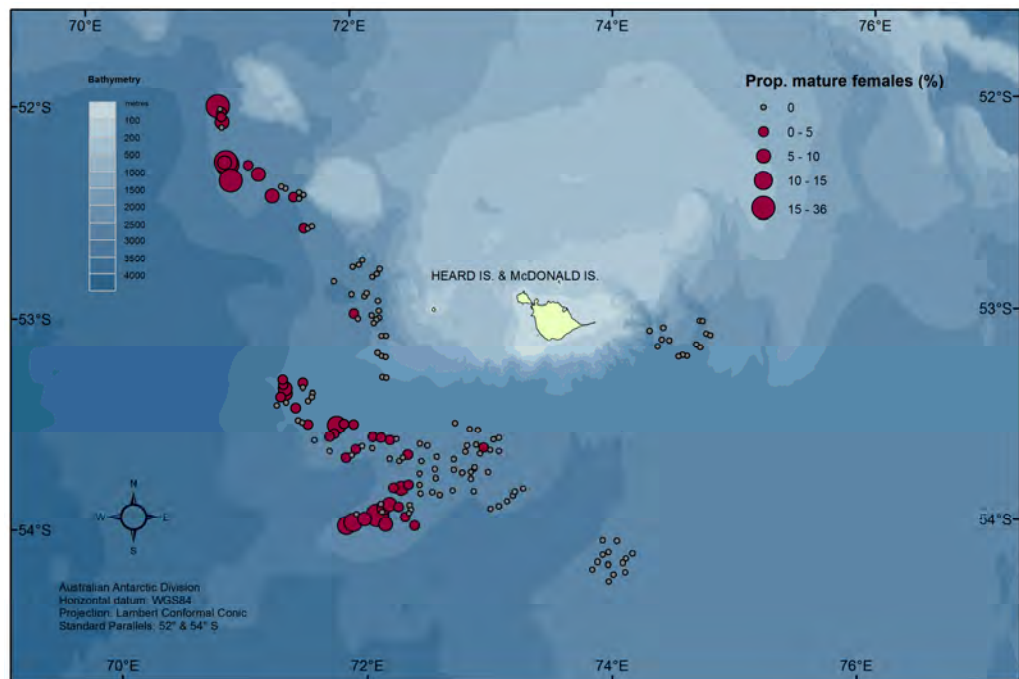
Dr Dirk Welsford

**Appendix 3. Age length key (proportions at age by 50 mm length bin) for Patagonian toothfish at Heard Island and the McDonald Islands used to convert size at maturity to age at maturity, derived from commercially caught samples, 1997-2011**

