



UNIVERSITY *of*
TASMANIA



IMAS
INSTITUTE FOR MARINE & ANTARCTIC STUDIES

Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour

Justin Bell, Jeremy Lyle, Jayson Semmens, Cynthia Awruch, David Moreno, Suzie Currie, Andrea Morash, Jeff Ross and Neville Barrett

February 2016

FRDC Project No 2013/008



FRDC

FISHERIES RESEARCH &
DEVELOPMENT CORPORATION



Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour

**Justin Bell, Jeremy Lyle, Jayson Semmens, Cynthia Awruch, David Moreno,
Suzie Currie, Andrea Morash, Jeff Ross and Neville Barrett**

February 2016

FRDC Project No 2013/008

© 2016 Fisheries Research and Development Corporation.
All rights reserved.

ISBN 978-1-86295-851-7 (print) 978-1-86295-853-1 (electronic)

Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour

2013/008

2016

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Fisheries Research and Development Corporation and the Institute for Marine and Antarctic Studies, University of Tasmania.

This publication (and any information sourced from it) should be attributed to Bell, J.D., Lyle J.M., Semmens, J.M., Awruch, C., Moreno, D., Currie, S., Morash, A., Ross, J., Barrett, N., 2016, *Movement, habitat utilisation and population status of the endangered Maugean skate and implications for fishing and aquaculture operations in Macquarie Harbour*, Fisheries Research and Development Corporation Project No. 2013/008. Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, January. CC BY 3.0]

Creative Commons licence

All material in this publication is licensed under a Creative Commons Attribution 3.0 Australia Licence, save for content supplied by third parties, logos and the Commonwealth Coat of Arms.



Creative Commons Attribution 3.0 Australia Licence is a standard form licence agreement that allows you to copy, distribute, transmit and adapt this publication provided you attribute the work. A summary of the licence terms is available from creativecommons.org/licenses/by/3.0/au/deed.en. The full licence terms are available from creativecommons.org/licenses/by/3.0/au/legalcode.

Inquiries regarding the licence and any use of this document should be sent to: frdc@frdc.com.au

Disclaimer

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a readers particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the FRDC.

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

Researcher Contact Details

Name: Jeremy Lyle
Address: Nubeena Crescent,
Taroona, Tasmania, 7053
Phone: 0362 277 255
Fax: 0362 278 035
Email: Jeremy.Lyle@utas.edu.au

FRDC Contact Details

Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6285 0400
Fax: 02 6285 0499
Email: frdc@frdc.com.au
Web: www.frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Acknowledgments	v
Abbreviations	vi
Executive Summary	vii
Introduction	1
Objectives	5
Methods	6
Acoustic telemetry	6
VR2 deployment and retrieval	6
Tagging	6
Movement modelling	8
Range testing.....	10
Biological sampling.....	11
Endocrinology.....	12
Respirometry.....	12
Age determination	13
Population estimation	13
Water chemistry.....	13
Ethics and permits.....	13
Results	14
Water chemistry.....	14
Maugean Skate.....	19
General	19
Movement and behaviour.....	20
Diet.....	36
Reproduction	38
Respirometry	45
Age and growth.....	46
Population estimation.....	48
Salmonids	49
Movement	49
Survival	54
Discussion	56
Distribution, habitat utilisation and movement of Maugean Skate.....	56
Biology and population size of Maugean Skate	57
Diet.....	57
Reproduction.....	58
Respirometry.....	59
Age and growth.....	59
Population size	60
Spatial and temporal dispersal pattern of salmonid escapees	61
Potential impacts of marine farming operations on the Maugean Skate population	62
Strategies to reduce Maugean Skate interactions with gillnetting	63
Conclusion	66

Implications	68
Recommendations	70
Extension and Adoption	71
Project coverage.....	71
Appendices 1: Range testing	73
Appendix 2: Individual depth utilisation	76
Appendix 3: Project staff	79
Appendix 4: References	80

List of Tables

Table 1: Multiple pairwise Kolmogorov-Smirnov for regional difference in the length distribution of Maugean Skate.....	20
Table 2: Summary statistics for each acoustically tagged Maugean Skate, Atlantic Salmon and Rainbow Trout.....	23
Table 3: Mean/median day, and night, depth distribution with standard deviation in parentheses.	32
Table 4: The significance values (p) of multiple pairwise Mann-Whitney test for variation in the activity of Maugean Skate between dawn, day, dusk and night.	33
Table 5: Welch two sample t-test (not assuming equal variance) of Maugean Skate depth usage in the Liberty Point region immediately prior to and after the recharge event in late July 2014.	33
Table 6: Dietary composition as measured by the IRI, percent contributions by weight, number and frequency of occurrence for 57 Maugean Skate.	37
Table 7: Logistic regression of the influence of size and sex on sexual maturation.	39
Table 8: Age estimates derived from Maugean Skate vertebrae.	47
Table 9: Tag-recaptured individuals that had been at liberty for >6 months.....	47
Table 10: Maugean Skate population estimates using Chao Lower Bound and Huggins models of tag-recapture data.	49
Table 11: The number of days that 90, 75, 50, 25 and 10% of salmonids ‘survived’ following release.	55

List of Figures

Figure 1: Maugean Skate following capture (A) and in situ (B).	4
Figure 2: VR2 (red circle) names and locations, water quality monitoring stations (triangles), fishing locations (blue) and location terminology in Macquarie Harbour.....	7
Figure 3: Regional breakdown of Macquarie Harbour.....	9
Figure 4: Gordon River flow above the Franklin River junction during the study period.	14

Figure 5: Vertical salinity (ppt) at depth profiles at the four water quality monitoring sites in Macquarie Harbour during the study period.	15
Figure 6: Vertical temperature (°C) at depth profiles at the four water quality monitoring sites in Macquarie Harbour during the study period.	16
Figure 7: Vertical dissolved oxygen concentration (% saturation) at depth profiles at the four water quality monitoring sites in Macquarie Harbour during the study period.	17
Figure 8: Variation in temperature and dissolved oxygen throughout the study period at a site at (A) 22 m depth, (B) 17 m and (C) 7 m depth off Liberty Point during the study period.	18
Figure 9: Length frequency distribution of Maugean Skate captured in both the present and previous studies (Lyle <i>et al.</i> , 2014).	19
Figure 10: Comparison of the length frequency distribution of male and female Maugean Skate captured in the present study and in a general gillnet project by Lyle <i>et al.</i> (2014) undertaken between November 2011 and February 2013.	20
Figure 11: Regional presence-absence plots for tagged Maugean Skate in Macquarie Harbour.	27
Figure 12: Utilisation distributions of selected Maugean Skate displaying differing home ranges.	28
Figure 13: Utilisation distributions (50% and 95%) for all Maugean Skate.	29
Figure 14: Utilisation distributions of all tagged Maugean Skate created from the combined probability distribution retrieved from the BBMM of each individual skate.	29
Figure 15: Frequency of detections by depth for all tagged Maugean Skate combined.	31
Figure 16: Diel depth utilisation (detections) for all Maugean Skate combined.	31
Figure 17: Diel variation in activity for Maugean Skate.	33
Figure 18: Depth utilisation (detections) of Maugean Skate with >500 detections in the week before and after the recharge event that occurred on the 29 th July 2014.	34
Figure 19: GAM of depth utilisation of selected Maugean Skate in the Liberty Point region.	35
Figure 20: Depth utilisation of MS05 throughout the duration of the entire study highlighting the change in behaviour following the DO recharge that occurred in late July 2014.	36
Figure 21: Number of dietary items by major prey group present in Maugean Skate stomachs.	37
Figure 22: Ultrasound of a large, mature Maugean Skate ovary. Arrows indicate follicles. Grid squares are 10 mm.	38
Figure 23: Bias plot of MFD as measured using ultrasound and post dissection with callipers.	38
Figure 24: Maturity ogives of male (A) and female (B) Maugean Skate.	40
Figure 25: Relationship between hormone levels and total length of male Maugean skate (n=49).	40
Figure 26: Relationship between hormone levels and total length of female Maugean skate (n = 51).	41
Figure 27: Seasonal MFD (determined by ultrasonography) for Maugean Skate.	42
Figure 28: Reproductive system and dissected ovary of a 719 mm female Maugean Skate.	43
Figure 29: Seasonal variation in hormone levels of adult female Maugean Skate.	44
Figure 30: Seasonal variation in testosterone levels of adult male Maugean Skate.	45
Figure 31: Mean metabolic rate (MO ₂) of 20% (blue) and 55% (orange) DO (%O ₂) treatment skate correlated with the DO level in the sealed respirometer as oxygen is consumed.	46
Figure 32: Longitudinal section of a Maugean Skate vertebrae.	47

Figure 33: von Bertalanffy growth model of Maugean Skate length at age.....	48
Figure 34: BBMMs for Atlantic Salmon that were at liberty for longer than one month.	50
Figure 35: Frequency of salmonid detections by the two main curtains in Macquarie Harbour (refer to Figure 1 for receiver locations).	51
Figure 36: Regional utilisation of Macquarie Harbour by Atlantic Salmon.....	51
Figure 37: BBMMs for Rainbow Trout that were at liberty for the longest periods of time.....	53
Figure 38: Regional utilisation of Macquarie Harbour by Rainbow Trout.	54
Figure 39: Survival rate and residency within Macquarie Harbour of salmonids after the simulated release event. Mortalities include fish captured in recreational gillnets.	55
Figure 40: Management changes (indicated in grey and yellow) to recreational gillnet usage implemented in November 2015.	65
Figure 41: Newspaper article describing the present study.	72

Acknowledgments

Firstly, we wish to thank Adam Main from the Tasmanian Salmonid Growers Association. Adam played a pivotal role in facilitating the current project, was a valuable member of the project steering committee and acted as a liaison between the research team and industry. We also gratefully acknowledge the cooperation and practical assistance provided by Tassal, Petuna and Huon Aquaculture for their assistance with deployment and retrieval of acoustic receivers, for welcoming us on their vessels and lease sites and providing Atlantic Salmon (Tassal) and Rainbow Trout (Petuna) for tagging. Zac Wingfield (Tassal) was particularly helpful regarding logistics in Strahan and generously provided mooring materials and storage space.

We wish to express our gratitude to the steering committee members for their guidance and oversight throughout the study. The members were Peter Trott (World Wildlife Fund for Nature), Neil Stump (Tasmanian Seafood Industry Council), Mark Nikolai (Tasmanian Association for Recreational Fishing), Alastair Morton (Biodiversity Conservation Branch, DPIPWE), David Jarvis (Marine Resources Branch, DPIPWE), Eric Brain (Marine Framing Branch, DPIPWE) and Adam Main.

Edward Forbes and Kay Weltz provided invaluable assistance through all phases of this study, Graeme Ewing and Jaime McAllister also assisted with fieldwork often under trying circumstances. Killian Stehfest provided guidance and helpful discussion involving the analysis of acoustic telemetry data and Andrew Pender helped with the analysis of environmental data.

Finally, we wish to thank David Hutchings for providing helpful insights into the recreational fishing practices in Macquarie Harbour and a historic perspective about the area and fishery.

Funding for this study was provided by the Australian Government through the Fisheries Research and Development Corporation and the Institute for Marine and Antarctic Studies, University of Tasmania. Additional funding was obtained from the Winifred Violet Scott Charitable Trust, Holsworth Wildlife Research Endowment Natural Sciences and Engineering Research Council Discovery Program (Canada).

Abbreviations

AIC	Akaike information criterion
BBMM	Brownian Bridge Movement Model
DPIPWE	Department of Primary Industries, Parks, Water and Environment
DO	Dissolved oxygen
E₂	17 β -estradiol
EPBC Act	Environmental Protection and Biodiversity Conservation Act (Commonwealth)
FO	Frequency of occurrence
Hb	Haemoglobin
Hct	Haematocrit
IRI	Index of relative importance
MFD	Maximum follicle diameter
MFPRP	Marine Farming Planning Review Panel
MHRA	Macquarie Harbour Recreation Association
MO₂	Metabolic rate
PIT	Passive integrated transponder
P₄	Progesterone
RBC	Red blood cells
T	Testosterone
TL	Total length
UD	Utilisation distribution
VR2	Vemco VR2w 69kHz acoustic monitoring receiver

Executive Summary

The present study, undertaken by the Institute for Marine and Antarctic Studies, represents the first major investigation of the ecology and biology of the endangered Maugean Skate (*Zearaja maugeana*). Maugean Skate are only known from two estuarine systems located on the west coast of Tasmania (Macquarie Harbour and Bathurst Harbour), suggesting that the species has one of most restricted distributions of any elasmobranch.

The Maugean Skate is afforded a degree of protection in the World Heritage listed Bathurst Harbour, a no-take marine protected area, although they appear to be rare in this location. In Macquarie Harbour they appear to be relatively abundant but are subjected to multiple impacts due to human activities: they are a by-catch in recreational gillnets, and salmonid aquaculture operations are widespread and expanding in Macquarie Harbour. It is not known how these activities, coupled with the environmental variability in Macquarie Harbour, impact on the Maugean Skate population.

Objectives

Due to the issues outlined above, the present study aimed to:

1. determine the distribution, habitat utilisation and movement of the Maugean Skate in Macquarie Harbour;
2. determine the key biological characteristics of Maugean Skate, including population size, reproductive dynamics and feeding habits;
3. describe the spatial and temporal dispersal patterns of aquaculture escapees;
4. assess the potential impacts of current and proposed marine farming operations on the Maugean Skate population; and
5. evaluate strategies to reduce the probability of encountering Maugean Skate whilst fishing (gillnetting) for escapees

Methodology

To address the above objectives, an extensive array of acoustic receivers was placed throughout Macquarie Harbour. This array comprised several curtains to assess harbour wide movements, along with a high density of receivers amongst the marine farms and in areas where Maugean Skate are abundant and recreational gillnetting is common. A total of 58 Maugean Skate were acoustically tagged at multiple locations in the harbour and, to simulate a salmonid escape event, 30 Atlantic Salmon and 30 Rainbow Trout were acoustically tagged and released. To address the remaining objectives, seasonal biological sampling was conducted over a period of 15 months. Reproductive status was assessed using non-destructive techniques (endocrinology and ultrasonography) and stomach lavage was used to investigate diet. A preliminary assessment of metabolic response to varying levels of dissolved oxygen was also undertaken experimentally. All skate were PIT tagged prior to release and population size estimated using tag recapture rates throughout the study period.

Key findings

Maugean Skate generally displayed a high degree of site fidelity, with 50% and 95% utilisation distributions generally <3 and <10 km² respectively. Many individuals showed an affinity for the Liberty Point/Table Head region, which is located in the central, south western side of Macquarie Harbour. Over half of the tagged skate left their core home range for brief periods (days to weeks) during the study period with all but five (15%) returning to their home range. These latter individuals relocated to new sites, often after a period of relatively extensive movement throughout the harbour.

Although several skate were detected at the entrance to Macquarie Harbour all were re-detected inside the harbour afterwards with no evidence to suggest long-term movement out of the estuary. None of the skate were detected at the Gordon River mouth suggesting they do not venture into the main tributary that feeds the harbour.

Based on the number of detections, Maugean Skate spent the 85% of their time at 6–12 m depth, although they were detected from 0.6 m to >55 m, albeit rarely, indicating they are not restricted to their preferred depth range. Skate depth utilisation appears to be dictated by water chemistry with shallow waters having low salinity and high temperature variability, whereas deeper waters are stable in terms of temperature and salinity but have low concentrations of dissolved oxygen (DO) (<20%). Low DO concentrations appear limiting for the skate and presumably their prey. Waters in their preferred depth range tend to have relatively stable temperature (12–15 °C), salinity (18–27 ppt) and generally retained moderate dissolved oxygen concentrations (>30%).

Maugean Skate were more active during the night and moved into shallower water, which possibly represents nocturnal foraging behaviour. Maugean Skate have a restricted diet dominated by three groups of epibenthic crustaceans, namely crabs, carid shrimp and mysids. Fish represented a minor prey item. While there was no evidence of pellet feeding, this cannot be ruled out since sampling was conducted some distance away from the farm lease sites and skate tend to have small home ranges.

Males and females matured at significantly different sizes; 50% maturity was attained at 632 mm TL in males (based on clasper size and condition) and 662 mm TL in females (based on maximum follicle diameter). Endocrinology generally confirmed these estimates, with females >680 mm displaying an increased levels of testosterone and progesterone and males >620 mm displaying an increase in testosterone. Maximum follicle diameter and hormone levels (testosterone, progesterone and 17 β -estradiol) of mature females were highly variable seasonally suggesting an asynchronous, discontinuous reproductive cycle in which a proportion of the population is reproductively active while the remainder are in a resting phase at any given time of year. There was, however, some evidence that reproductive activity may be reduced during summer.

Preliminary estimates of age from sectioned vertebrae for thirteen Maugean Skate suggest that the species is relatively short lived (maximum age observed of 11 years) but may live to about 15+ years. Maximum age (and size) is a useful proxy for productivity and our results suggest that Maugean Skate are probably relatively productive.

The population of Maugean Skate is possibly one of the smallest of any chondrichthyan species. The species is only known from two Tasmanian estuaries and when considered in the context of their preferred habitat (predominantly 5–15 m) means they probably also have one of the smallest distributions of any chondrichthyan. The best estimate of the population size in Macquarie Harbour was in the order of 3000 individuals. There are, however, potential biases in this assessment that suggest it may be an underestimate and thus a feasible minimum possible population size.

Atlantic Salmon and Rainbow Trout dispersed rapidly upon release, moving widely and generally randomly throughout Macquarie Harbour. Several Rainbow Trout and a single Atlantic Salmon showed an affinity for regions near aquaculture leases suggesting at least some of these individuals were feeding on aquaculture overfeed. The vast majority did not, however, survive or remain in the harbour for longer than about two months following release. As a general observation, Rainbow Trout tended to survive slightly longer than Atlantic Salmon. While about 25% of the escapees were recaptured by recreational fishers (in gillnets), most are assumed to have died of natural causes (starvation and possibly predation). About 20% of the salmonids did, however, leave the harbour either by moving out to sea (mainly Atlantic Salmon) or entering the Gordon River (Rainbow Trout). The fate of these individuals could not be assessed.

Implications for marine farm operations

Direct interactions between Maugean Skate and aquaculture operations appear to be limited. The aquaculture industry expansion strategy in Macquarie Harbour involves the location of new lease sites into the deeper regions, which given the Maugean Skate's preference for shallower depths means that there is minimal overlap between core skate habitat and the marine farm lease sites. Furthermore, this study provided no evidence of feeding on fish pellet overfeed by the skate. It is not possible, however, to completely discount this occurring, noting that skate are capable of moving throughout the entire harbour, including into the deepest areas.

There may, however, be indirect interactions, for instance the production of organic wastes associated with marine farming operations increases biological oxygen demand and acts to reduce DO as well as enriching of the pelagic environment through the excretion of dissolved nutrients (e.g. ammonium and nitrate). Between 2009 and mid-2014 there was a downward trend in DO levels of the deep-waters (>15m) of Macquarie Harbour. As this period corresponded with a major expansion of salmon aquaculture in the harbour as well as historically low river flow, any causal attribution for this decline is uncertain. The influence of bottom DO in determining suitable skate habitat is uncertain but it is highly likely that any reductions in bottom DO, regardless of cause, will negatively influence the area of core habitat (preferred depths).

The environmental health of Macquarie Harbour, in particular levels of DO in the bottom waters, is likely to represent a crucial factor in the future well-being of the Maugean Skate population. The aquaculture industry along with other human activities impacting on Macquarie Harbour (mining, hydro-electricity generation [river flows], coastal development) all play a role in shaping the environmental conditions of this unique system. The maintenance of best environmental practices by the aquaculture industry supported by effective monitoring and environmental management policies represent essential requirements if industry and Maugean Skate populations are to coexist in perpetuity.

Implications for gillnetting

Maugean Skate are a relatively common by-catch in gillnets. Recreational fishers tend to set their nets close to shore in relatively shallow water to target salmonids or on the shallow sand flats in the lower reaches to target flounder. Nevertheless recreational gillnets were observed in areas where Maugean Skate are abundant, particularly in the Table Head and Liberty Point regions where it can be difficult to avoid waters >5 m due to the drop-off close to shore. The majority of the Maugean Skate captured in gillnets are likely to be alive, however, mortalities do occur as a result of predation and potentially due to prolonged exposure to unfavourable environmental conditions whilst restrained by the gillnet.

There are several management options that could be implemented, either singularly or in combination, to reduce the likelihood of fishery interactions with Maugean Skate, but only a prohibition on gillnetting will effectively eliminate interactions. Given that a ban is unlikely in the short to medium term, management must balance the risk of interactions occurring, and their consequences, against the size and productivity of the population to ensure that fishing induced mortality does not exceed the rate at which the population is able to regenerate. A number of changes specifically aimed at reducing the impacts of fishing on Maugean Skate populations were implemented in late 2015. Of these, limiting gillnetting mainly to waters shallower than 5 m and the closure of areas around Table Head and Liberty Point are anticipated to reduce (but not eliminate) the likelihood of Maugean Skate interactions with gillnets.

Keywords

Maugean Skate, *Zearaja maugeana*, Macquarie Harbour, telemetry, gillnet bycatch, salmonid aquaculture, salmonid escapees.

Introduction

The expansion of marine farming in Macquarie Harbour represents a key element of the aquaculture industry's strategy to significantly increase salmonid production in Tasmania. In considering the planning application, the Marine Farming Planning Review Panel identified that research is required to properly understand the ecological effects of the farming operations, including potential impacts on the endangered Maugean Skate (*Zearaja maugeana*), a species restricted to Macquarie Harbour and one other western Tasmanian estuary (Bathurst Harbour). Further, recreational gillnetting is common in Macquarie Harbour and there has been some concern expressed regarding the impacts of this activity on bycatch, including Maugean Skate. The present study was developed to provide an assessment of the potential impacts of fishing and marine farming on the Maugean Skate and a scientific basis to mitigate and/or manage these impacts.

Maugean Skate

The Maugean Skate, pictured in Figure 1, was first discovered scientifically in 1988 and is known from only two localities, Macquarie Harbour (western Tasmania) and Bathurst Harbour (south-western Tasmania). The total range of the species is thought to be no more than 100 km² and the population size has been estimated to be in the order of 1000 individuals (Last and Gledhill, 2007). In the absence of stock discrimination studies it is also considered likely that individuals in Macquarie and Bathurst Harbours comprise separate populations (IUCN, 2007).

The population in Bathurst Harbour appears to be small; in relation to research surveys of the area only four individuals have been captured (using gillnets) and another individual observed while diving (Last and Gledhill, 2007). No Maugean Skate have been observed in Bathurst Harbour since initial surveys were conducted in the late 1980s, this is despite more than 30 hours of dive surveys spent searching for the species (Treloar *et al.*, 2013). In contrast, the population in Macquarie Harbour appears to be larger, with the species readily captured throughout the harbour and at times in relatively high numbers (Lyle *et al.*, 2014). Based on its limited geographic range the species has been listed as endangered under the Threatened Species Protection Act (Tasmania), the Environmental Protection and Biodiversity Conservation Act (Commonwealth) and is listed on the International Union for the Conservation of Nature Red List of Threatened Species (Gledhill and Last, 2005). According to the listings, the main potential threats to the Maugean Skate in Macquarie Harbour are heavy metal pollution (in the sediments) from historic mining operations, incidental capture in fishing activities (in particular gillnets), the introduction of non-native marine species, and an increase in eco-tourism.

The Maugean Skate is a relatively small species with two known, larger, sister species, *Z. nasutus* from New Zealand and *Z. chilensis* from Chile. Both these species inhabit the continental slope. The ancestral form of this lineage probably inhabited the continental slope of Gondwana during the late Mesozoic until the separation of Australia, New Zealand and South America led to speciation (Last and Yearsley, 2002). Maugean Skate were presumably more widespread in southern Australia with the reason for their isolation to the two estuaries systems unknown but most likely related to intermittent warming and cooling during the Pleistocene (Last and Gledhill, 2007). Interestingly, the Maugean Skate appears to be the only Rajoid known to permanently inhabit estuarine waters (Last and Gledhill, 2007).

Skates belong to the order Rajiformes and have been shown to be prone to the effects of fishing, particularly in the north Atlantic (reviewed by Dulvy and Forest (2010)). The lineage is typically moderately long lived (Licandeo *et al.*, 2006; Matta and Gunderson, 2007; Francis and Gallagher, 2009) and attains sexual maturity at a late age (Henderson *et al.*, 2004; Matta and Gunderson, 2007; Colonello *et al.*, 2012). Although oviparous and moderately fecund (Holden, 1975; Ebert, 2005; Parent *et al.*, 2008), many species are reproductively active for only a few years (discussed by Ebert (2005)), with their egg capsules susceptible to predation (Cox and Koob, 1993; Lucifora and Garcia, 2004; Hoff, 2009). The conservative life history and low productivity that is typical of many skate species means that populations will be susceptible to fishing and other pressures. An understanding of

the life history characteristics and responses to external population pressures are thus essential in the development of effective strategies to manage these impacts.

The most studied member of the *Zearaja* genus is *Z. chilensis*. This species has been the target of artisanal fisheries since the 1970s and is also a by-product of industrial fishing fleets (Bustamante *et al.*, 2012). Landings of *Z. chilensis* averaged 2663 t from 1990 to 2007 in Chile alone but it is also landed in Argentina and Uruguay (Bustamante *et al.*, 2012). Studies have found *Z. chilensis* to be relatively long lived (max 21 years), late in maturation (14 years for females) and with a low reproductive rate (Licandeo *et al.*, 2006). Populations have declined over time, most likely as a consequence of high fishing pressure (Licandeo *et al.*, 2006; Bustamante *et al.*, 2012). Basic reproductive biology and ageing information are also available for *Z. nasutus* from New Zealand. This species is relatively short lived (max 9 years) and despite fisheries retaining them more often than previously, there is no evidence that stocks are being overfished (Francis *et al.*, 2001). Apart from the original description of the species virtually nothing has been published on the life history of the Maugean Skate.

Macquarie Harbour

Macquarie Harbour is a large embayment of 276 km² on the central west coast of Tasmania. High freshwater input from the Gordon-Franklin River catchment and, to a lesser extent, the King River catchment and several smaller tributaries results in heavily tannin stained surface waters. This minimises light penetration resulting in low levels of primary productivity (Edgar *et al.*, 1999). The waters of Macquarie Harbour tend to remain highly stratified and there are three distinct physio-chemical layers (Creswell *et al.*, 1989): the upper layer is predominantly freshwater and has high seasonal temperature variation; the middle layer has a brackish salinity, minimal temperature variation and low dissolved oxygen; and the bottom layer, is almost marine with salinities exceeding 31 ppt and oxygen levels increasing with depth. This bottom layer is replenished by flood tides pushing over the shallow entrance and remains below central layer due to the increased density that comes with elevated salinity. This bottom layer is not, however, truly marine due to the mixing that occurs as the seawater makes its way into the harbour.

Macquarie Harbour has been subjected to considerable alteration due to human activities for more than a century. Activities and impacts include:

- Widespread mining throughout the catchment has occurred for >100 years and has resulted in greatly reduced water quality (Carpenter *et al.*, 1991);
- Damming of the Gordon, Huon and King rivers for hydroelectricity generation has altered the hydrology of the system (Carpenter *et al.*, 1991);
- Large-scale salmonid aquaculture occurs within the harbour;
- Commercial fishing has taken place since the development of Strahan, with approximately 20 commercial fishers active at the start of the 20th century (Ware, 1908) when landings were valued at ~£3000 (\$520,000 in present day terms). A low level of commercial fishing activity still occurs in the area but is now directed primarily at the removal of salmonid escapees;
- Recreational fishing is a common activity within the harbour with fishers using hook and line and gillnets. Hook and line was once the preferred method with fishers targeting cod (*Pseudophysis* spp.) and Rock Ling (*Genypterus tigerinus*) along with gillnetting for Flounder (*Rhombosolea tapirina*). Apparent declines in the availability of these species and growth of the marine farming sector has resulted in gillnets being used increasingly to target escapees. A survey of recreational gillnetting found that 'skate' were occasionally reported as by-catch in Macquarie Harbour (Lyle and Tracey, 2012). Since the only other species of skate (Thornback Skate, *Dentiraja lemprieri*) encountered in area is very infrequently captured (Lyle *et al.*, 2014), it is likely that the majority were Maugean Skate. There is a small portion

of the World Heritage Area (South of an imaginary line between Charcoal Burners Bluff and Gordon Point) that is closed to gillnetting. It is not known how frequently Maugean Skate are captured by line fishing but it is likely they would be captured by this fishing gear.

Salmonid aquaculture

Salmonid aquaculture has a relatively short history in Tasmania with trial and small scale ventures occurring throughout the 1960s and 70s. Full scale Atlantic Salmon (*Salmo salar*) aquaculture developed through the 1980s and by 1989 Tasmania was exporting considerable quantities of Atlantic Salmon to Japan. In recent years domestic demand has increased and most of the Atlantic Salmon and Rainbow Trout (*Onchorhynchus mykiss*) produced in Tasmania is consumed within Australia. Salmonid aquaculture production in the 2012/13 financial year was 41,762 tonnes, valued at \$489 million (Anon, 2014) and now exceeds terrestrial farming in terms of value in Tasmania.

Macquarie Harbour has gradually become a favoured site for farming salmonids as the highly stratified water means that fish are able to move regularly between fresh and brackish water within the cages. This is advantageous since many of the parasites and diseases that plague the industry are unable to cope with this highly variable environment. For instance, amoebic gill disease, caused by *Neoparamoeba perurans* (Young *et al.*, 2007), is particularly problematic in purely marine waters and can prove fatal to salmonids if not treated. The best treatment is bathing fish in freshwater but doing so costs the aquaculture industry millions of dollars annually. Due to the freshwater surface layer in Macquarie Harbour this process is unnecessary thereby considerably reducing the costs of production.

A key component of the Tasmanian salmonid industry's strategic plan to double production by 2030 includes a substantial increase in production levels in Macquarie Harbour. In November 2011 the three major aquaculture companies, Tassal, Petuna and Huon Aquaculture lodged an Environmental Impact Statement to facilitate an amendment to the Macquarie Harbour Marine Farming Development Plan that would increase the aquaculture lease area from 2% to 3.3% of Macquarie Harbour. The independent Marine Farming Planning Review Panel (MFPRP) reviewed the proposal and identified, among other issues, that research was required to better understand the ecological effects of the proposed farming operations, including potential impacts on the Maugean skate (MFPRP 2012). Specifically, the MFPRP identified a need to describe the distribution, abundance and general ecology of the species, such information being a precursor to determining the nature of interactions with marine farming operations. Furthermore, the MFPRP noted the need to better understand the potential effects on the Maugean skate of efforts to recover escapees through fish-downs using gillnets. This concern linked to the more general population risk posed by gillnetting, noting that skate are taken incidentally in commercial and recreational gillnets (Lyle *et al.*, 2014) and that recreational gillnetting activity is strongly associated with targeting of escapees (Lyle and Tracey 2012).

State approval for the amendment was granted in May 2012. The amended Macquarie Harbour Marine Farming Development Plan was then provided to the Commonwealth for consideration under the EPBC Act. In October 2012 the Federal Minister for Sustainability, Environment, Water, Population and Communities announced that the proposed action was not a controlled action under the EPBC Act provided it was taken in accordance with the manner described in his decision document. This decision was made under section 75 and 77A of the EPBC Act and outlined the manner in which the action was to be undertaken.

The current project was developed in response to the MFPRP recommendations and with the full recognition by the salmonid industry that they have an environmental responsibility to identify and act to minimise any impacts that may be linked to marine farming on this unique species. There is also a need to better understand the level of risk posed by fishing on the Maugean skate population, especially since much of the gillnet effort in Macquarie Harbour is related to the targeting of escapees.



Figure 1: Maugean Skate following capture (A) and in situ (B).

Objectives

- 1 Determine the distribution, habitat utilisation and movement of the Maugean Skate in Macquarie Harbour.
- 2 Determine the key biological characteristics of Maugean Skate, including population size, reproductive dynamics and feeding habits.
- 3 Describe the spatial and temporal dispersal patterns of salmonid escapees in Macquarie Harbour.
- 4 Assess the potential impacts of current and proposed marine farming operations on the Maugean Skate population.
- 5 Evaluate strategies to reduce the probability of encountering Maugean Skate whilst fishing (gillnetting) for escapees.

Methods

Acoustic telemetry

Acoustic telemetry, comprising an array of omnidirectional acoustic receivers designed to detect, and record, individually coded acoustic tags (Heupel *et al.*, 2006) implanted in Maugean Skate and ‘escapee’ salmonids, was used to examine the distribution, habitat usage and movement of these species within Macquarie Harbour.

VR2 deployment and retrieval

The acoustic array comprised 57 passive acoustic receivers (Vemco VR2w-69kHz) deployed throughout Macquarie Harbour (Figure 2). The layout of the array was based on several considerations that would best enable the study objectives to be achieved. Acoustic curtains were located in the upper, mid and lower reaches of the system to investigate large scale movements throughout the harbour. A high density of receivers was located in the Table Head/Liberty Point region as this covered the area where the majority of the aquaculture activity was located prior to the start of the industry expansion. It also represented an area known to contain high densities of Maugean Skate (Lyle *et al.*, 2014) and to attract a considerable amount of recreational gillnetting activity. In addition, receivers were placed strategically in the upper (Kelly’s Basin, Farm Cove and Rum Point) and lower (Swan Basin and Long Bay) reaches of the harbour where previous studies have shown Maugean Skate are moderately abundant (Lyle *et al.*, 2014). Recreational gillnetting is also particularly common in the lower reaches due to close proximity to the township of Strahan. Additional receivers were positioned at the mouth of the Gordon River, in the main channels leading to Macquarie Heads (MH01 and CC01) and at the ocean entrance to the harbour, Macquarie Heads (MH02).

Most receivers were attached to a rope with cable ties ~1 m above a mooring block. A sub-surface buoy ensured the receivers remained erect in the water column. Receivers at the Gordon River mouth (GR01) and in the channel heading toward Macquarie Heads (MH01) were attached to pylons and angled towards the deeper areas in both cases. The receiver at the entrance of Macquarie Harbour (MH02) and receivers located on aquaculture farms were weighted and hung mid-water beneath a jetty and marine farm infrastructure, respectively.

The array was deployed in late October and early November 2013 and was retrieved in early February 2015. Receivers LP02, LP03 and SP03 (Figure 2) were unable to be retrieved meaning the effective array and subsequent analyses was based on 54 receivers.

Tagging

Most of the Maugean Skate for tagging were captured by gillnets deployed in depths ranging from 1–19 m in three separate regions (Liberty Point/Table Head, Swan Basin and Kelly’s Basin/Rum Point). Gillnets were set during the daytime and retrieved within 2–3 hours to maximise the likelihood that any Maugean Skate captured were in excellent condition (Lyle *et al.*, 2014). The gillnets were standard monofilament graball nets (50 m long by 33 mesh drop, 114 mm stretched mesh) of the type commonly used by recreational fishers. A small number of Maugean Skate were also captured by demersal longline when targeting Whitespotted Dogfish (*Squalus acanthias*) for an associated postgraduate project.

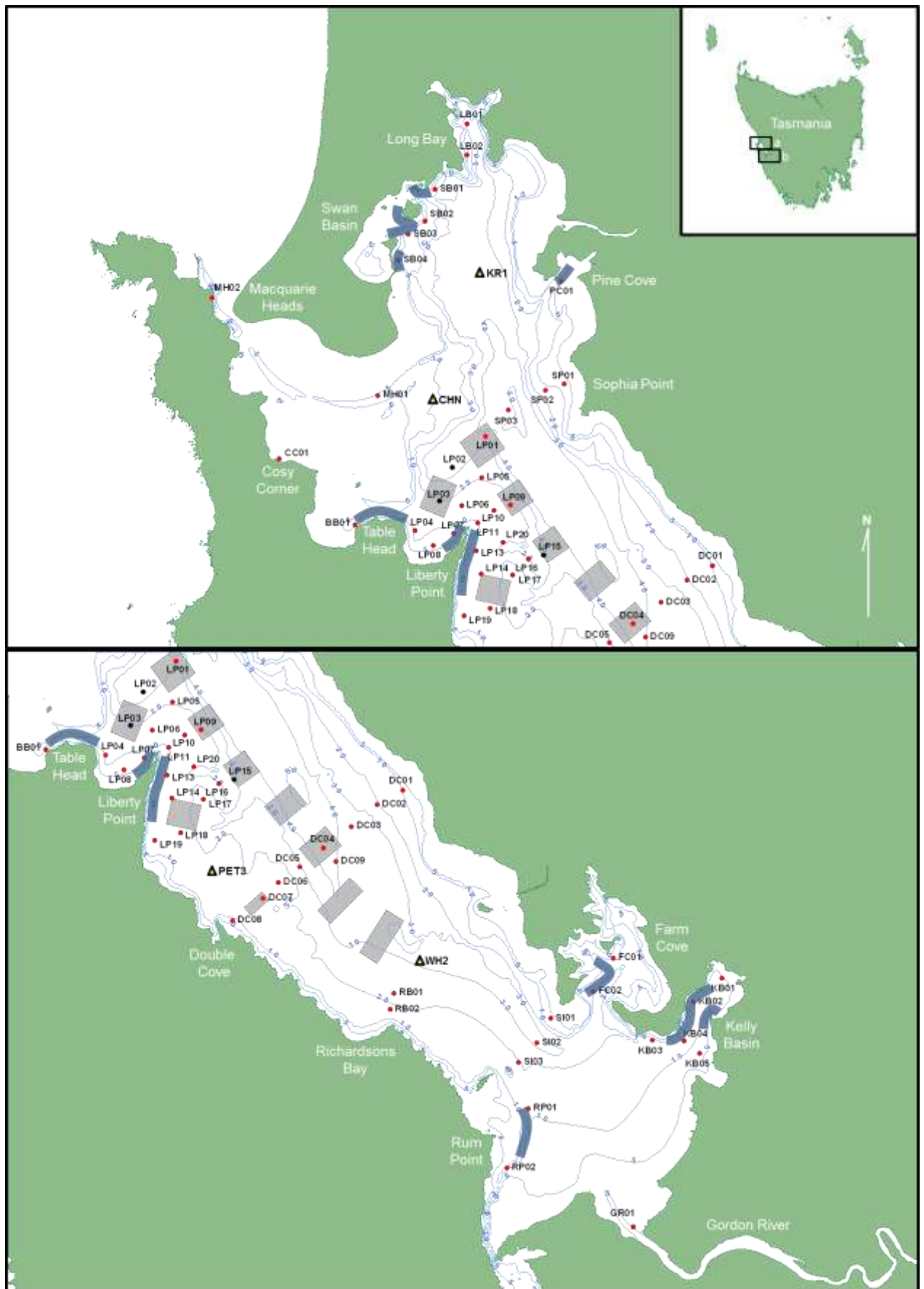


Figure 2: VR2 (red circle) names and locations, water quality monitoring stations (triangles), fishing locations (blue) and location terminology in Macquarie Harbour. Black circles indicate VR2's that were not successfully retrieved.

When captured, skate were immediately placed into a 250 L tub with water that had been pumped from ~10 m depth, thereby ensuring the water chemistry of the holding tub was similar to that of the depth from which the majority of fish were captured. Once the net was cleared, skate were inverted to induce tonic immobility, a 1–2 cm incision was made between the pelvic and pectoral girdles, and an acoustic transmitter was inserted into the peritoneal cavity. A passive integrated transponder (PIT) tag was also inserted before the incision was closed with sutures. Skate were then released near an acoustic receiver to ensure that the tag was operational.

Forty Maugean Skate were tagged between the 30th October and 6th November 2013 with a further 18 individuals tagged on the 17th and 18th of February 2014. The initial 40 Maugean Skate were tagged with V9P-2H transmitters (9 mm diameter, 47 mm length and 3.5 g in water, and 151 dB re 1uPa @ 1m power output), which have a pressure sensor for measuring depth. In the second group, eight individuals were tagged with V13-1H transmitters (recycled from recaptured tagged salmonids, refer below) and ten skate were tagged with V9AP-2L accelerometer/pressure transmitters (9 mm diameter, 46 mm length and 3.3 g in water, and 145 dB re 1uPa @ 1m power output). The V9AP transmitters measure acceleration at a rate of 5 Hz in three (forward (y), lateral (z) and vertical (x)) axes over a 20 s sampling interval. Static acceleration (that due to the earth's gravity) is filtered out of the data, leaving only the acceleration from the motion of the tagged animal (dynamic acceleration). The root mean square (RMS) or resultant vector of the x, z, y dynamic acceleration is calculated (e.g., $\sqrt{x^2 + z^2 + y^2}$), averaged over the sampling period and then stored in memory. This RMS value is then transmitted during the next transmission cycle and provides a relative measure of activity. Pressure was also transmitted every third transmission. All V9 transmitters had a transmission delay of 160–200 seconds.

Salmonids were captured by dipnet from their aquaculture lease cages (30 Atlantic Salmon and 30 Rainbow Trout) and tagged using similar procedures to the skate with the following variations: fish were anaesthetised in a bath of Aqui-S™ (0.03 ml·L⁻¹) until sufficiently subdued (when the fish can be handled without response) and a dilute solution (0.015 ml·L⁻¹) of Aqui-S™ was pumped across the fish's gills via the mouth to maintain an anaesthetised state during the surgery; the acoustic transmitters implanted were V13-1H (13 mm diameter, 36 mm length and 6 g in water, and 153 dB re 1uPa @ 1m power output), and have a transmission delay of 60–120 seconds but do not contain a pressure sensor; fish were retained in a water bath until they had regained consciousness (usually within 5 minutes) and displayed regular opercular movement and responses to stimuli; and the fish were released at the aquaculture lease site, rather than near an acoustic receiver. Release locations were 42°18'58.42''S, 145°23'01.74''E for Atlantic Salmon and 42°19'50.82''S, 145°21'32.14''E for Rainbow Trout (chart datum WGS84). The salmonids were also tagged with a standard t-bar tag to aid their identification if recaptured by recreational fishers. Posters promoting the reporting of recaptured salmonids were placed at key fishing access points throughout the harbour.

All acoustic transmitter types had an expected battery life of one year.

Movement modelling

Presence/absence plots, along with summaries of the frequency of days individuals were detected, the number of receivers detecting individual skate/fish daily, summaries of variation in daily depth and acceleration (where available), were used to describe the movement of each skate/fish individually and also to ensure that the individual was still alive. If an individual was assessed to have died, the time and date of the event were identified and any further detections were removed from subsequent analysis. In order to summarise and visualise presence/absence data, Macquarie Harbour was divided into five regions (Figure 3) and the presence by region was assessed on a daily basis for each tagged animal. Since individuals may be detected in multiple regions on a given day, the 'jitter' option in R was used when plotting the data. This function randomly distributes each data point vertically and thus ensures that if multiple regions were visited on a given day each of the regions will be visible in the data plot.