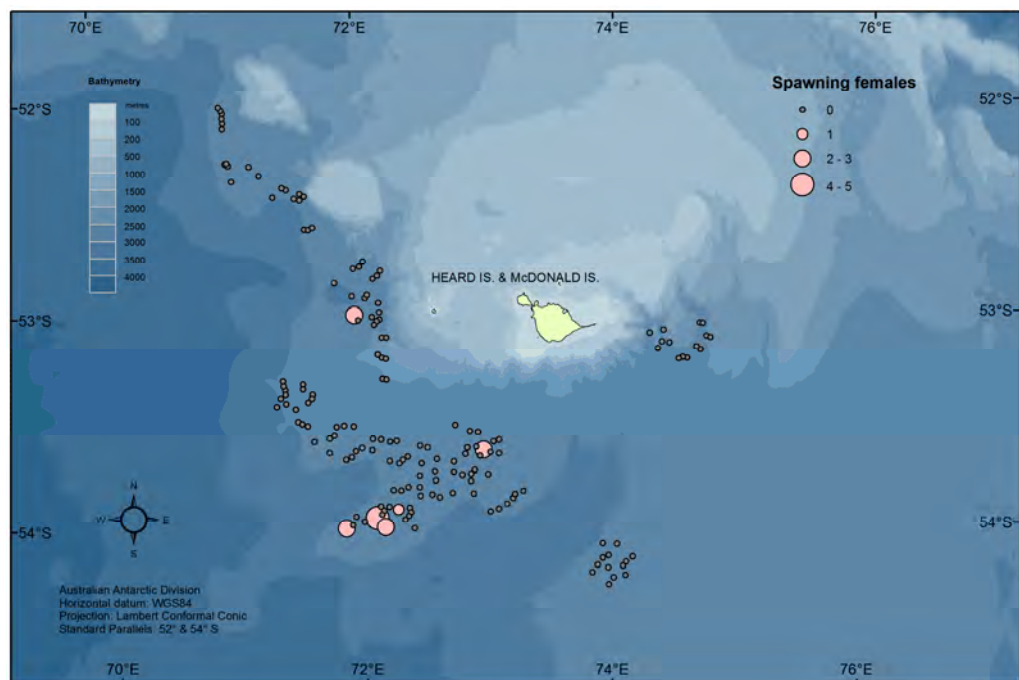
Age	Length Class (mm)																								
	250	300	350	400	450	500	550	600	650	700	750	800	850	900	950	1000	1050	1100	1150	1200	1250	1300	1350	1400	1450
1	0.06	0.02	0.03	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.69	0.46	0.18	0.08	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.25	0.49	0.59	0.31	0.12	0.06	0.02	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.03	0.15	0.45	0.34	0.20	0.10	0.04	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.04	0.09	0.35	0.37	0.21	0.12	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.01	0.03	0.12	0.20	0.28	0.20	0.12	0.06	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.03	0.09	0.21	0.30	0.23	0.12	0.05	0.03	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.02	0.03	0.11	0.17	0.24	0.20	0.14	0.06	0.03	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.11	0.19	0.22	0.22	0.11	0.05	0.03	0.02	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.03	0.11	0.18	0.22	0.18	0.11	0.08	0.02	0.03	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.04	0.11	0.14	0.18	0.18	0.10	0.07	0.04	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.05	0.09	0.20	0.20	0.18	0.09	0.10	0.03	0.06	0.03	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.06	0.13	0.15	0.19	0.18	0.15	0.05	0.05	0.04	0.04	0.01	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01	0.02	0.06	0.12	0.16	0.19	0.11	0.17	0.09	0.03	0.07	0.02	0.02	0.06	0.03	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.07	0.09	0.15	0.18	0.14	0.17	0.09	0.09	0.07	0.02	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.08	0.12	0.11	0.16	0.18	0.09	0.11	0.08	0.06	0.00	0.03	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.03	0.07	0.10	0.15	0.12	0.16	0.13	0.12	0.08	0.14	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.03	0.04	0.08	0.14	0.12	0.14	0.10	0.08	0.08	0.11	0.13	0.06
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.02	0.04	0.06	0.06	0.11	0.12	0.21	0.08	0.09	0.07	0.12
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.05	0.05	0.08	0.12	0.15	0.10	0.17	0.00	0.06
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.03	0.08	0.09	0.08	0.21	0.14	0.13	0.06
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.02	0.04	0.08	0.15	0.06	0.23	0.12
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.02	0.05	0.10	0.09	0.17	0.10
24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.04	0.04	0.01	0.08	0.09	0.10	0.12
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.03	0.00	0.00	0.03	0.07	0.06
26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03	0.02	0.00	0.03	0.03	0.33



**Appendix 4. Locations of sampling by the Antarctic Chieftain, June – August 2011, and levels of reproductive development recorded.**



**Figure A4.1** Midpoint locations of longlines set from the Antarctic Chieftain in winter 2011 and proportions of females sampled per line that had gonads assessed as macroscopic stage 3 or above. Bathymetry data courtesy of the Australian Antarctic Data Centre (AADC).



**Figure A4.2** Midpoint locations of longlines set from the Antarctic Chieftain in winter 2011 and numbers of females sampled per line that had gonads assessed as macroscopic stage 4 (spawning). Bathymetry data courtesy of the AADC.