A variety of modelling techniques were investigated to describe the movement, habitat utilisation and home range of the Maugean Skate and salmonids. The first, and simplest, was minimum convex polygons, which describe the minimum total area occupied by the individual. These, however, have a tendency to overestimate the home range of animals (Burgman and Fox, 2003) and were therefore abandoned. To overcome the limitations of minimum convex polygons, it has become increasingly popular to model the probability distribution, which is commonly referred to as utilisation distribution (UD). We applied kernel utilisation distribution (KUD) and Brownian bridge movement models (BBMM) using the R packages 'BBMM' v.3.0 (Nielson *et al.*, 2013) and 'move' v.1.4.496 (Kranstauber and Smolla, 2014). BBMMs were created using the 50% detection range calculated using range testing results (see below) and, following power analysis, five hours was selected as an appropriate time lag (the period of time between successive detections for which to estimate the UD). Longer time lags did create bridges during longer movements but also created implausibly large UD's while animals were most likely still within the vicinity of their core home range. As the VR2 array was relatively sparse throughout Macquarie Harbour, BBMM were more effective in describing UD as they assign probability to where individuals could be when they are not being detected. Therefore, KUD are not provided herein.

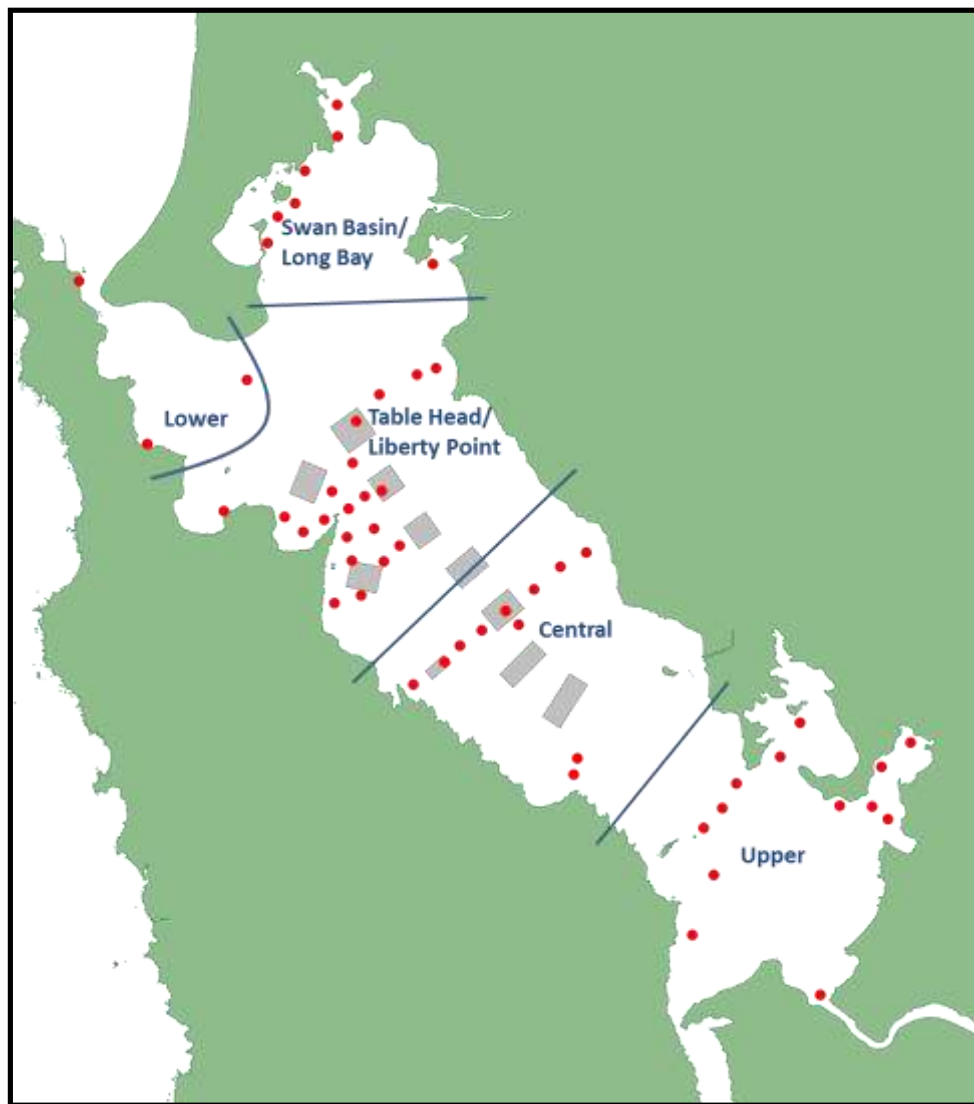


Figure 3: Regional breakdown of Macquarie Harbour.

Diel patterns in depth utilisation and activity in Maugean Skate were also explored. This was achieved by calculating sunrise and sunset times for each receiver using the ‘maptools’ package v.0.8–36 (Bivand and Lewin-Koh, 2015) in R. Dawn was assigned as between one hour before and one hour after sunrise and dusk one hour before and one hour after sunset. Day was the period between dawn and dusk and night the period between dusk and dawn.

For salmonids, BBMMs were also calculated with a five hour time lag, however, their highly mobile behaviour meant that they spent a great deal of time outside of detection range so, to prevent overly large unrealistic UD, position averaging was used to estimate their position at ten minute intervals while they were travelling between receivers (e.g. Simpfendorfer *et al.* (2002)). To describe survival and residency of the salmonids within Macquarie Harbour, the proportion that were active (i.e. being detected) and present in Macquarie Harbour was assessed through time. Some individuals died within the detection range of a receiver, however, for the remainder, we deemed them to have died at the time of their last detection or to have exited the harbour at the time of their last detection at the entrance to the harbour (MH02) or the Gordon River (GR01). This approach was considered appropriate as both species were highly active and typically detected on multiple receivers each day whilst alive. The likelihood of an individual still being alive and within the harbour but remaining undetected for long periods was, therefore, assumed to be very low. Fish that were reported as recaptures by recreational fishers were classed to have died on the day that they were captured. Fish that appear to have died within three days of tagging were eliminated from this analysis as their death was likely to be associated with tagging.

Range testing

Range testing involves testing the distance that VR2 receivers are able to detect acoustic tags. Range testing should preferably be carried out using a variety of methods, throughout the duration of the study and throughout the study area to enable spatial and temporal variation in detection range to be identified (Kessel *et al.*, 2014).

In this study the following range testing methods were utilised:

- *Sentinel tags* – twelve sentinel tags (V16-4H; transmission delay 500–700 seconds; 153 dB re 1uPa @ 1m power output) were deployed near receivers throughout Macquarie Harbour for the entire study duration (DC08, FC02, GR01, KB02, KB04, LB02, LP01, LP07, LP20, RB02, RP01 and SB03). Most sentinels were located ~120 m from the receiver, exceptions included GR01 and KB02 (~200 m from the receivers) and LP01 (~360 m from the receiver).
- *Boat-based range testing* – this was carried out at Macquarie Heads (MH02) to determine the likelihood of detecting a tagged animal should it leave, or return to, Macquarie Harbour. This was achieved by drifting with the current at a variety of distances from the receiver with a range test tag (V9-2H with a transmission delay of 12 seconds and 151 dB re 1uPa @ 1m power output) hanging as deep as possible without it becoming snagged on the bottom.
- *Single range test tag at a variety of distances from a receiver* – during seasonal sampling, a VR2 was deployed and a range test tag (V9-2H) was placed at a variety of distances from the receiver. Additionally, this was often carried out within detection range of receivers within the acoustic array and the distance between tag and receiver was calculated to enable these data to be included in the analyses.

In all cases range test tags had identical transmission power to those inserted into skate to ensure results were representative.

The quality of the range testing carried out was assessed according to the ranking system described in Kessel *et al.* (2014) and is based on the following criteria: how it is conducted; whether it is considered to be variable; the source of range testing data; the type and number of methods used; the duration of range testing; the number of receivers assessed; the types of tags used; and, the number of variables that were assessed for their influence on detection range.

Spatial and temporal variation in the proportion of successful detections at various distances was investigated using logistic regression in base R. Temporal variation was tested by converting dates to Julian day and treating this as a numeric variable in the logistic model. In terms of spatial variation, categorical generalised linear models compare one category to each subsequent category. Kelly's Basin was selected as the comparison location as it had the shortest detection range.

Biological sampling

Biological sampling was undertaken at three monthly intervals between February 2014 and February 2015 (i.e. five seasonal samples) to investigate reproductive seasonality and changes in the dietary composition of Maugean Skate. When possible, sampling took place in the Swan Basin, Liberty Point/Table Head and Kelly's Basin regions to ensure regional variation could be identified. It was not, however, possible to sample at Kelly's Basin during winter 2014 due to adverse weather conditions. Sampling took place with identical gear and methods to those used when capturing skate for tagging.

When a skate was captured, it was measured, scanned for a PIT tag (tagging procedure detailed later) and then placed in a 250 L tub of oxygenated water that had been pumped from ~10 m depth. After the net was retrieved, female skate were placed in a shallow secondary tub of oxygenated water and examined using an ultrasound (BCF Easy-Scan, Series 2) to determine maximum follicle diameter (MFD). Clasper length (from cloaca to distal end) and clasper calcification (un-calcified, partially-calcified or fully-calcified) were recorded for males. Following reproductive assessment, both sexes were treated identically. Fish were placed in a dampened towel, inverted, and a 0.5–1 ml blood sample was taken from the caudal vein immediately posterior to the cloaca with a 22 gauge heparinised syringe. Blood was stored on crushed ice until processing (described in detail later). A PIT tag was then inserted into the musculature of the right anterior pectoral fin and the tag scanned to ensure it was functional and to record the tag number. Betadine antiseptic cream was applied to the wound and the skate was either released or returned to the oxygenated tub of water to recover before gastric lavage was used to extract their stomach contents (Nelson and Ross, 1995). Stomach contents were initially stored on ice and then frozen until laboratory examination.

The number, weight and size (carapace length of shrimp and carapace width of crabs) of Maugean Skate dietary items was recorded to the lowest taxonomic level possible. An index of relative importance (Pinkas, 1971) was calculate using the equation:

$$IRI = (N + M) * FO,$$

where N is the numerical percentage, M is the mass percentage and FO is the frequency of occurrence of the particular prey item.

To validate ultrasonography, 12 Whitespotted Skate (*Dipturus cervia*) and one Thornback Skate (*Dentiraja lemprieri*) were purchased from a commercial fisher and MFD was estimated with the ultrasound before dissecting each fish and measuring the largest ovum with Vernier callipers. During this process assistance was provided by an experienced ultrasonographer who specialises in identifying the reproductive condition of fish for the aquaculture industry. Additionally, seven female Maugean Skate that were euthanised for respirometry experiments (refer below) were used to assess the accuracy of the ultrasound method.

The size at which Maugean Skate mature and whether maturation was sexually dimorphic was investigated using binomial logistic regression with the 'glm' function in base R. Male skate were considered mature when they had fully calcified claspers and females were considered mature if the MFD was greater than or equal to 5 mm. Variation in MFD was investigated to determine whether there was any obvious reproductive seasonality.

Endocrinology

Blood samples were centrifuged at 12,000 rpm for 5 min and plasma extracted with a micropipette before freezing until analysis. Blood plasma levels of testosterone (T), 17 β -estradiol (E₂) and progesterone (P₄) were measured in both sexes by radioimmunoassay. Plasma samples (200 μ l) were extracted twice with ethyl acetate (1 ml), and 100 μ l aliquots were transferred to assay tubes and evaporated before addition of an assay buffer. Testosterone antiserum was purchased from Novus Biologicals[®] (U.S.A) and reconstituted in 10 ml of phosgel buffer. Estradiol and P₄ antisera were purchased from Sigma-Aldrich (Australia) and reconstituted by adding 5 ml of Tris buffer (pH 8, 0.1 M HCl). Testosterone [1,2,6,7-³H], E₂ [1,2,6,7-³H] and P₄ [1,2,6,7-³H] were purchased from Perkin-Elmer (Australia) and 50 μ l of T, E₂ and P₄ were diluted in 5 ml of 100% ethanol and kept as stock for the assay. Duplicate standards (0–800 pg per tube authentic T, E₂ and P₄ in ethanol) and sample extracts were evaporated. 200 μ l of the reconstituted T antiserum, diluted 1:10 in assay buffer (containing 0.1% of gelatin and 0.01% of thiomersal), 100 μ l of the reconstituted P₄ antiserum (diluted 1:250) and E₂ antiserum (diluted 1:30000), and 50 μ l of the T, P₄ and E₂ stock, were added to each tube. Bound and free fractions were then separated using dextran-coated charcoal and aliquots of the supernatants counted in a Beckman LS 5801 liquid scintillation counter. All assays were validated by the evaluation of the slope of serially diluted extractions of plasma against the assay standards. The extraction efficiency was determined from recovery of ³H-labelled steroid added to 200 μ l pooled aliquots of plasma and assay values were corrected accordingly. Extraction efficiency was 93% (T), 87% (P₄) and 83% (E₂). The detection limit for all assays was 0.04 ng (ml plasma)⁻¹. Intra- and inter-assay variability was determined by including replicates of three levels of commercially available human control serum (CON4, CON5 and CON6 DPC) in each assay. Inter-assay variability was 8% (T), 9% (E₂) and 11% (P₄) and intra-assay variability was <5% for all hormones.

Respirometry

Experimental Design

In order to examine physiological responses to varying levels of DO, a sample of thirteen Maugean Skate were captured by gillnet in ~10 m of water at Table Head and Swan Basin during November 2014. They were then transported to a temporary onshore laboratory and held in aquaria containing harbour water drawn from ~10 m depths. Six skate were transferred into a sealed 1200 l tank containing water with 55% DO, which was generally consistent with the DO at the depths the animals were captured, and seven were transferred into a sealed 1200 l tank containing water with 20% DO, representative of areas of low DO in the harbour. Both tanks had a pump placed in the bottom to circulate the water. Skate were held in their respective treatments (20% or 55% DO) for ~24 hours. A blood sample was then taken (see method described previously) and the skate were placed individually into an open respirometer to recover for ~1 hour. The respirometer was then sealed and metabolic rate (MO₂) was measured using an HQ40d dissolved oxygen meter and LDO probe (HACH), until MO₂ was close to zero. The skate was then removed from the respirometer, another blood sample taken and the individual placed back into its respective holding treatment for a further ~24 hours. Following this, a final blood sample was taken and the individual euthanised and dissected. An array of tissue samples were removed and stored in liquid nitrogen and later in a -80°C freezer for later analysis.

Biochemical analyses

Haematocrit (Hct) was measured in duplicate immediately after each blood sample was drawn using a SpinCrit Microhaematocrit centrifuge (SpinCrit.). Haemoglobin (Hb) was measured using a HemoCue[®] Hb 201+ system (Ängelholm) in accordance with manufacturer's instructions, and whole blood glucose and lactate concentrations (~5 μ l blood each) were determined respectively using a OneTouch Ultra2 glucometer (LifeScan, Milpitas, California) and a Lactate Pro[™] (Arkray Global Business, Inc.) handheld meter. The remaining blood sample was then spun at 13,000 rpm for 4 min at 4°C to separate plasma and red blood cells (RBCs). The buffy coat was discarded and plasma and red blood cells were immediately placed in separate cryovials in liquid nitrogen for later analysis. Long-term storage was at -80°C.

Age determination

Maugean Skate euthanised at the completion of the respirometry experiments enabled post cranial vertebrae to be extracted for age determination. Excess tissue was removed with a scalpel and remaining tissue dissolved by immersing vertebrae in bleach (sodium hypochlorite 42 g L⁻¹; sodium hydroxide 9 g L⁻¹) for 5–10 mins. Vertebrae were then embedded in polyester resin and sectioned longitudinally at ~300 µm. Sections were mounted on microscope slides and a cover slip applied with polyester resin. Sections were viewed with transmitted light under a dissecting microscope at 25–100 times magnification. Alternating translucent and opaque regions were assumed to represent annual growth and each estimate was assigned a readability ranking using a five stage system based on Morison *et al.*, (1998). The von Bertalanffy growth model was fit to the length at age data using the 'nls' function in R.

Population estimation

Tag-recapture histories were created for each individual PIT tagged Maugean Skate. Population size was estimated using closed population models (Otis *et al.*, 1978) within the R packages Rcapture v1.4-2 (Baillargeon and Rivest, 2007) and RMark version v.2.1.12 (Laake, 2013) that acts as an interface with program MARK v.6.2 (White and Burnham, 1999). Acoustically tagged individuals that had died soon after tagging (indicated by acoustic telemetry) were removed from this analysis.

Water chemistry

Dissolved oxygen (DO), salinity and temperature loggers (Onset HOBO U26-001 Dissolved Oxygen Logger), positioned approximately one metre from the bottom, were mounted on the receiver infrastructure at LP05, LP06 and LP07 (Figure 2). These loggers were downloaded and batteries replaced on each sampling trip to Macquarie Harbour to provide a continuous record of DO, salinity and temperature at three depths throughout the study period. Only two dissolved oxygen replacement caps were able to be sourced for the winter 2014 trip and as a consequence only caps on LP05 and LP06 were replaced. This meant that the dataset for LP07 was incomplete (three months of missing data). However, this was the shallowest location and previous downloads had indicated that it was permanently in relatively mixed and oxygenated water.

In addition, on each seasonal sampling trip vertical water chemistry profiles (DO, temperature and salinity by depth) were taken at a variety of fixed sites around the harbour, and in the vicinity of where fishing was conducted. The logger used was a Sonde (YSI 6600 multi-parameter water quality monitor) and the individual parameters were determined at one metre or greater intervals between the surface and bottom. Additionally, the aquaculture industry has accumulated a large dataset of water quality in Macquarie Harbour to which we were granted access for the present study. These data comprise monthly vertical water column profiles from a variety of sites throughout the harbour. For our purposes, we chose to utilise the data from four sites; two being in depths and locations relatively close to where the majority of acoustic receivers were located, and the other two being in deeper waters and located in the northern and southern extremities of the harbour (Figure 2).

Ethics and permits

All procedures were undertaken with University of Tasmania Animal Ethics Committee approval (permit A13468) and scientific permits 13125 and 14139 issued under Section 14 of the Living Marine Resources Management Act 1995 and permits TFA 13982, 14019, and 14253 issued under Regulation 4 of the Threatened Species Protection Regulations 2006 and Section 29 of the Nature Conservation Act 2002. These latter permits covered the capture, possession, tagging and biological sampling of an endangered species, the deployment of research fishing gear and the deployment of moorings within Macquarie Harbour, including the World Heritage Area.

Results

Water chemistry

The water chemistry of Macquarie Harbour is complex with a distinct halocline occurring below the freshwater surface layer, driven by the flows of the Gordon River (Figure 4), typically occurring in the 4–8 m depth range (Figure 5). The precise depth at which the halocline occurred varied temporally but was relatively consistent throughout the harbour. The depth of the halocline was linked with Gordon River flows but since both the Gordon and Franklin rivers are dammed variability in the river flows was not entirely representative of seasonal rainfall. For instance, river flows were as high in summer 2013/14 as they were for much of the winter of 2014 (Figure 4). Flows were much reduced during the summer of 2014/15 and were likely to be more representative of normal conditions as the Gordon dam had been lowered intentionally during the previous year. While the release of water for hydro-electricity is responsible for large scale trends, daily flows were variable throughout the year, presumably as a result of rainfall in the catchment below the dams and in the catchments of undammed tributaries. In winter there were a series of spikes in river flow, the greatest of which was during a significant flood event that occurred in late July 2014.

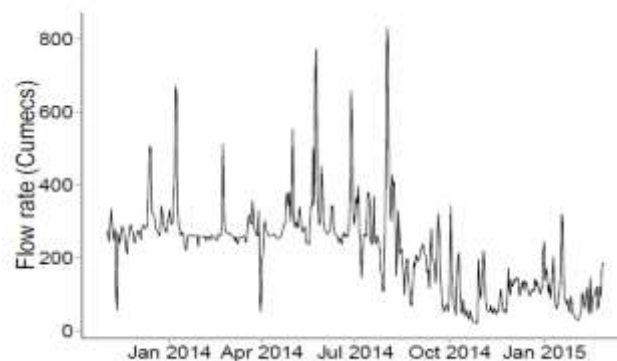


Figure 4: Gordon River flow above the Franklin River junction during the study period.

Temperatures were very stable below the halocline, ranging between 13–16 °C throughout the year and throughout the harbour (Figure 6). Salinity increased to about 30 ppt in depths greater than 15 m (Figure 5) while DO declined with depth (Figure 7). Although there was some temporal variation in these parameters, vertical water chemistry profiles were remarkably similar between the upper and lower reaches of Macquarie Harbour at any given time. Above the halocline, salinity and temperature were highly variable whereas DO remained relatively high throughout the year (Figures 5, 6 and 7).

At each of the three fixed Liberty Point monitoring stations there was notable variation in bottom DO throughout the year. The shallowest station (LP07 – 7 m depth) had very high variability (Figure 8a), even over short time periods, because it was at a depth close to that of the halocline. At this site, DO was high (generally >80%) for much of the year dropping to around 60% for a period of time between January and April 2014 (Figure 8a). The modelled mean daily DO showed no obvious trend at this depth (Figure 8a). At the two deeper sites (LP06 – 17 m and LP05 – 22 m) bottom DO fluctuated considerably (0–100%) but was frequently below 10% saturation (Figure 8b, c). There was a general decline in DO at these sites from November 2013 until the 29th July 2014 at which time there was a major storm event that recharged DO levels. At LP06, DO increased from 12.8% at 18:00 on the 29th July to 72.4% at 04:30 on the 30th of July (i.e. over a period of 10.5 hours). Throughout the remainder of the study period DO declined gradually and by February 2015 bottom DO at LP06 had returned to levels similar to those prior to the recharge event. At LP05 DO increased to ~50% immediately after the storm before declining throughout the remainder of the study but was still elevated (25%) at the end of the study some six months later. Salinity and temperature remained relatively stable (temperature 13–15 °C and salinity 24–27 ppt) at these deeper sites throughout the entire study with the recharge event and other periods of unstable weather, causing only brief and relatively minor fluctuations in both parameters.

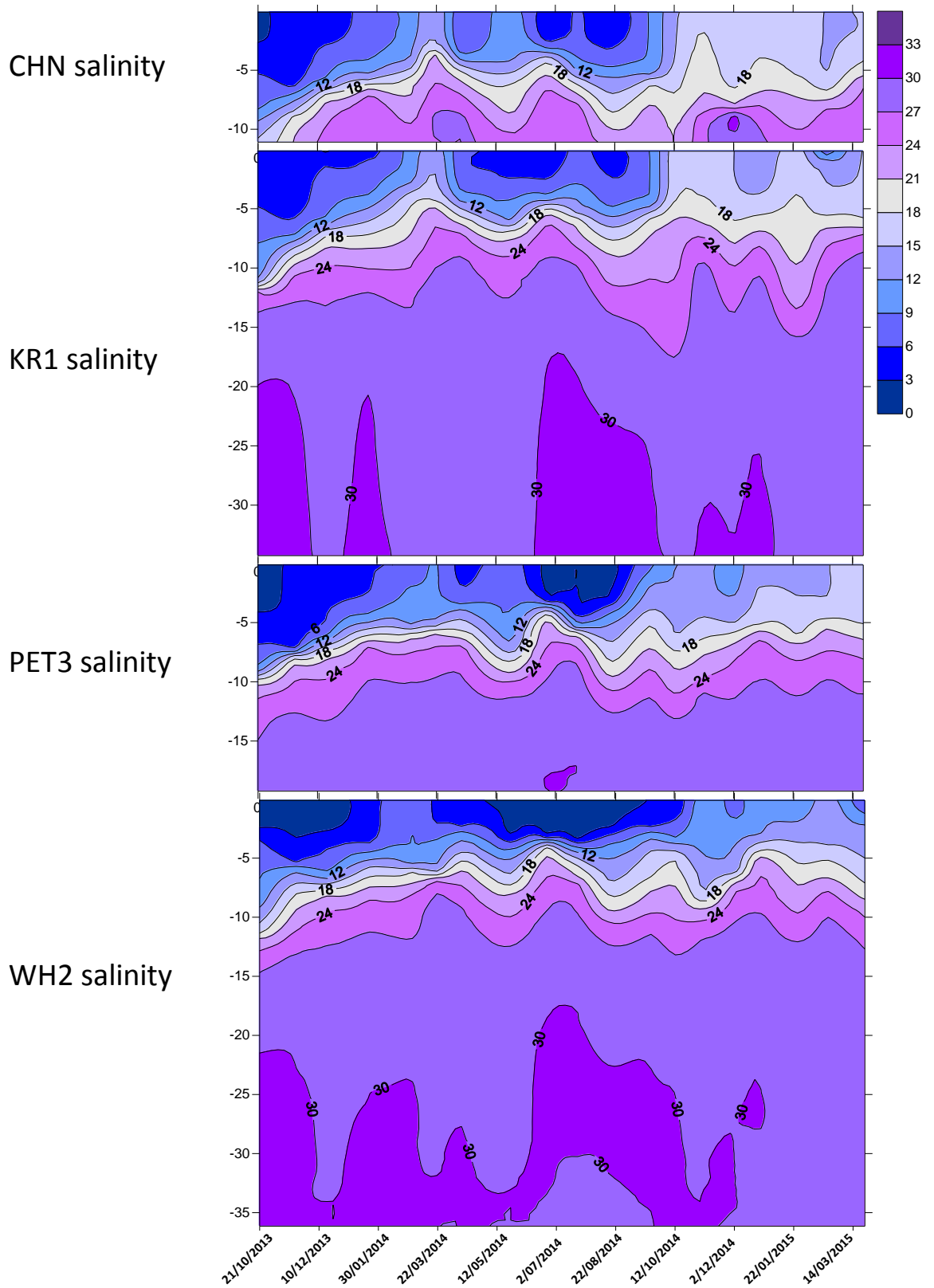


Figure 5: Vertical salinity (ppt) at depth profiles at the four water quality monitoring sites in Macquarie Harbour during the study period.

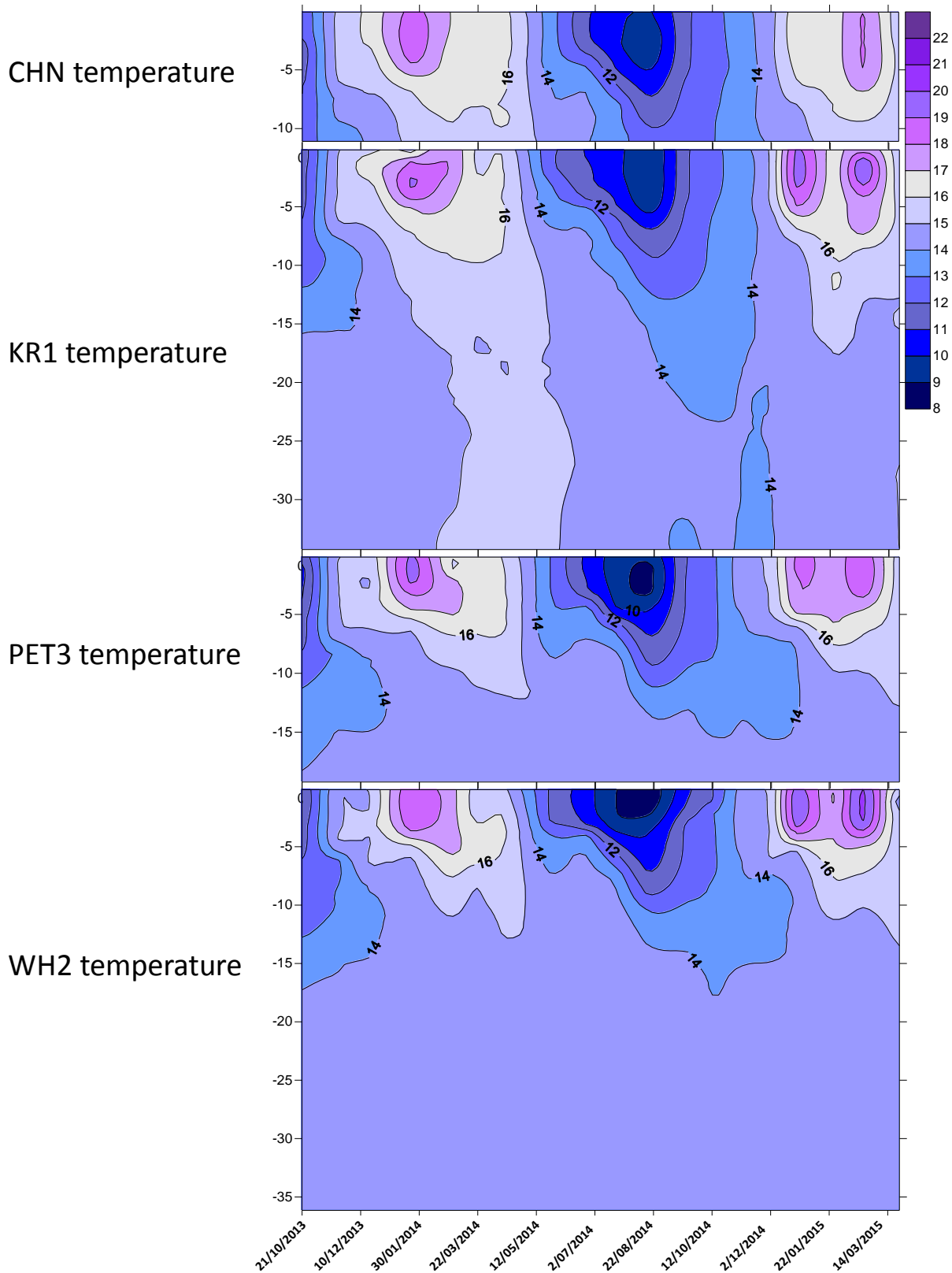


Figure 6: Vertical temperature ($^{\circ}\text{C}$) at depth profiles at the four water quality monitoring sites in Macquarie Harbour during the study period.

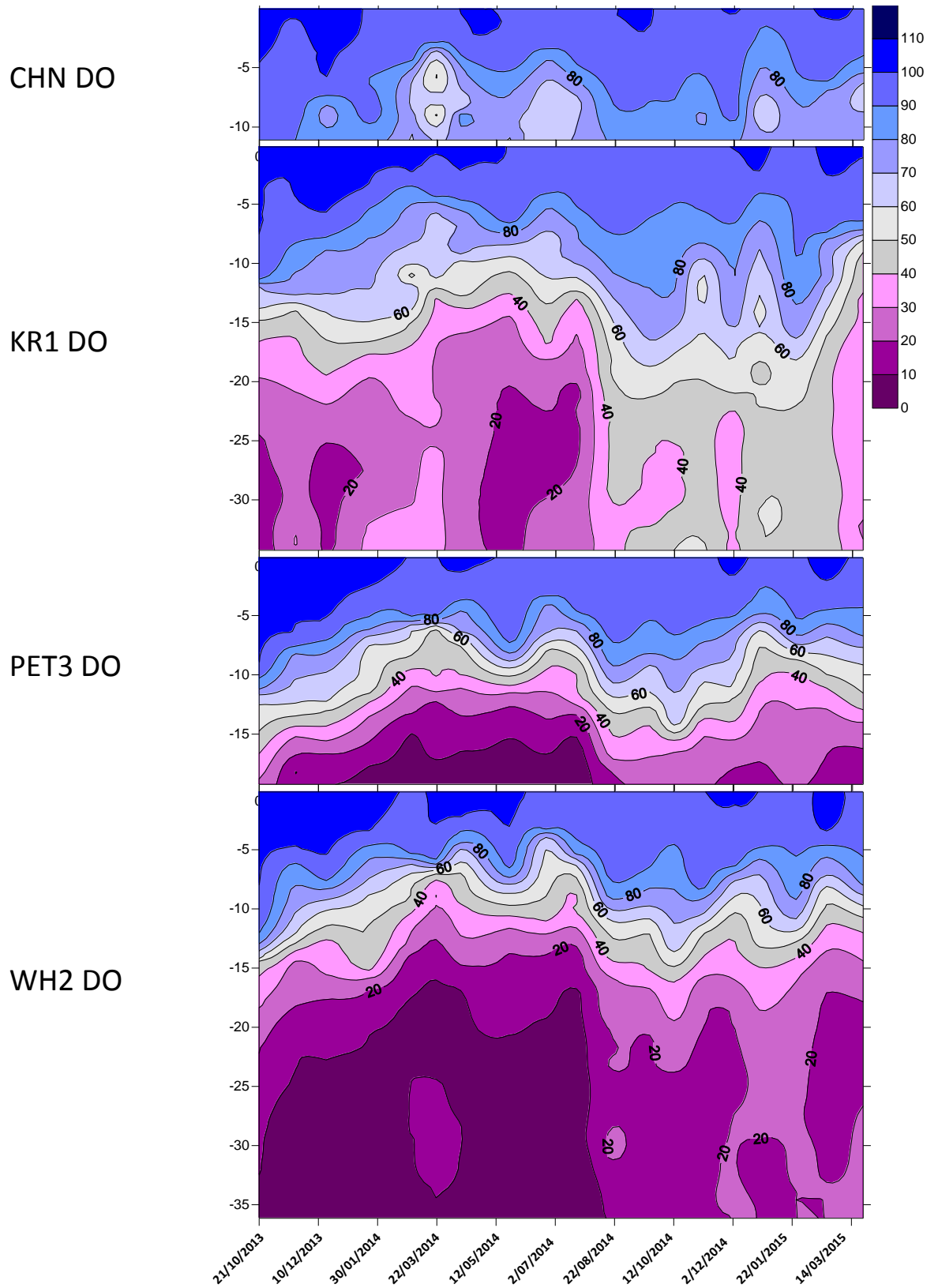


Figure 7: Vertical dissolved oxygen concentration (% saturation) at depth profiles at the four water quality monitoring sites in Macquarie Harbour during the study period.

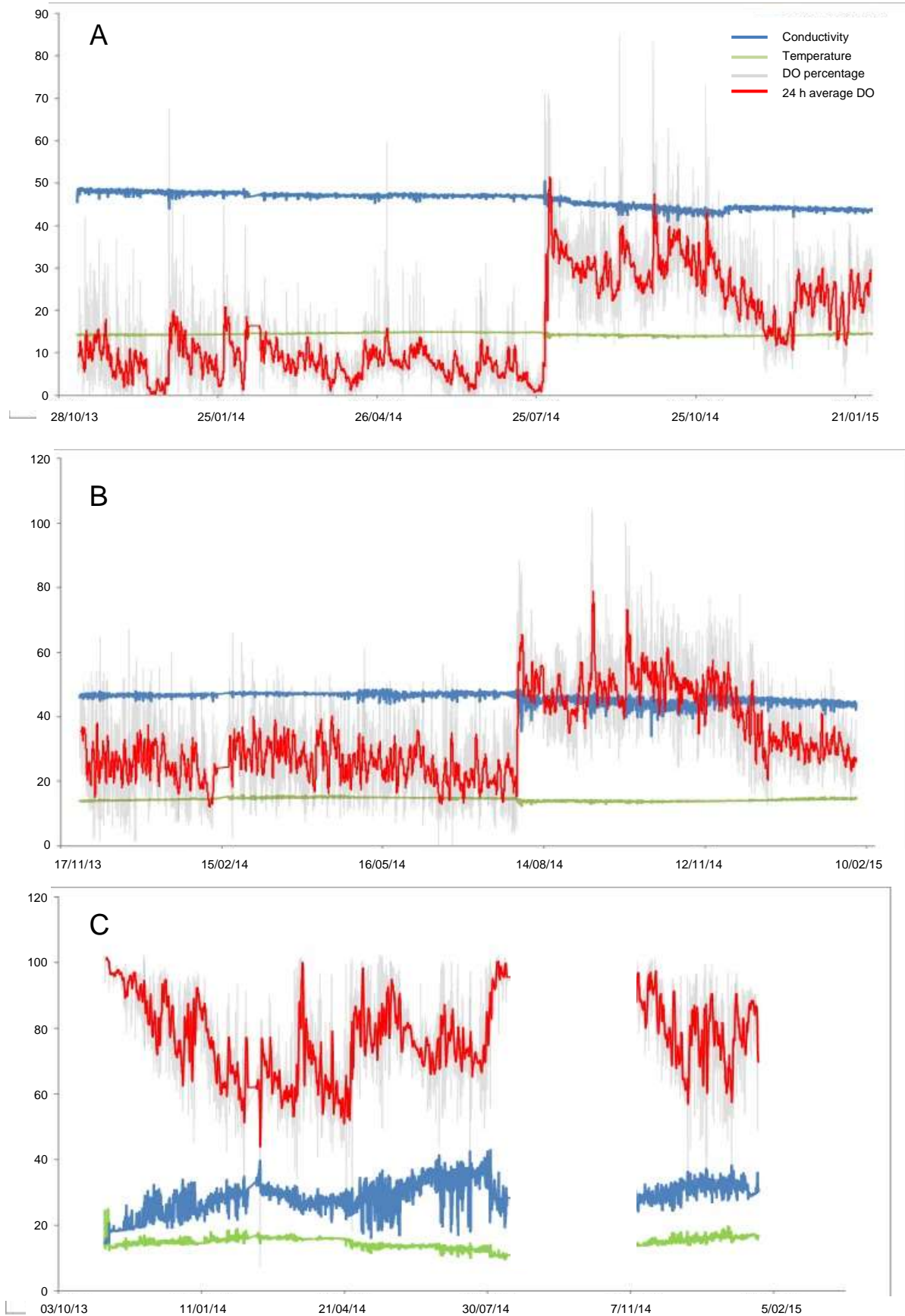


Figure 8: Variation in temperature and dissolved oxygen throughout the study period at a site at (A) 22 m depth, (B) 17 m and (C) 7 m depth off Liberty Point during the study period.
 Note: in Figure C the oxygen sensor cap was not functional between early August and early November.

Maugean Skate

General

In the present study, 270 Maugean Skate were captured for tagging and during seasonal biological sampling phase. In addition, data were available for 177 individuals that were captured in a previous gillnet study using the same fishing methods (Lyle *et al.*, 2014). Both sexes were captured in roughly equivalent quantities (49.5% female, 50.5% male).

The smallest individual captured was 327 mm TL while the largest male and female Maugean Skate were 739 and 870 mm TL, respectively. The size composition of the combined sample was unimodal and skewed to the right, with a clear mode in the 600–750 mm range (Figure 9). Individuals <500 mm were rare indicating a degree of selectivity by sampling equipment. The length distribution of females was significantly different (generally larger) than that of males (Kolmogorov-Smirnov test; $D=0.393$, $p<0.001$). There was no significant difference in the length distribution of skate captured in the present study and by Lyle *et al.*, (2014) ($D = 0.112$, $p= 0.175$); however, when the sexes were investigated individually (Figure 10), the length distribution of males was significantly different (generally smaller) in the earlier study ($D = 0.234$, $p=0.023$) but there was no difference in females.

Sufficient data existed to investigate regional differences in size compositions between the upper harbour (Kelly's Basin, Farm Cove and Rum Point), Table Head/Liberty Point, and Swan Basin/Long Bay regions (Figure 3). There was a significant regional difference in length distributions (Table 1) with individuals from the upper harbour tending to be smaller (median 588.5 mm) than those from the other two regions, which were not different and both had a median length of 675–676 mm.

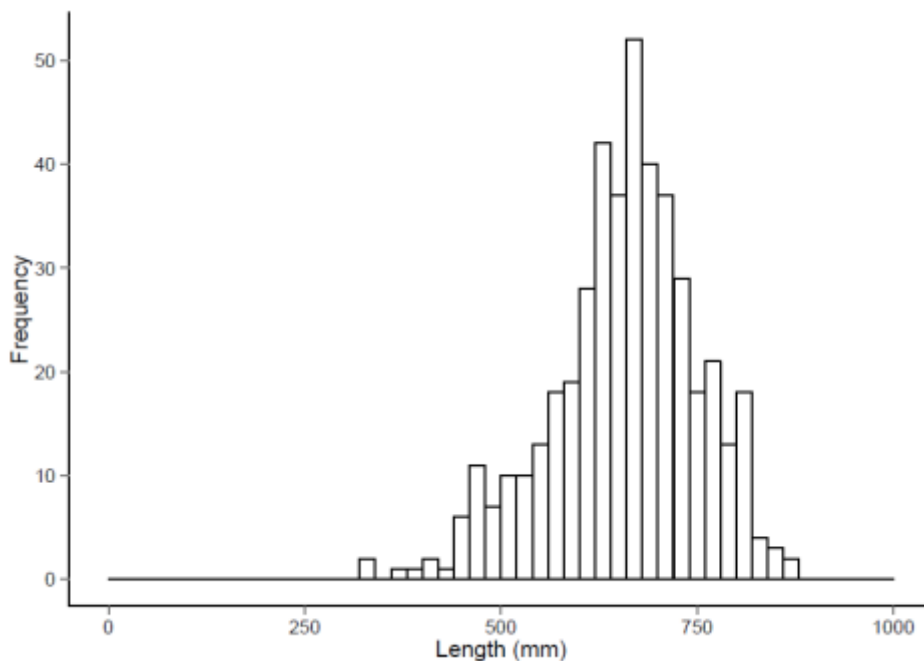


Figure 9: Length frequency distribution of Maugean Skate captured in both the present and previous studies (Lyle *et al.*, 2014).

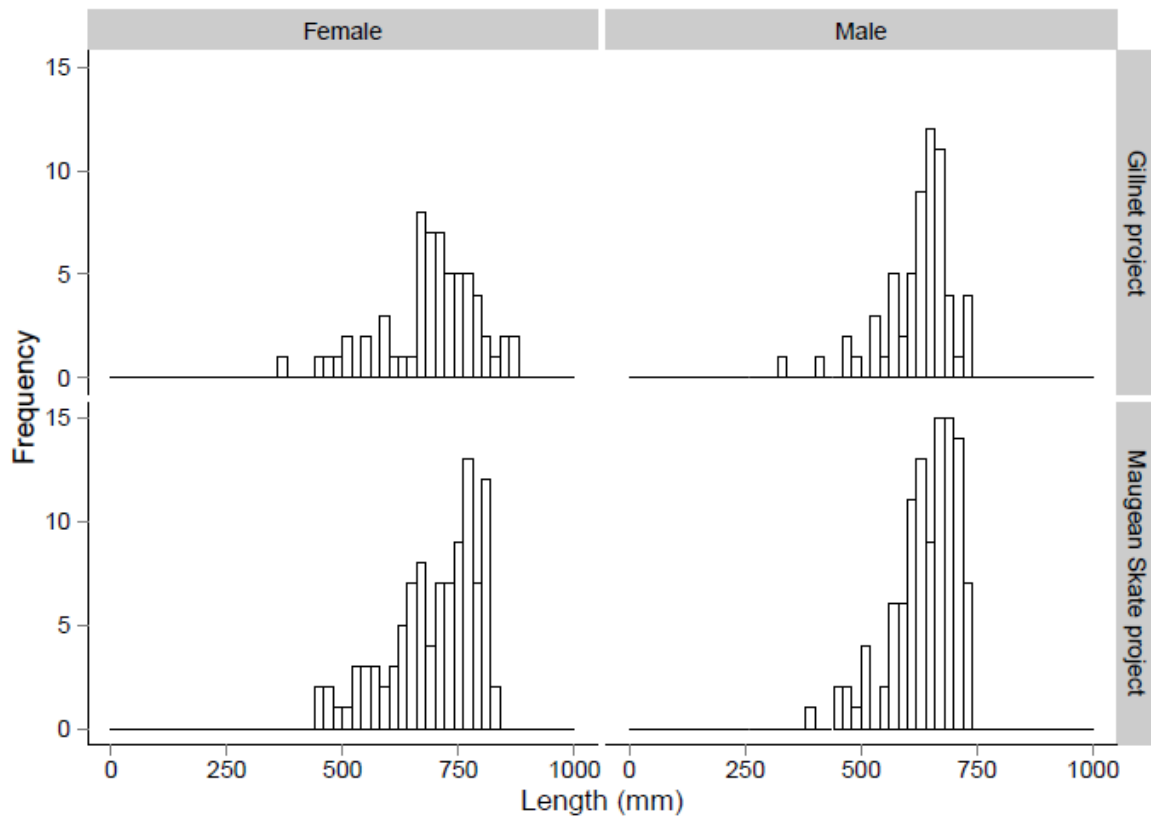


Figure 10: Comparison of the length frequency distribution of male and female Maugean Skate captured in the present study and in a general gillnet project by Lyle *et al.* (2014) undertaken between November 2011 and February 2013.