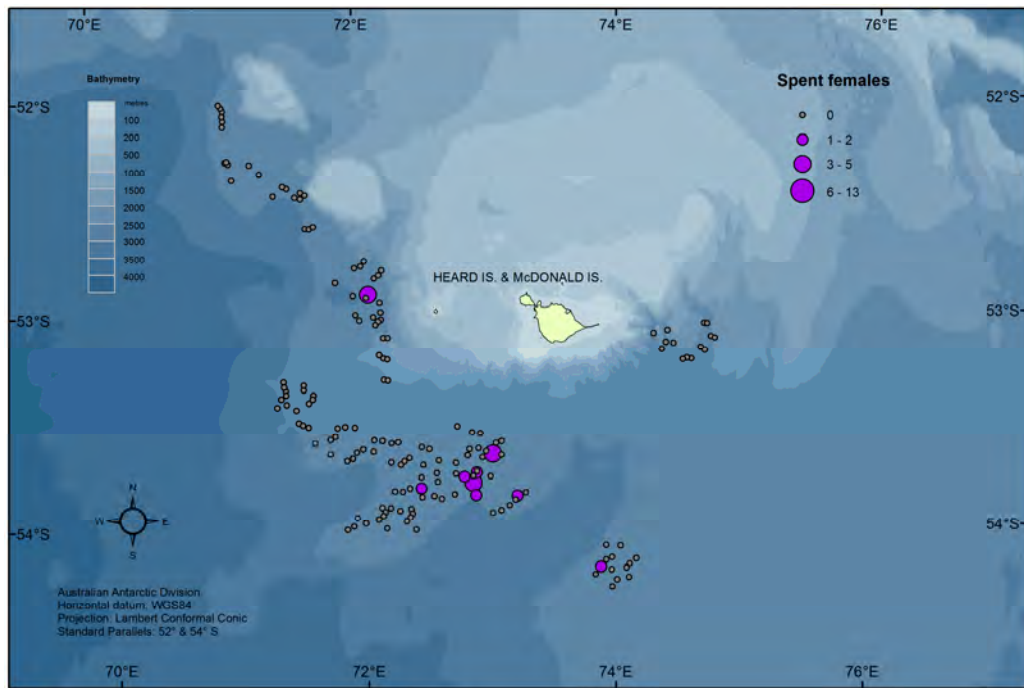


Figure A4.3 Midpoint locations of longlines set from the Antarctic Chieftain in winter 2011 and numbers of females sampled per line that had gonads assessed as macroscopic stage 5 (spent). Bathymetry data courtesy of the AADC.

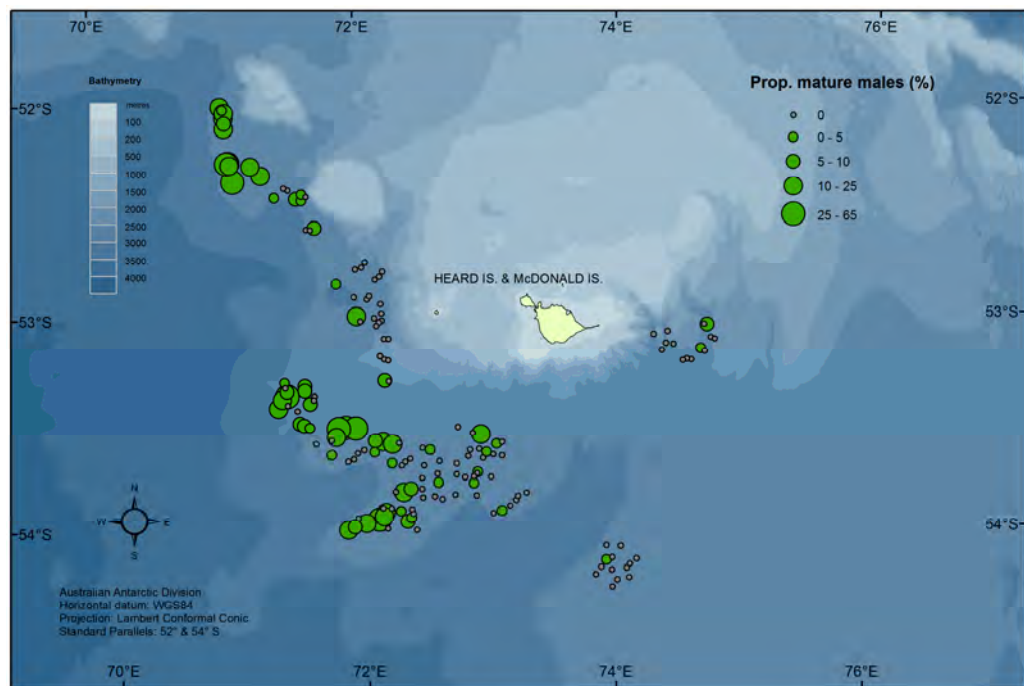


Figure A4.4 Midpoint locations of longlines set from the Antarctic Chieftain in winter 2011 and proportions of males sampled per line that had gonads assessed as macroscopic stage 3 or above (mature). Bathymetry data courtesy of the AADC.