Table 1: Multiple pairwise Kolmogorov-Smirnov for regional difference in the length distribution of Maugean Skate.

	Upper harbour	Swan Basin/Long Bay
Swan Basin/Long Bay	<0.001***	–
Table Head/Liberty Point	<0.001***	0.710

Movement and behaviour

During the study there were 1,198,124 valid detections from the tagged Maugean Skate. *Supplementary information providing detailed information for individual skate (and salmonids) is available upon request.*

Following release, many skate initially displayed unusually erratic behaviour, presumably as a response to capture and tagging (extreme examples include MS06, MS22, MS24, MS41, MS54; individual presence absence plots are available as supplementary information). As a consequence the first two weeks of telemetry data have been excluded from the analyses, minimising any potential biases introduced by this behaviour. This also eliminated data for any skate that had died in this period. Mortalities that occurred after two weeks were considered unlikely to have been a direct result of tagging and data from these individuals has been reported up until the date of their death. In total, ten skate were judged to have died as a consequence of tagging, 24 died or ceased to be detected (moved outside of the detection range of the array) at some stage during the study period while 24 individuals were effectively detected throughout the study period (Table 2). Thirty four Maugean

Skate were detected relatively frequently for more than a month (Figure 11), twenty of which were detected on >50% of days for more than five months. Individuals tagged in the upper reaches of the harbour were detected relatively infrequently reflecting the paucity of receiver coverage in this region. Presence/absence plots, BBMMs and movement summaries for each individual are located in supplementary material. All plots should be interpreted by taking into account the quantity of data used in analyses, information that is summarised in Table 2.

Appendix 1 details the analysis of range testing data. In summary, these analyses identified that 50% detection probability was at 405 m, which was subsequently used for BBMMs. There was no significant difference in detection range either spatially or temporally so this single value was used for all analyses. Sentinel tags indicated that there was no major change in detection efficiency throughout the study, other than a brief decrease in detection efficiency during the storm that caused the recharge of DO. Range testing at the entrance to Macquarie Harbour indicated that it was unlikely that a tagged animal could leave the harbour without being detected.

All skate showed a relatively high degree of site fidelity (presence/absence in Figure 11, UD examples in Figure 12) with 50% UDs mostly <3 km² and the 95% UD of most skate <10 km² (Figure 13). When the UDs of all tagged skate are combined they, as a whole, indicate a clear affinity for the inshore areas of Liberty Point and Table Head (Figure 14). Furthermore, the combined total UD overlapped the total marine farming lease area by 9.27 km². However, when the 95% and 99% UDs are considered the area of overlap was reduced to just 1.14 and 1.20 km², respectively, suggesting that much of areas of overlap are relatively unimportant to skate. The fact that aquaculture cages also only take up a small proportion of a lease implies that any direct overlap between skate distribution and aquaculture farming is small and, for the skate, likely to be transient.

More than half of the individuals (55%) for which sufficient data were available (i.e. the twenty that were detected on >50% of days for >5 months) left the general area of their home range at some stage during the study period, sometimes for several weeks. Most returned to the area where they were initially tagged and the UD estimated by the BBMM suggests that the home range of almost all individuals is relatively small (Figure 13). Several fish did, however, relocate to new areas often after a period of moving throughout the harbour, presumably having found suitable alternative habitat. Examples include MS22 which moved from Swan Basin to Farm Cove (almost 30 km) soon after tagging and remained in that area for the duration of the study. Similarly, MS25 moved from Swan Basin to the Liberty Point/Table Head area (~10 km) and remained in that area. Notably, five individuals relocated to the lower reaches of the harbour and were regularly detected by the receiver in the channel that leads to the harbour entrance (MH01). These skate relocated from both Liberty Point/Table Head (MS02 and MS46) and Swan Basin (MS16, MS19 and MS20). As there were few receivers in the lower reaches detailed movement information was unavailable in this area, although the skate were detected regularly at MH01 and only occasionally at Cosy Corner (CC01) (~3 km away) and at the entrance to the harbour (MH02) (~6 km away).

Description of fine scale movement patterns of individual skate was possible for the Liberty Point/Table Head region due to the high density of receivers in this area. A variety of individual home ranges were identifiable in this area (individual UD presented in the supplementary information): MS01, MS03, MS10, MS12, MS45, MS50, MS52, MS54 and MS55 predominantly inhabited the Table Head region; MS09, MS48 and MS49 inhabited the bay between Table Head and Liberty Point; MS04, MS05 and MS07 spent most of the time on the eastern side of Liberty Point; MS14 and MS17 resided on the western side of Liberty Point; MS40, MS41 and MS57 resided to the north of Liberty Point; MS43 ranged throughout the entire Table Head and Liberty Point region; and MS51 was broad ranging and regularly moved between Table Head and Double Cove and occasionally as far as Richardson's Bay (RB01), covering much of the south-western shore. The behaviour of MS51 meant that the BBMM with a five hour lag period did not adequately describe its UD as movement between areas generally took longer than five hours and thus bridges between them did not form (Figure 12E). Increasing the lag period resulted in the UD being unrealistically overinflated. In order to address this issue, position averaging at five hour intervals was used prior to BBMM and bridges formed between

Liberty Point and Double Cove, and Double Cove and Richardson's Bay, producing a more realistic description of this skate's behaviour and UD (Figure 12F).

Although the frequency of detections were less continuous through time in the other regions it was nonetheless evident that many individual skate tended to remain within the general regions that they were tagged. For instance, skate tagged in Kelly's Basin regularly travelled north into Farm Cove and occasionally across the harbour to Rum Point but were not detected moving further down the harbour. Many of these fish were detected infrequently, for example MS29 and MS38 were only detected on 27–28% of days at liberty indicating that they spent a large proportion of the time in habitats outside of the detection range of the VR2s. By contrast, skate tagged in Swan Basin tended to display a higher degree of site fidelity than those at Kelly's Basin and were frequently detected throughout the study period.

There was no evidence for the movement of any Maugean Skate out of Macquarie Harbour via the entrance and none of the tagged skate were detected at the Gordon River mouth (GR01), indicating that they do not utilise the river system. Two individuals were detected by the receiver at Macquarie Heads (MH02): MS16 was detected there on six separate occasions and on one of these occasions was detected consistently over a three day period; MS21 was detected briefly at Macquarie Heads on two occasions. Both skate were detected by receivers inside the harbour after each visit, confirming that they had not left the harbour permanently.

Table 2: Summary statistics for each acoustically tagged Maugean Skate, Atlantic Salmon and Rainbow Trout.

MS refers to Maugean Skate, AS refers to Atlantic Salmon and RT refers to Rainbow Trout. Number of receivers refers to the number of receivers that detected each individual fish, days at liberty refers to the number of days between when the fish was tagged and its last detection and percentage of days detected refers to how many days the animal was detected between the day it was tagged and the day of its last detection.

Fish	Length	Sex	Date tagged	Date of last detection	Number of receivers	Number of detections	Last receiver	Days at liberty	Percentage of days detected	Comment
MS01	730	F	30/10/2013	4/11/2014	29	32125	LP04	370	83.51	
MS02	705	F	30/10/2013	3/04/2014	8	4261	MH01	155	56.13	
MS03	725	F	30/10/2013	4/11/2014	35	50246	LP01	370	81.35	
MS04	690	M	30/10/2013	4/11/2014	12	119866	LP13	371	99.73	
MS05	775	F	30/10/2013	4/11/2014	22	60478	LP18	370	93.78	
MS06	720	F	30/10/2013	17/11/2013	13	1246	LP18	18	83.33	Died 17/11/2013
MS07	685	M	30/10/2013	4/11/2014	12	114820	LP11	371	99.19	
MS08	720	F	30/10/2013	18/11/2013	13	884	PC01	20	70.00	
MS09	555	M	30/10/2013	18/09/2014	7	26798	CC01	324	44.44	
MS10	645	F	30/10/2013	4/11/2014	9	7270	LP04	370	48.65	
MS11	658	M	30/10/2013	17/11/2013	5	1425	CC01	18	88.89	Died 17/11/2013
MS12	510	F	30/10/2013	3/11/2014	5	49492	BB01	370	84.86	
MS13	710	F	30/10/2013	12/11/2013	6	837	BB01	13	100.00	Died 12/11/2013
MS14	625	F	30/10/2013	26/04/2014	10	60143	LP07	178	100.00	Died 26/04/2014
MS15	530	F	30/10/2013	6/11/2013	7	491	DC04	7	42.86	Died 06/11/2013
MS16	665	F	31/10/2013	3/11/2014	7	8394	MH01	369	37.40	
MS17	605	F	31/10/2013	5/11/2014	10	93571	LP07	371	88.68	
MS18	675	M	31/10/2013	6/07/2014	5	2630	MH01	248	8.06	
MS19	705	F	31/10/2013	9/03/2014	4	2059	MH01	129	30.23	
MS20	620	M	31/10/2013	1/11/2014	11	5167	MH01	366	18.85	
MS21	680	F	31/10/2013	19/01/2015	9	10126	MH01	446	25.78	
MS22	675	F	31/10/2013	5/11/2014	12	85419	FC02	371	98.92	
MS23	585	M	31/10/2013	9/11/2013	8	1016	LP06	10	60.00	
MS24	650	F	31/10/2013	15/11/2013	19	1177	KB03	15	73.33	Died 15/11/2013
MS25	780	F	31/10/2013	21/09/2014	20	2449	LP08	326	26.69	
MS26	760	F	31/10/2013	5/11/2014	4	93105	SB03	371	96.23	
MS28	620	M	5/11/2013							Died immediately
MS29	490	M	5/11/2013	9/11/2014	8	7718	FC01	369	28.18	

Fish	Length	Sex	Date tagged	Date of last detection	Number of receivers	Number of detections	Last receiver	Days at liberty	Percentage of days detected	Comment
MS30	808	F	5/11/2013	30/11/2013	7	3194	FC02	25	88.00	
MS31	640	M	5/11/2013							Died immediately
MS32	620	F	5/11/2013							Died immediately
MS33	450	F	5/11/2013							Died immediately
MS34	630	M	5/11/2013							Died immediately
MS35	565	F	5/11/2013	3/12/2013	6	916	RP02	28	53.57	
MS36	630	F	5/11/2013	23/11/2013	8	710	FC01	18	66.67	
MS37	610	M	5/11/2013							Died immediately
MS38	600	M	5/11/2013	8/08/2014	8	7050	KB04	276	27.17	
MS39	580	M	5/11/2013							Died immediately
MS40	753	F	6/11/2013	11/11/2014	10	120726	LP11	370	99.73	
MS41	677	M	6/11/2013	11/12/2013	12	9050	LP08	36	100.00	Died 12/12/2013
MS42	713	M	17/02/2014	7/04/2014	4	28110	BB01	49	93.88	Died 7/04/2014
MS43	638	M	17/02/2014	27/08/2014	14	27585	LP01	192	60.94	
MS44	619	M	17/02/2014	28/02/2014	4	6129	LP07	11	100.00	
MS45	809	F	17/02/2014	25/05/2014	4	31600	BB01	97	89.69	
MS46	692	M	17/02/2014	7/02/2015	11	9102	MH01	355	37.18	
MS47	814	F	17/02/2014	7/05/2014	26	2598	CC01	79	41.77	
MS48	746	F	17/02/2014	4/04/2014	20	3636	LP04	47	70.21	
MS49	705	M	17/02/2014	16/05/2014	12	7069	CC01	88	55.68	
MS50	667	M	17/02/2014	21/08/2014	5	14089	LP08	185	44.32	
MS51	702	F	17/02/2014	29/01/2015	17	9576	LP11	347	53.31	
MS52	695	M	17/02/2014	1/02/2015	9	15332	BB01	350	70.29	
MS53	615	M	17/02/2014	1/02/2015	16	9798	BB01	350	43.71	Died 15/03/2014
MS54	754	F	17/02/2014	26/05/2014	10	8438	BB01	99	71.72	
MS55	482	F	17/02/2014	1/02/2015	18	17654	BB01	349	74.21	
MS56	702	M	17/02/2014	21/02/2014	3	542	LP04	4	100.00	
MS57	608	M	17/02/2014	5/05/2014	9	18281	LP07	77	98.70	Died 5/05/2014
MS58	NA	F	18/02/2014	12/04/2014	8	1049	LP05	53	30.19	Died 15/04/2014
MS59	540	F	18/02/2014	12/04/2014	9	2677	SB01	53	39.62	Died 12/04/2014

Fish	Length	Sex	Date tagged	Date of last detection	Number of receivers	Number of detections	Last receiver	Days at liberty	Percentage of days detected	Comment
AS01	595		31/10/2013	31/10/2013	1	59	DC03	1	100.00	Died on 31/10/2013
AS02	585		31/10/2013	3/11/2013	10	190	DC02	3	100.00	
AS03	685		31/10/2013	20/11/2013	41	8245	LB02	20	100.00	Caught by fisher on 20/11/2013
AS04	680		31/10/2013	26/11/2013	42	6249	MH02	26	100.00	Left the harbour
AS05	670		31/10/2013	31/12/2013	49	14620	MH02	61	90.32	Left the harbour
AS06	580		31/10/2013		0	0				
AS07	630		31/10/2013	10/11/2013	27	1714	BB01	10	100.00	Caught by fisher on 10/11/2013
AS08	620		31/10/2013		0	0				
AS09	625		31/10/2013	3/11/2013	15	946	LP09	3	100.00	
AS11	630		31/10/2013	7/11/2013	20	2153	MH01	8	100.00	
AS12	550		31/10/2013		0	0				
AS13	670		31/10/2013	20/11/2013	46	4049	LP06	21	100.00	Caught by fisher on 21/11/2013
AS14	610		31/10/2013	6/11/2013	12	1374	BB01	6	100.00	Caught by fisher on 6/11/2013
AS15	660		31/10/2013	15/11/2013	30	3414	MH02	15	100.00	Left the harbour
AS16	670		31/10/2013	15/12/2013	49	9561	DC05	46	93.48	
AS17	620		31/10/2013	2/11/2013	11	200	SB02	3	100.00	
AS18	675		31/10/2013	6/12/2013	49	5518	MH02	37	100.00	Left the harbour
AS19	640		31/10/2013	7/11/2013	18	2445	LP05	7	85.71	
AS20	690		31/10/2013	3/11/2013	21	1160	SB02	3	100.00	
AS21	670		31/10/2013	7/02/2014	11	9557	SP03	99	65.98	
AS22	660		31/10/2013	11/11/2013	36	2551	MH02	12	100.00	Left the harbour
AS23	640		31/10/2013	19/11/2013	27	3840	SP03	20	95.00	Caught by fisher on 20/11/2013
AS24	640		31/10/2013		0	0				
AS25	680		31/10/2013	30/11/2013	37	6227	MH01	30	100.00	
AS26	690		31/10/2013	5/12/2013	51	10876	MH02	35	100.00	Left the harbour
AS27	695		31/10/2013	2/11/2013	10	495	LP16	2	100.00	Died on the 2/11/2013
AS28	630		31/10/2013	1/12/2013	45	5925	SB01	31	87.10	Caught by fisher on 1/12/2013
AS29	660		31/10/2013	30/11/2013	38	5567	MH01	30	100.00	
AS30	680		31/10/2013	8/11/2013	32	2080	MH01	8	100.00	
AS31	695		31/10/2013	4/11/2013	12	906	LP01	4	100.00	

Fish	Length	Sex	Date tagged	Date of last detection	Number of receivers	Number of detections	Last receiver	Days at liberty	Percentage of days detected	Comment
RT01	540		1/11/2013	31/12/2013	39	8201	LP11	60	68.33	Died on the 31/12/2013
RT02	450		1/11/2013	3/11/2013	5	204	LP14	3	100.00	Died on the 3/11/2013
RT03	480		1/11/2013	10/03/2014	46	20720	LP01	130	74.05	
RT04	500		1/11/2013	17/12/2013	47	7226	MH01	47	95.83	
RT05	450		1/11/2013	25/11/2013	17	553	SB02	24	76.00	Caught by fisher on 26/11/2013
RT06	540		1/11/2013	4/12/2013	31	9367	BB01	34	97.06	
RT07	440		1/11/2013	30/11/2013	29	8249	GR01	30	100.00	Caught by fisher on 24/04/2014* ¹
RT08	480		1/11/2013	11/08/2014	49	8748	GR01	284	23.86	Went up Gordon River
RT09	480		1/11/2013	30/11/2013	29	2631	RB02	30	83.87	
RT10	480		1/11/2013	9/11/2013	28	1304	GR01	8	88.89	Went up Gordon River
RT11	500		1/11/2013	11/11/2013	13	7871	LP07	10	100.00	Caught by fisher on 11/11/2013
RT12	490		1/11/2013	28/11/2013	30	2302	DC07	28	100.00	
RT13	420		1/11/2013	11/11/2013	16	2652	SB04	10	100.00	
RT14	450		1/11/2013	14/03/2014	45	22863	MH02	134	88.15	Left the harbour
RT15	500		1/11/2013	9/11/2013	19	3289	LP04	8	100.00	Caught by fisher on 9/11/2013
RT16	510		1/11/2013	1/12/2013	18	4375	DC08	31	93.55	
RT17	520		1/11/2013	10/12/2013	30	9996	LB01	39	87.50	Caught by fisher on 10/12/2013
RT18	480		1/11/2013	17/12/2013	46	11138	LP14	46	95.74	
RT19	430		1/11/2013	8/12/2013	27	7767	LP01	38	100.00	Caught by fisher on 7/04/2014*
RT20	450		1/11/2013	17/12/2013	41	6356	BB01	46	74.47	Caught by fisher on 17/12/2013
RT21	500		1/11/2013	9/11/2013	20	1962	LP04	8	100.00	
RT22	460		1/11/2013	2/02/2014	32	3411	DC08	94	33.68	
RT23	400		1/11/2013	4/11/2013	10	861	LP04	3	100.00	
RT24	510		1/11/2013	4/01/2014	35	10434	BB01	65	87.88	Caught by fisher on 9/01/2014
RT25	460		1/11/2013	9/02/2014	17	649	DC01	100	20.79	
RT26	460		1/11/2013	5/11/2013	13	1838	LP04	5	100.00	
RT27	480		1/11/2013	22/12/2013	44	6384	KB05	52	86.54	
RT28	430		1/11/2013	18/12/2013	37	5403	MH01	47	100.00	
RT29	510		1/11/2013	19/11/2013	24	4794	CC01	19	100.00	
RT30	500		1/11/2013	5/11/2013	13	759	BB01	4	100.00	

¹ * Recapture dates of these individuals do not align with telemetry data; however, both represented credible reports so have been included in the analyses.



Figure 11: Regional presence-absence plots for tagged Maugean Skate in Macquarie Harbour.

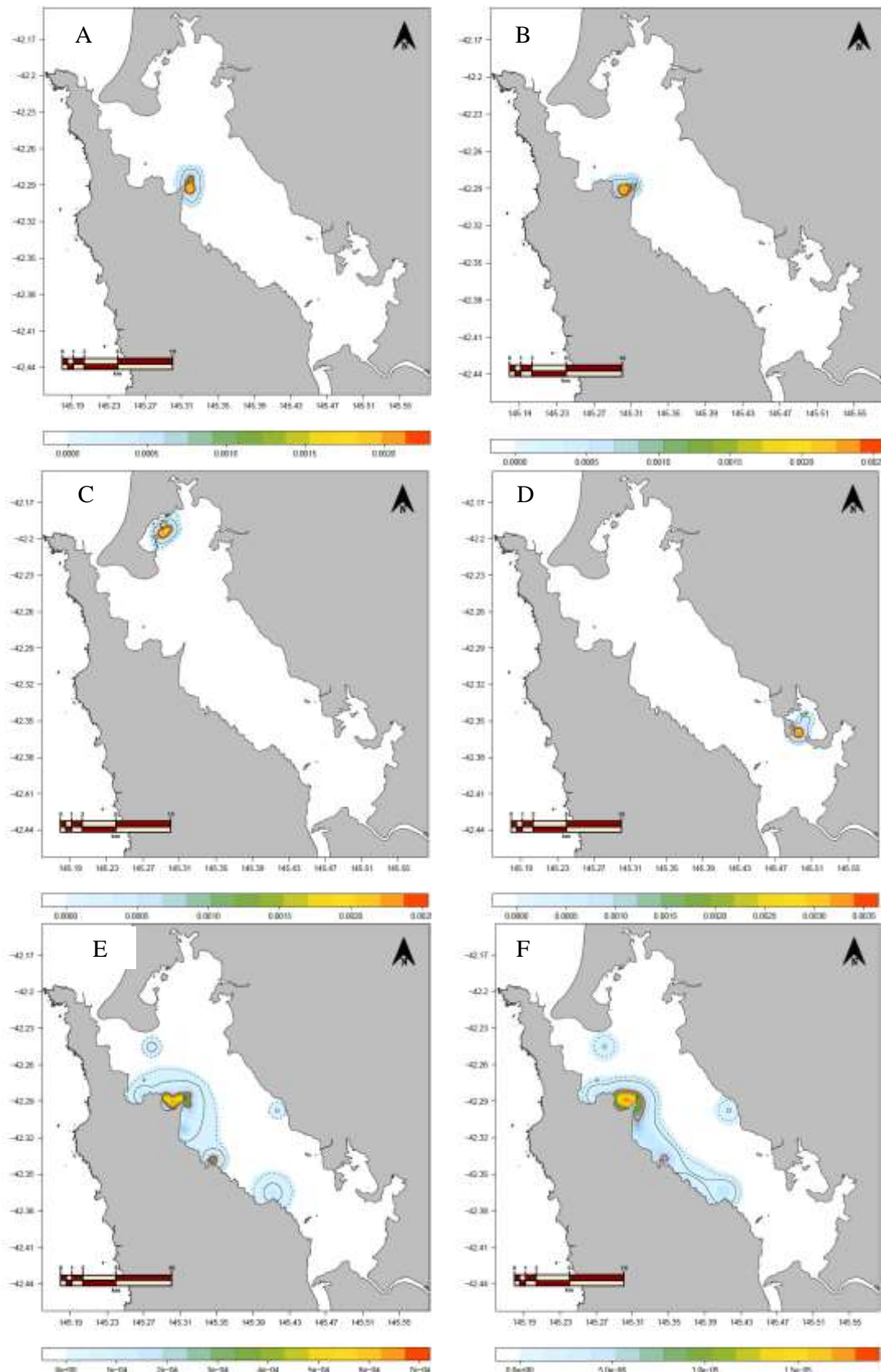


Figure 12: Utilisation distributions of selected Maugean Skate displaying differing home ranges.

MS04 (A) and MS17 (B) inhabited different areas in the Liberty Point/Table Head region, MS26 (C) solely inhabited the Swan Basin region and MS22 (D) was tagged at Swan Basin but moved to Farm Cove soon after where it remained for the duration of the study. MS51 (E, F) had the largest UD of any skate, regularly travelling along the south western shoreline. Initial BBMM (E) did not perform adequately so position averaging was used to more effectively model its UD (F). 50% UD (red line), 95% UD solid black line, 99% UD (dashed black line).

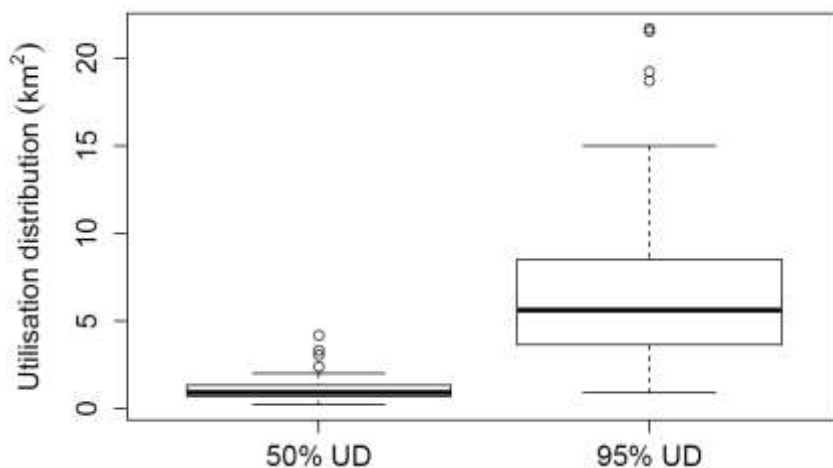


Figure 13: Utilisation distributions (50% and 95%) for all Maugean Skate.

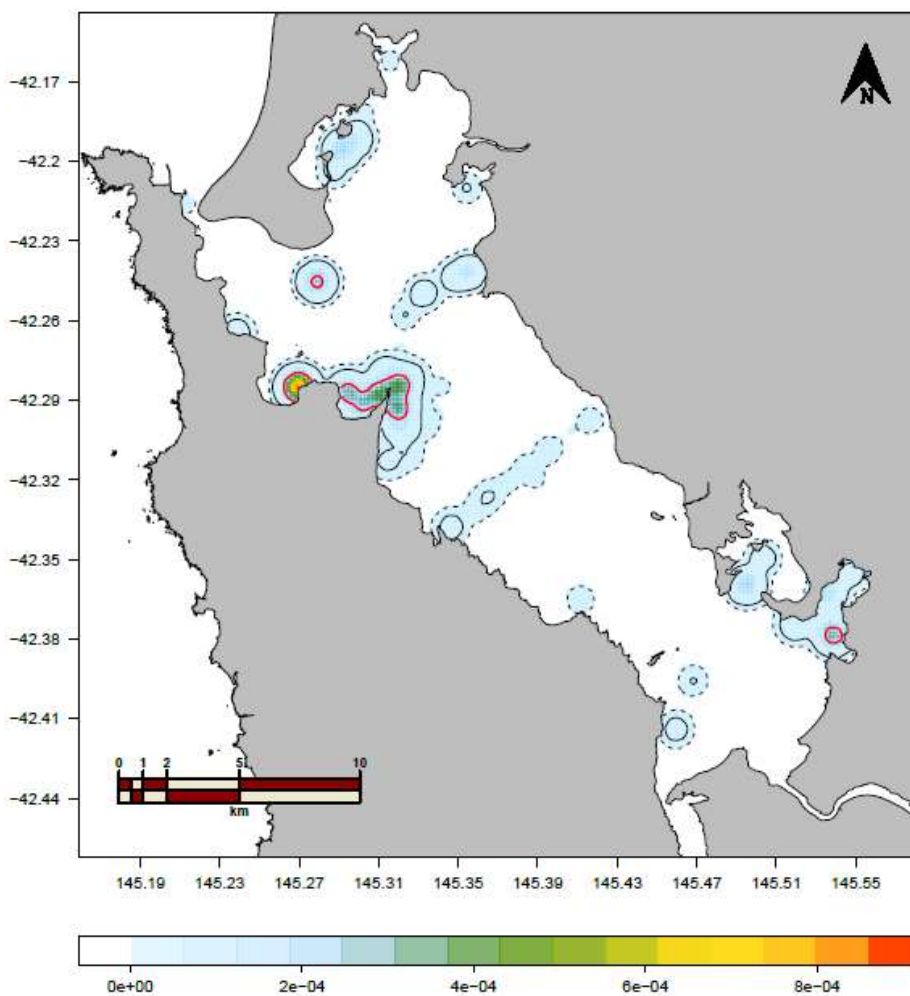


Figure 14: Utilisation distributions of all tagged Maugean Skate created from the combined probability distribution retrieved from the BBMM of each individual skate. 50% UD (red line), 95% UD solid black line, 99% UD (dashed black line).

Depth utilisation

Maugean Skate exhibited a strong preference for the shallower regions of Macquarie Harbour (Figure 15), with 95% of detections being at <15 m depth and 85% of detections occurring in the 6–12 m depth range. Around 55% (156 km²) of Macquarie Harbour is <15 m but only 24% (70 km²) is in their preferred depth range of 5–15 m. The acoustic array was estimated to cover 13.3% of the harbour's area in the 5–15 m depth range based on a detection range of 405 m. Skate were, however, detected from 0.6 m to >55 m, effectively encompassing almost the entire depth range of the harbour.

When skate were detected at depths >20 m they were generally moving between locations and only spent brief periods of time at these depths. One individual (MS20) was notable in that it was detected for almost a month (March/April 2014) by some of the deepest receivers in the array (LP01, SP02, SP03, DC03, DC04 and LP21). Many of the detections during this period were in >40 m and several days were spent in >50 m. In May, this individual took up residency in the lower reaches of the harbour in the vicinity of MH01 where its behaviour resembled that of other skate in the area.

It is possible that the significance of the shallower depths may have been underrepresented in the data. Around 31% of Macquarie Harbour is <5 m and represented by large shallow sand flats especially in the upper and lower reaches (Figure 2). These are areas that could not be covered by the acoustic array for logistic and safety reasons. As noted previously, a number of tagged skate that inhabited the upper and lower reaches of the harbour were frequently out of the range of the receivers and it is feasible that some of this time may have been spent in the shallow areas.

As a general rule, Maugean Skate exhibited a diel pattern that involved movement from deeper water during the day into shallower water at night (Welch t-test, $t = 95.39$, $df = 929160$, $p = <0.001$); Figure 16), although the overall mean difference in depth was only 0.59 m. Notably, there was greater variability in depths at night compared to during the day, inferring movement at night. Based on data pooled over the entire study period, it was evident that 36 skate (82% of individuals with depth tags) demonstrated this behaviour, with a significant difference in depths between day and night (Table 3; see Appendix 2 for individual depth usage boxplots). There were three individuals (7%) for which the depth differences were not significant and five (11%) for which the pattern was reversed, inhabiting significantly shallower water during the day. Due to the large number of data points and typically large range of depths occupied, where differences were significant the actual differences in mean/median day and night depth usage was typically small (Table 3).

The level of activity, based on an analysis of accelerometer data, varied throughout the day (Kruskal-Wallis $\chi^2 = 3391.7$, $df = 3$, $p = <0.001$). Skate were most active at night and least active during the day, with intermediate levels of activity during the dawn and dusk periods (Table 4; Figure 17), which is consistent with the inferences noted above and based on depth variability.

On the 29th July 2014 there was a large storm on the west coast of Tasmania. This resulted in a dramatic change to the water chemistry of the harbour and, in particular, a recharge of DO concentration in deeper waters (detailed in the 'water chemistry' section). For instance at 17 m (LP06) the dissolved oxygen concentration increased from 12.8% to 72.4% over a 10.5 hour period. In an attempt to assess the impact that this dramatic environmental event had on the skate, the behaviour of a group of five individuals that were detected more than 500 times in the week prior to and in the week following this event was examined. Each of the five skate moved into significantly deeper water for the week immediately after the recharge event (Table 5; Figure 20) with four of these subsequently returning to the depths and areas that they had occupied prior to the storm event (Figure 21). By contrast, the other individual remained in the deeper water (Figure 22), having relocated into a deeper water habitat to the north east of Liberty Point (note: this skate was generally detected by the same receivers as it had been prior to the storm). The immediate reaction of moving into deeper water may have been a response to the physical disturbance due to the storm, due to decreased salinity in their preferred depth range and/or increased DO in deeper water that effectively expanded the range of favourable habitat. Potentially supporting the latter possibility, each of the skate had been moving into progressively shallower water up until the recharge event (Figure 19), a trend that corresponded with,

and may have been correlated with, the gradual decline in bottom DO in their preferred depth range between November 2013 and the recharge event. However, despite DO remaining at elevated levels for several months after the storm (Figure 10) the usage of the deeper waters tended to be quite brief for most of the skate. This response matches the results of the physiology experiments (see physiology section), as the skate's main mechanism for coping with low DO appears to be a change from an aerobic to an anaerobic metabolic pathway. However this is not a long term strategy, as the lactate build up from glycolysis will eventually cause damage if not removed or metabolised via aerobic metabolism. While this strategy would allow them to move through areas of low DO over relatively short time periods, it would not allow them to be site attached in low DO water for long periods and as such it would make sense that they would shift to shallower, higher DO water.

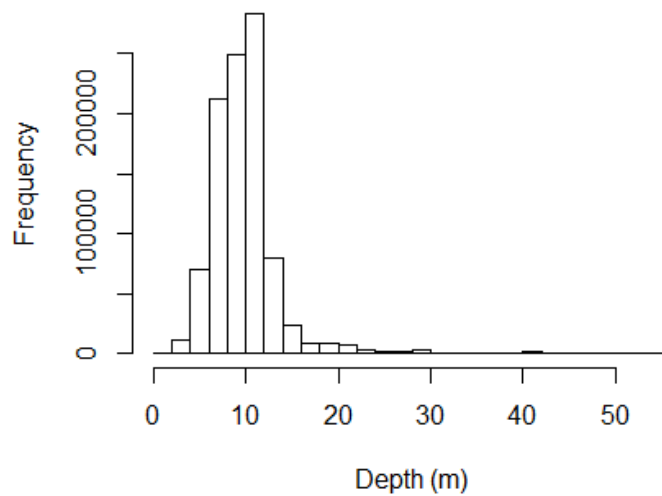


Figure 15: Frequency of detections by depth for all tagged Maugean Skate combined.

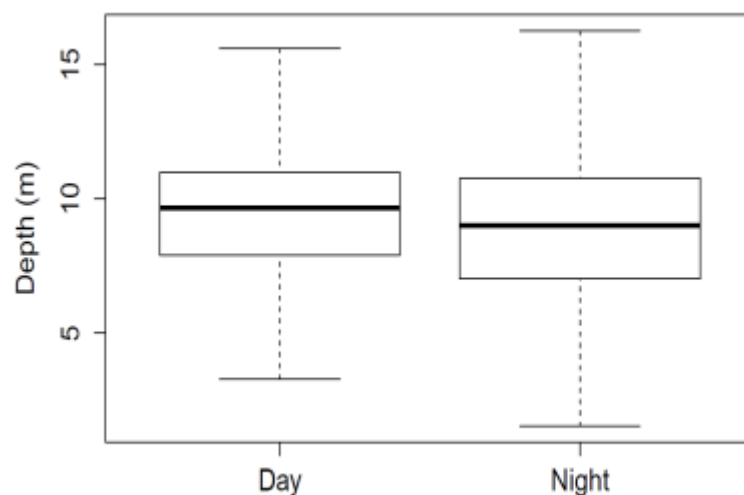


Figure 16: Diel depth utilisation (detections) for all Maugean Skate combined.

Table 3: Mean/median day, and night, depth distribution with standard deviation in parentheses.

When homoscedasticity could not be achieved, the median is provided as this is used in the Mann-Whitney test and is most likely to be representative. The test used to assess dial difference (M-W = Mann-Whitney test, TT = T-test, and TT(log) = T-test on log-transformed depth data) is indicated along with the alpha value.

Skate	Mean/median daily depth	Mean/median nightly depth	Test	<i>p</i>
MS01	9.45 (2.67)	9.23 (3.04)	M-W	<0.001***
MS02	7.25 (3.29)	8.35 (2.93)	M-W	<0.001***
MS03	8.57 (2.74)	8.57 (3.03)	M-W	0.004**
MS04	9.48 (1.97)	8.76 (1.91)	TT	<0.001***
MS05	13.87 (4.06)	13.40 (3.84)	TT(log)	<0.001***
MS07	10.33 (1.64)	8.79 (2.31)	M-W	<0.001***
MS08	11.86 (2.01)	11.42 (1.18)	M-W	<0.001***
MS09	8.35 (1.81)	8.13 (1.84)	M-W	<0.001***
MS10	9.06 (3.59)	8.85 (3.30)	TT	<0.010*
MS12	7.47 (1.74)	6.37 (2.07)	M-W	<0.001***
MS16	9.29 (3.47)	5.89 (3.06)	TT	<0.001***
MS17	9.45 (1.63)	7.91 (1.67)	M-W	<0.001***
MS18	13.85 (4.68)	12.53 (2.26)	M-W	<0.001***
MS19	9.88 (3.73)	8.98 (3.44)	TT	<0.001***
MS20	9.67 (17.33)	9.01 (14.23)	M-W	<0.001***
MS21	10.99 (4.03)	9.01 (4.98)	M-W	<0.001***
MS22	7.47 (1.55)	6.81 (2.09)	M-W	<0.001***
MS23	13.40 (2.02)	13.63 (0.80)	M-W	0.280
MS25	11.19 (3.43)	9.65 (4.53)	M-W	<0.001***
MS26	10.55 (2.23)	10.33 (2.92)	M-W	<0.001***
MS29	8.79 (1.99)	7.38 (2.02)	TT	<0.001***
MS30	10.23 (1.32)	9.98 (1.27)	TT	<0.001***
MS35	11.83 (1.78)	10.25 (1.45)	TT	<0.001***
MS36	12.02 (1.83)	11.79 (1.31)	TT	0.054
MS38	8.13 (1.69)	7.58 (2.04)	M-W	<0.001***
MS40	10.77 (1.05)	10.98 (1.09)	M-W	<0.001***
MS47	10.43 (2.41)	10.01 (2.60)	TT	0.014*
MS48	10.99 (7.03)	10.77 (4.61)	M-W	<0.001***
MS49	9.89 (1.73)	8.13 (2.25)	M-W	<0.001***
MS51	9.23 (1.79)	9.45 (2.00)	M-W	0.006**
MS52	8.00 (1.35)	8.24 (1.30)	TT	<0.001***
MS53	8.57 (2.57)	8.13 (2.04)	M-W	<0.001***
MS54	7.65 (1.12)	7.62 (1.15)	TT	0.547
MS55	8.57 (2.46)	7.91 (2.55)	M-W	<0.001***
MS56	7.47 (2.07)	9.45 (1.34)	M-W	<0.001***
MS57	20.44 (11.48)	12.31 (11.92)	M-W	<0.001***

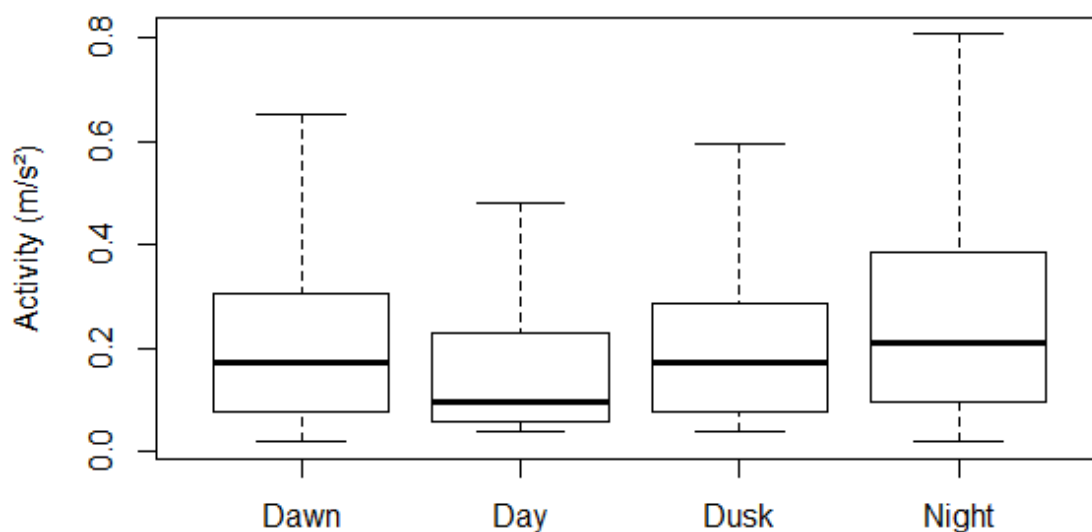


Figure 17: Diel variation in activity for Maugean Skate.

Table 4: The significance values (p) of multiple pairwise Mann-Whitney test for variation in the activity of Maugean Skate between dawn, day, dusk and night.

	Dawn	Day	Dusk
Day	<0.001***	-	-
Dusk	0.22	<0.001***	-
Night	<0.001***	<0.001***	<0.001***

Table 5: Welch two sample t-test (not assuming equal variance) of Maugean Skate depth usage in the Liberty Point region immediately prior to and after the recharge event in late July 2014.

	t value	df	p
MS04	-62.88	2474.70	<0.001***
MS05	-40.85	625.34	<0.001***
MS07	-8.66	872.80	<0.001***
MS17	-31.75	871.61	<0.001***
MS40	-39.53	2689.00	<0.001***

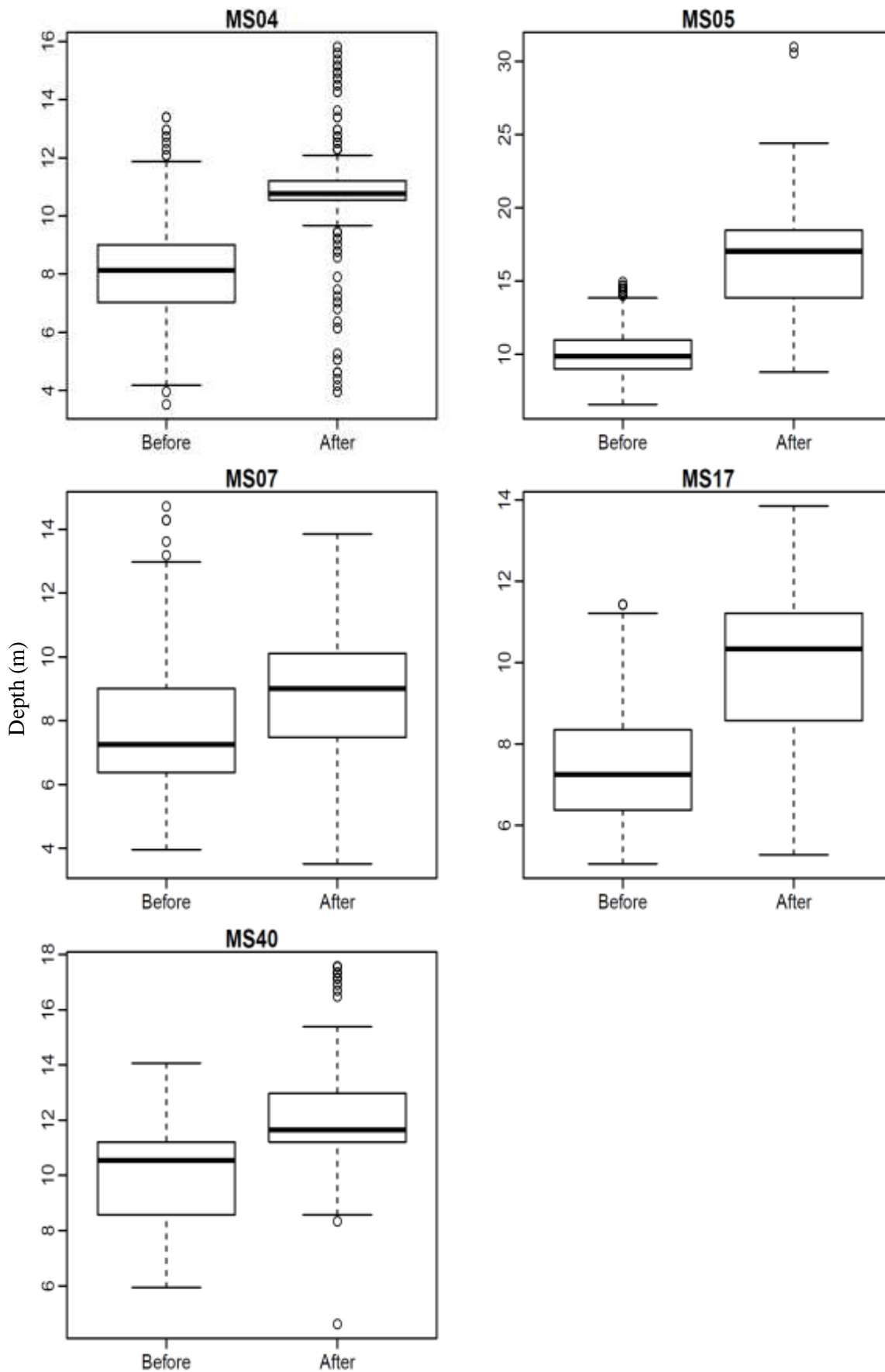


Figure 18: Depth utilisation (detections) of Maugean Skate with >500 detections in the week before and after the recharge event that occurred on the 29th July 2014.

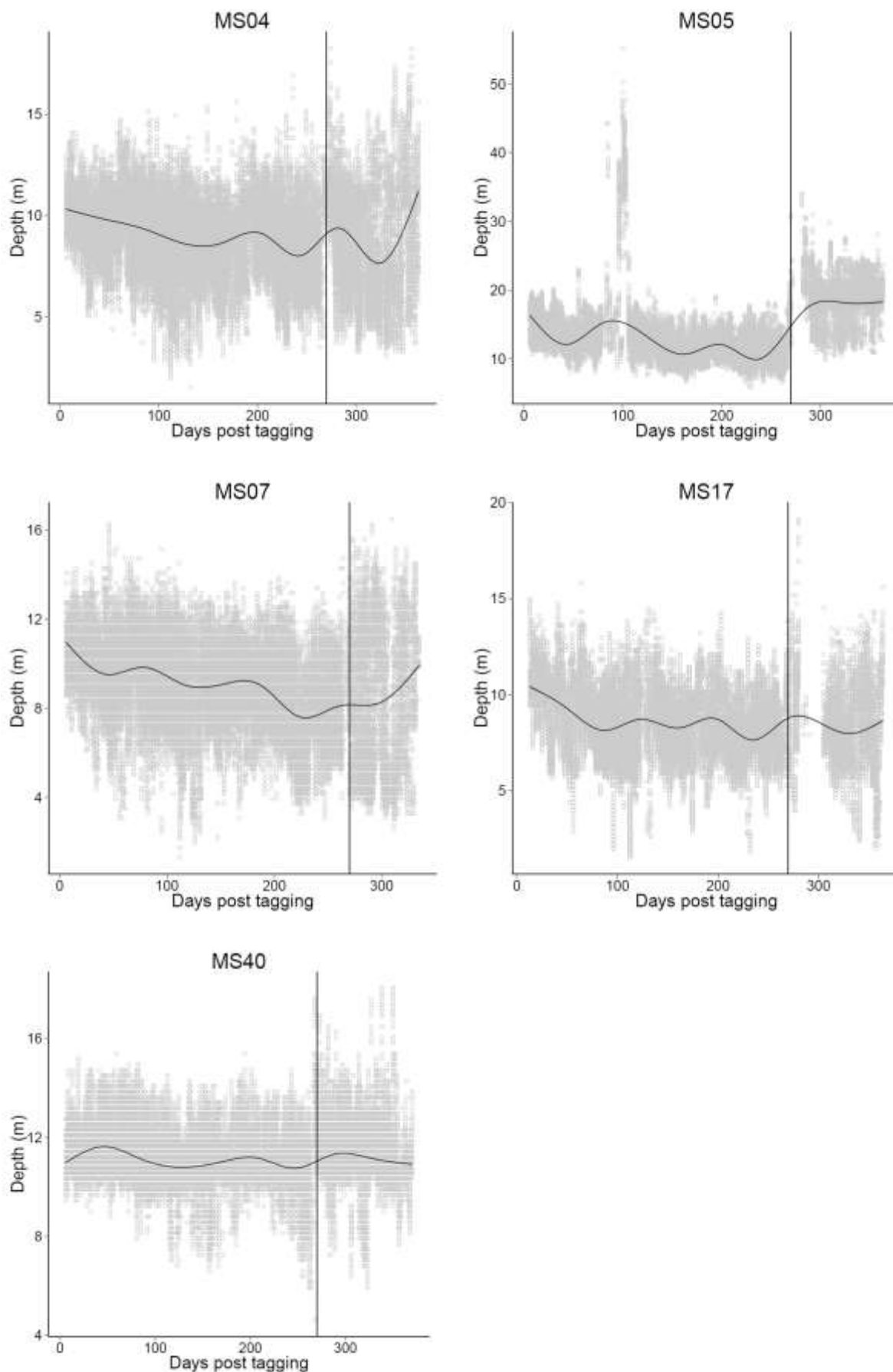


Figure 19: GAM of depth utilisation of selected Maugean Skate in the Liberty Point region. Recharge event on the 29th July 2014 occurred at day 270 and is indicated by the vertical line.

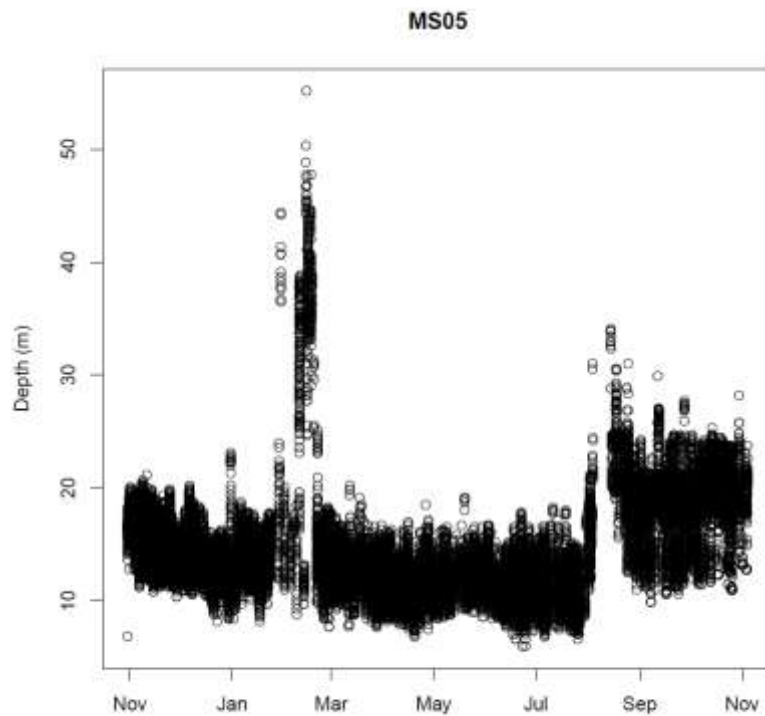


Figure 20: Depth utilisation of MS05 throughout the duration of the entire study highlighting the change in behaviour following the DO recharge that occurred in late July 2014.

Diet

Stomach contents were retrieved from 57 Maugean Skate with a further 24 (38%) being assessed as empty. Crustaceans were the most important dietary group, in terms of numbers, mass, frequency of occurrence (FO) and index of relative importance (IRI) (Table 6). Brachyurans (crabs) were the single most important crustacean, with *Paragrapsus gaimardii* the dominant species consumed. At least two other species of crabs were present in the stomach contents, albeit in low numbers. One of these was possibly *Helograpsus haswellianus* (based on coloration and this species being common in inshore habitats in Tasmania), whereas the other was only represented by leg parts and could not be identified. Most stomachs containing crabs had fewer than five individuals, with about half containing a single crab (Figure 21).

Carid shrimp were important in terms of numbers and FO, however their small size meant they did not contribute greatly in terms of mass or %IRI (Table 6). Most, if not all of the carid shrimp were *Palaemon* spp., probably *Palaemon dolospina* and *P. intermedius*, which are common in coastal Tasmania (Walker and Poore, 2003). Mysid shrimp were the most numerous of any prey group, despite having a low FO, but their small size meant they were relatively unimportant in terms of mass and IRI. Several skate appeared to have fed heavily on mysids, containing as many as 24 individuals (Figure 21) and few other prey items. Numerous mysid species are present in Tasmanian coastal waters (Fenton, 1985) and due to the crushed and digested state of most individuals it was not possible to confidently identify them to species.

Based on numbers and FO, teleosts represented a minor component of the diet but did represent a considerable contribution in terms of mass (37.3%), due mainly to a single large Jack Mackerel (*Trachurus declivis*). The remaining teleosts were smaller in size and fusiform in shape and generally in advanced stages of digestion. One fish was identified as an Australian Anchovy (*Engraulis australis*), the others may have also been anchovies or possibly 'whitebait' (predominantly *Galaxias* spp.), the latter being quite abundant in the harbour.

A single annelid worm, cephalopod (squid) and tunicate were also recorded in the stomach contents but none of these items contributed significantly to the diet (Table 6). Filamentous algae were present in the stomach contents of five individuals but were likely to have been ingested incidentally and have little or no nutritional value for the skate.

Table 6: Dietary composition as measured by the IRI, percent contributions by weight, number and frequency of occurrence for 57 Maugean Skate.

	IRI (%)	Weight (%)	Number (%)	Frequency of occurrence (%)
Crustacea				
Brachyura	58.5	47.4	31.4	66.7
<i>Paragrapsus gaimardii</i>		46.1	29.5	63.2
Unidentified sp. 1 (small)		0.6	1.4	5.3
Unidentified sp. 2 (large)		0.8	0.7	3.5
Caridea				
Palaemon spp.	25.6	12.1	27.6	57.9
Peracarida				
Mysida (unidentified)	10.2	1.5	35.8	24.6
Teleost	5.4	37.3	2.4	12.3
<i>Trachurus declivis</i>		32.0	0.3	1.8
<i>Engraulis australis</i>		0.7	0.3	1.8
Unidentified		4.6	1.7	8.8
Cephalopoda	0.0	0.1	0.3	1.8
Tunicata	0.0	0.6	0.3	1.8
Annelida	0.0	0.0	0.3	1.8
Chlorophyta	0.3	1.0	1.7	8.8

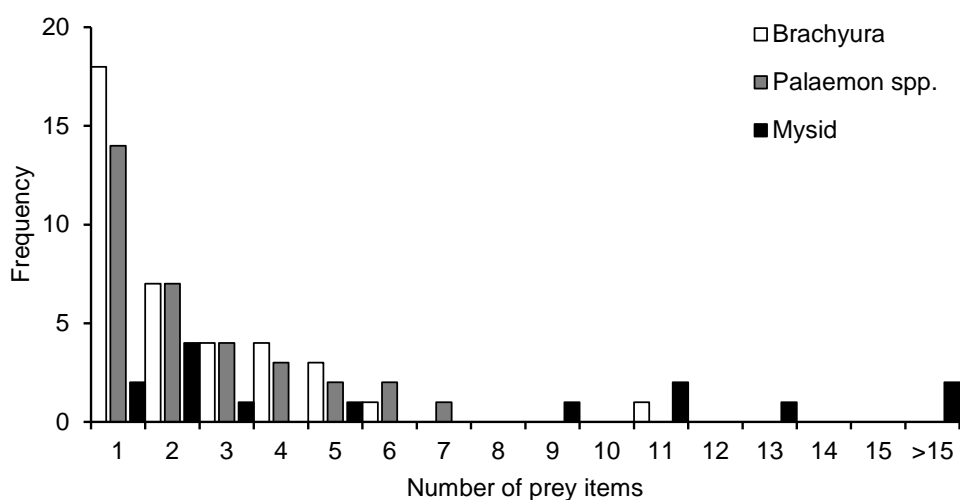


Figure 21: Number of dietary items by major prey group present in Maugean Skate stomachs.

Reproduction

Ultrasound validation

Ultrasonography proved relatively effective in identifying skate ovaries (Figure 22) and in predicting the MFD for most of the skate investigated in the validation study. There was a strong significant relationship between ultrasound MFD and MFD measured with callipers following dissection ($y = 1.01x + 0.78$, $R^2 = 0.86$, $p = <0.001$). In all but three instances the ultrasound estimate was accurate to within 2 mm of the calliper measured MFD (Figure 23). Maximum follicular diameter was underestimated on two occasions for individuals with large follicles (>20 mm) and overestimated for one skate that had no visible follicles. In relation to the largest discrepancy (13 mm underestimate), it was noted that the wings of this individual had entered rigor mortis and curled dramatically making it difficult to flatten the dorsal region to achieve a clear image.

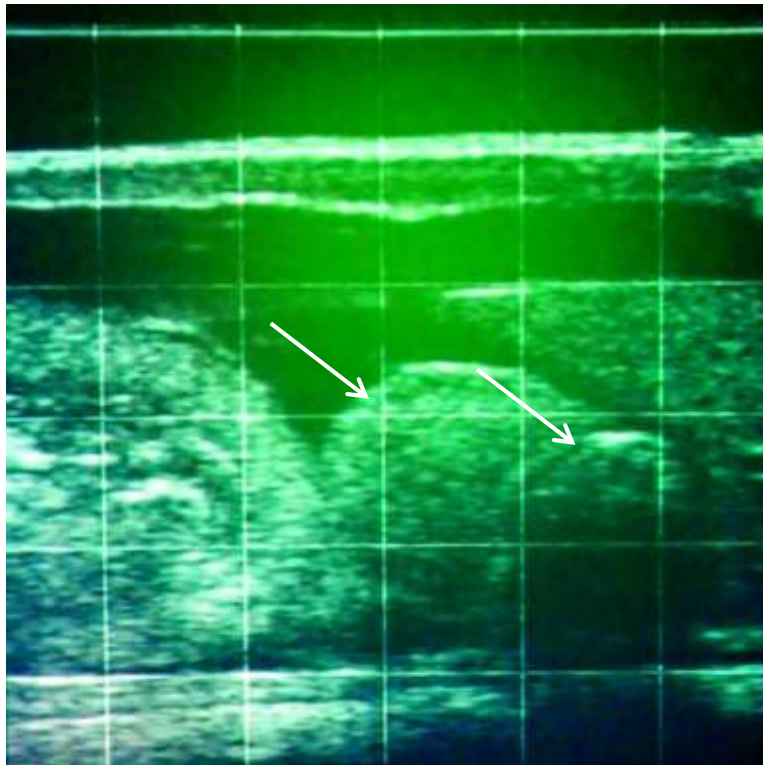


Figure 22: Ultrasound of a large, mature Maugean Skate ovary. Arrows indicate follicles. Grid squares are 10 mm.

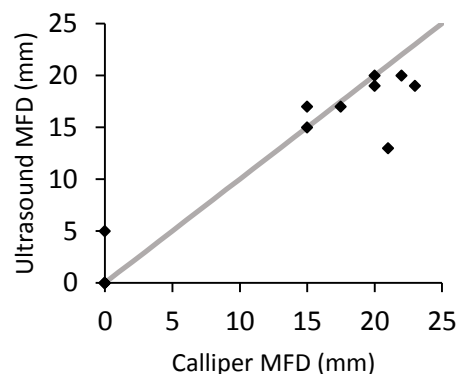


Figure 23: Bias plot of MFD as measured using ultrasound and post dissection with callipers.

Maturation

Size at maturity ogives based on clasper condition in males and MFD of females indicate that the sexes mature at significantly different sizes (Table 7), with males attaining 50% maturity at 632 mm TL and females at 662 mm TL (Figure 24). This represents 85.5 and 76.1% of the maximum recorded lengths for males and females, respectively.

Claspers increased rapidly in length as males reached lengths of between 600–630 mm, calcifying as they grew and resulting in a maturity ogive with very narrow confidence intervals and a steep slope. Endocrinology supported this result with elevated T and P₄ levels in males >620 mm TL (Figure 25). Testosterone is the primary reproductive hormone of males and did not exceed 123 ng.ml⁻¹ in males below 620 mm TL. Progesterone and E₂ did not exceed 1.4 and 5.6 ng.ml⁻¹, respectively in males below this size, although many larger males did have low T, E₂ and P₄ levels that were comparable to those for small individuals (Figure 25).

Endocrinology, while generally supporting the pattern of maturation against size determined using MFD suggested that maturation in females occurred at slightly larger size, with elevated T and E₂ concentrations in skate >680 mm TL (Figure 26). Below this size, T and E₂ levels were consistently less than 90 and 4.5 ng.ml⁻¹, respectively. There was no clear relationship between size and P₄ levels, although there were two obvious outliers. In oviparous species the main role of P₄ is to regulate and induce ovulation, with this hormone peaking in sexually mature females for a very short time just before ovulation (Callard *et al.*, 2005). It is possible that the individuals with exceptionally high P₄ levels may have been ovulating, suggesting that the smaller individual (650 mm TL) was sexually mature. For the seasonal analysis based on hormone levels this individual is treated as being mature along with females larger than 680 mm TL.

While it is possible that there was some error in assigning maturity based on MFDs determined using ultrasonography, dissection of three immature (450–616 mm) and six mature (672–819 mm) females support the size at maturity estimated herein. As a consequence a combination of MFD and TL should be used to accurately assess an individual's state of sexual maturity. This is because adult individuals in a resting phase do not possess enlarged follicles and appear as non-sexually reproductive (sexually immature). Similarly, a combination of hormone levels and TL should be used to distinguish between juvenile (sexually immature) and adult (sexually mature, either reproductively active or inactive) individuals. For instance, our data indicate that some large and putatively adult individuals (sexually mature but not active) had T, P₄ and E₂ levels that were comparable to much smaller (sexually immature) specimens. As such, the size at 50% maturity for females provided herein is less certain than that for males, although we are confident that the majority of females smaller than ~660 mm TL were immature whilst females larger than ~680 mm TL were mature.

Table 7: Logistic regression of the influence of size and sex on sexual maturation.

	Estimate	Std. error	Z value	<i>p</i>
Intercept	-41.24	6.40	-6.44	<0.001***
Length	0.06	0.01	6.52	<0.001***
Sex	2.01	0.66	3.031	0.002**

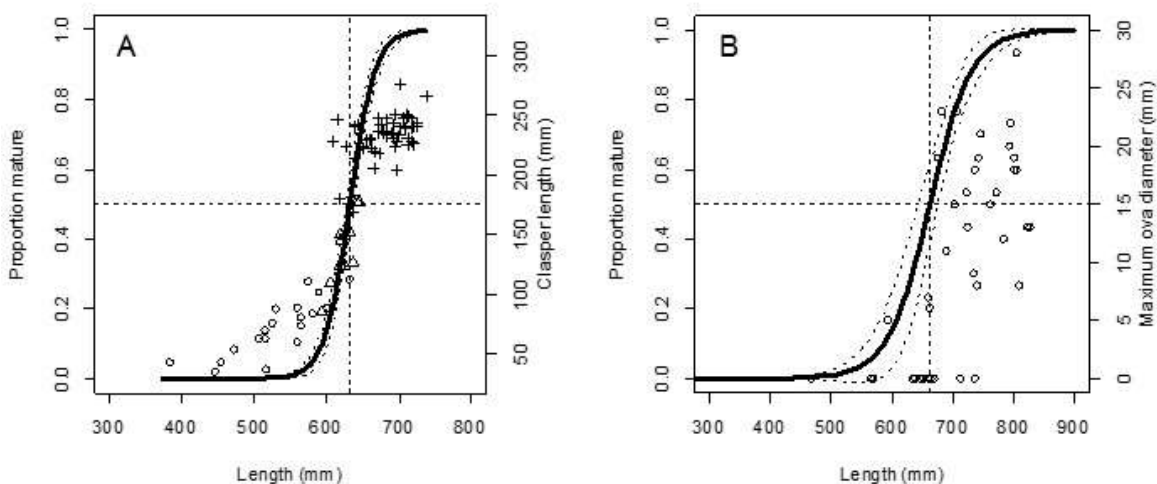


Figure 24: Maturity ogives of male (A) and female (B) Maugean Skate.

Clasper length and calcification (o – uncalcified, Δ – partially calcified, + – fully calcified) are provided on the male ogive (n=81) and MFD on the female ogive (n=49).

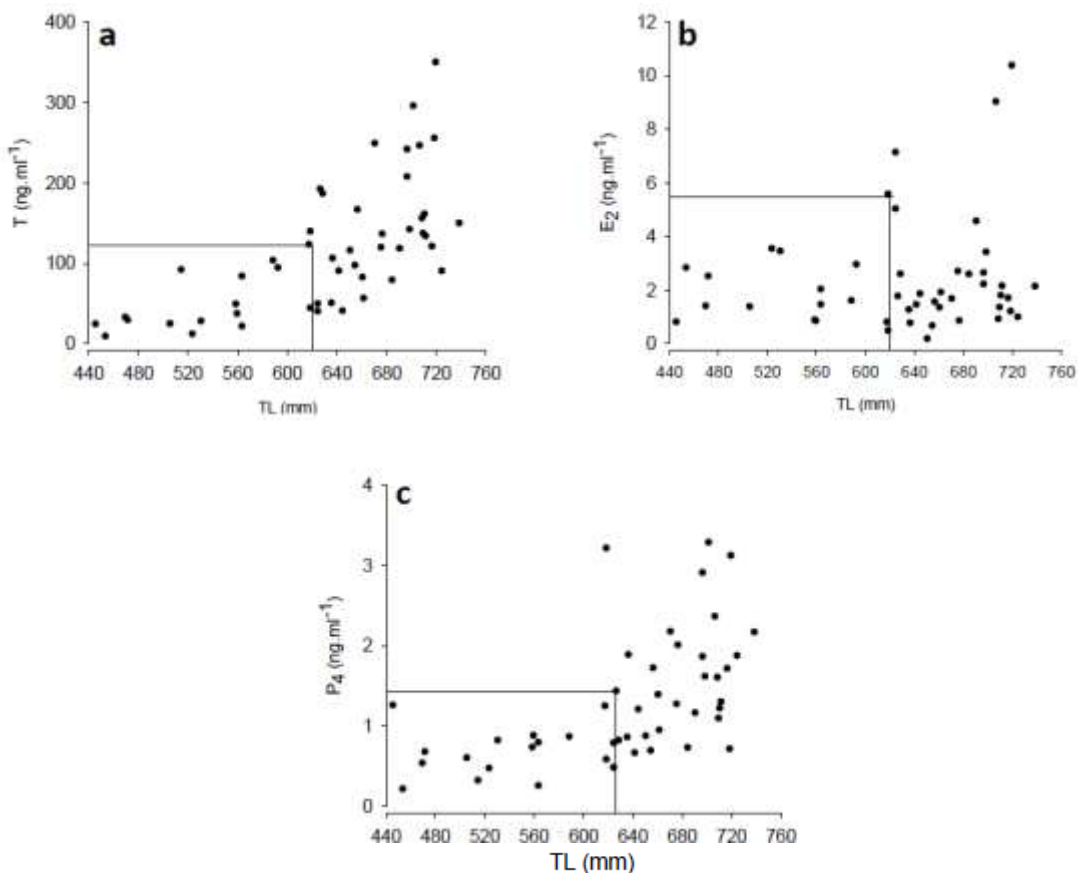


Figure 25: Relationship between hormone levels and total length of male Maugean skate (n=49). (a) Testosterone (T); (b) 17β-estradiol (E₂); (c) Progesterone (P₄).

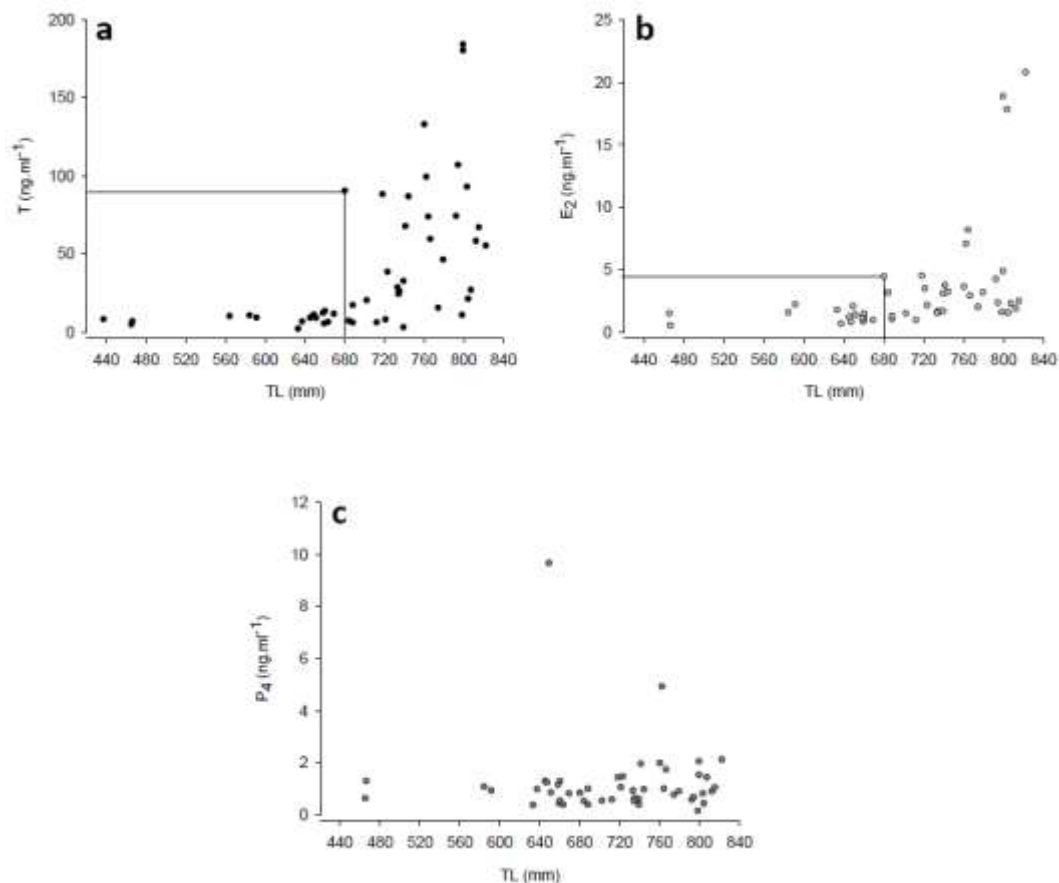


Figure 26: Relationship between hormone levels and total length of female Maugean skate ($n = 51$).
(a) Testosterone (T); (b) 17 β -estradiol (E₂); (c) Progesterone (P₄).

Reproductive seasonality

Seasonal variation in MFD provides some evidence that female Maugean Skate were reproductively active throughout the year but least active during summer (Figure 27). Females with enlarged follicles (>10 mm) were found during each season while individuals with relatively small follicles (5–10 mm) were recorded in spring and summer. Dissections of six mature females collected in spring (November 2014) revealed that only one individual was reproductively active, with eight enlarged and yolked follicles in each ovary (13–23 mm). The remaining five individuals had enlarged uteri and oviducal glands confirming that they were mature, however, they were clearly in a resting phase as their ovaries contained only small (<10 mm) un-yolked follicles (Figure 28).

Hormone levels in adult females also provided evidence for a range of developmental stages in each season (Figure 29). Although sample sizes were small, there was evidence that T levels tended to be lowest during summer, increasing and becoming more variable by winter and spring. This pattern suggests that follicles in at least some individuals were reaching final maturation stages prior to ovulation in the cooler months. The high levels of P₄ in two individuals, one sampled in autumn and the other in winter, suggest they were close to ovulating. The decline in E₂ levels after summer reflects the antagonistic effect between E₂ and P₄ and the finalisation of follicle maturation at this time. In combination, MFD, biological examination and hormone data support an asynchronous discontinuous reproductive cycle within the population, with reproductive activity occurring throughout the year. Our data do, however, suggest reduced reproductive activity during summer compared to other seasons of the year.

As the frequency of egg laying and duration of the active phase are unknown it is not possible to estimate fecundity. No skate were observed to be laying eggs when captured, nor were any *in utero* eggs observed by ultrasound.

Testosterone levels in adult males were lowest in winter and quite variable at other times of the year, suggesting that the males produce sperm in most seasons and the general increased levels between spring and autumn may reflect a protracted mating season (Figure 30).

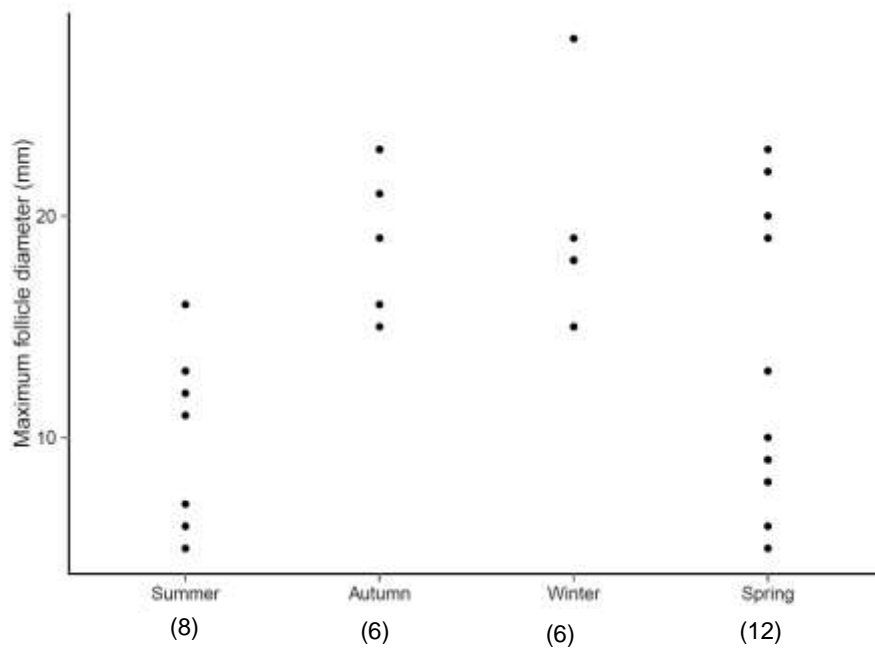


Figure 27: Seasonal MFD (determined by ultrasonography) for Maugean Skate. Only individuals with follicles able to be viewed by ultrasound are presented (i.e. not from dissection as these were only available for one season and introduce bias). Numbers in parentheses represent sample size

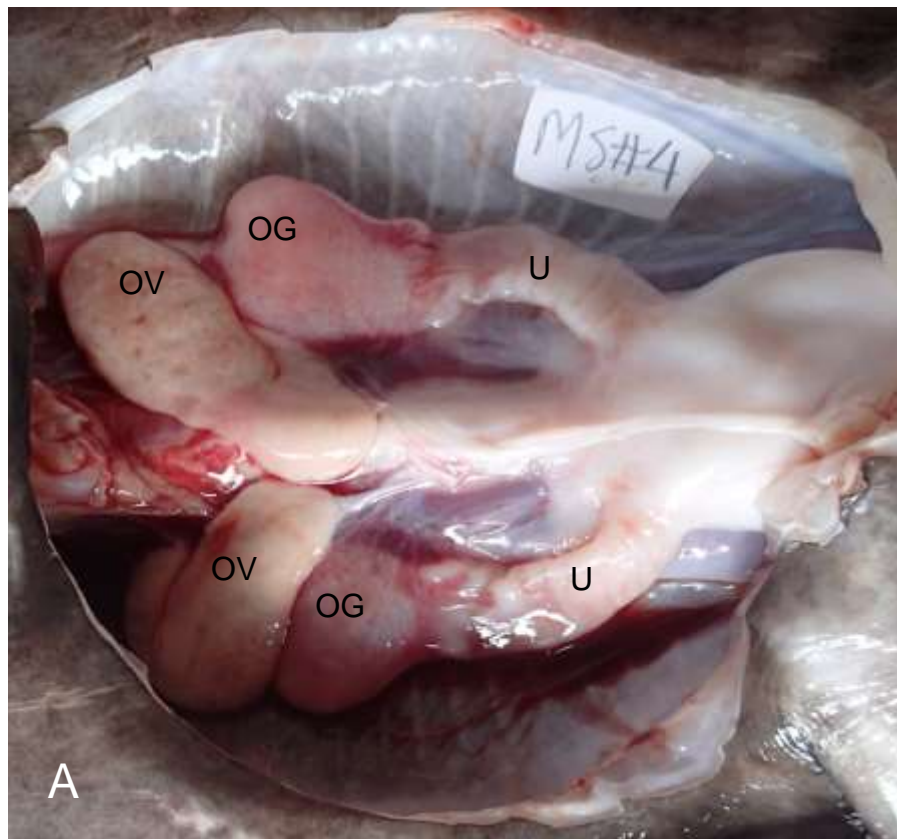


Figure 28: Reproductive system and dissected ovary of a 719 mm female Maugean Skate. U – uterus, OG – oviducal gland and OV – ovary in image A. The dissected ovary from the same skate is depicted in image B.

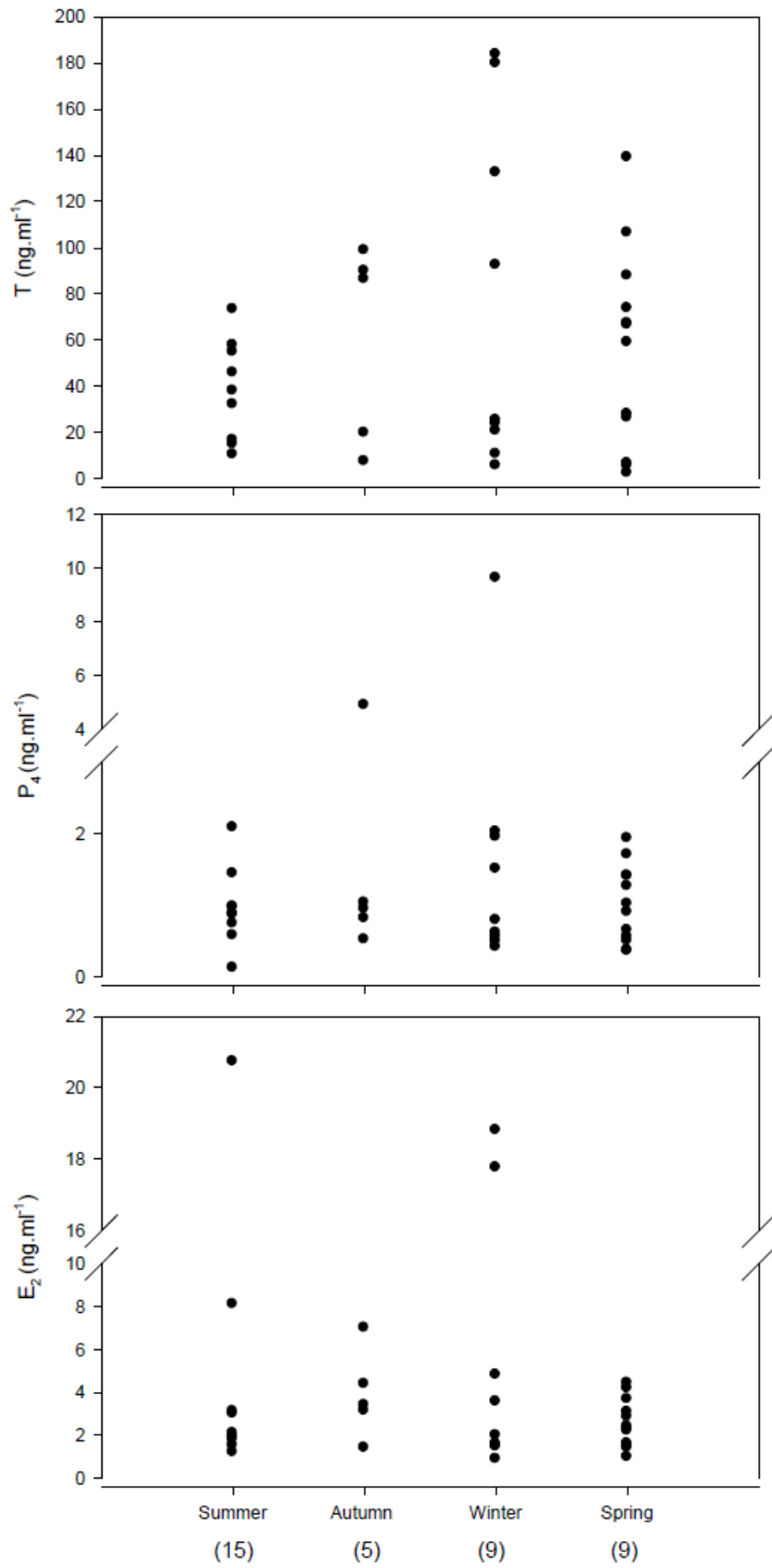


Figure 29: Seasonal variation in hormone levels of adult female Maugean Skate. Numbers in parentheses represent sample size.

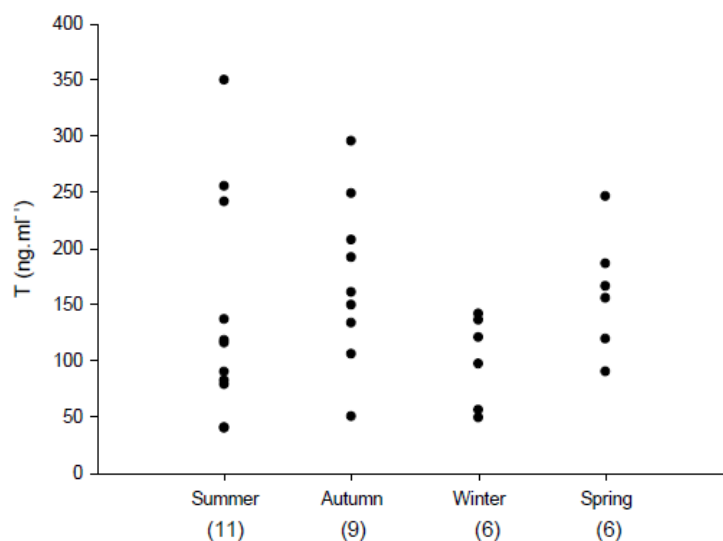


Figure 30: Seasonal variation in testosterone levels of adult male Maugean Skate. Numbers in parentheses represent sample size.

Respirometry

Although formal statistical analyses are yet to be completed², visual inspection of the relationship between MO_2 and DO (Figure 31) indicate that there was virtually no difference between treatments. This is not unexpected given the short acclimation period before skate were placed in the respirometer (~24 hours). However, there is an almost linear decline in MO_2 that is already encroaching on 0 at 25% DO. This strongly suggests that the skate switch to an anaerobic metabolic pathway, whereby metabolism of stored glycogen meets energetic demands, with an associated accumulation of lactate and metabolic protons.

This result is validated by the fact that whole blood lactate levels in the 20% DO treatment group were significantly higher at around 10 mmol L^{-1} , compared to those for the 55% DO group (controls), which were always "low" (this means that the concentration was $< 0.8 \text{ mmol}^{-1}$, as this is the lower limit of its range). There were no differences in Hct or Hb between the treatments, suggesting that they do not respond to low DO by increasing RBCs (Hct) or oxygen transport (Hb), but go completely anaerobic instead. Note, however, that the 20% DO animals had smaller spleens relative to their body size compared to the control group. Given that the spleen is one site of RBC production in elasmobranchs, this difference may in fact indicate RBC release into the circulation, despite Hct indicating otherwise.

² Note the physiological study was undertaken as a linked but independent project to the current study, the formal analysis of which is in progress. However, given the significance of the relationships between DO and metabolism in Maugean Skate we have chosen to present the preliminary findings in this report.

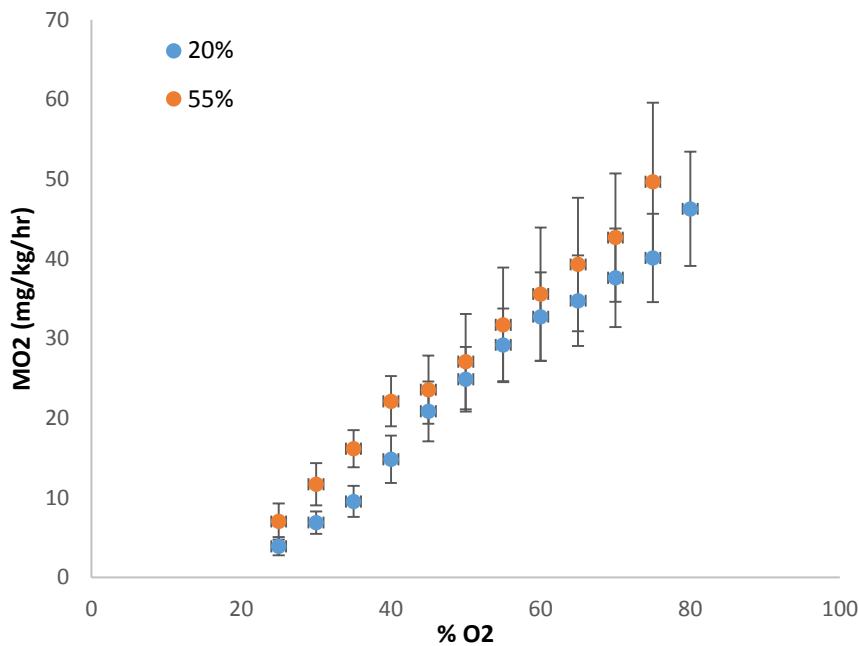


Figure 31: Mean metabolic rate (MO₂) of 20% (blue) and 55% (orange) DO (%O₂) treatment skate correlated with the DO level in the sealed respirometer as oxygen is consumed. Error bars represent standard error.

Age and growth

Opaque and translucent regions were apparent on Maugean Skate vertebral sections (Figure 32) and are likely to represent annual growth increments based on similar patterns observed in other skate species. A 'birth band' was relatively easily identified in most instances (Figure 32) and was often highlighted by a change in the angle of vertebral growth. Age estimates (based on ring counts) ranged from two to ten years (Table 8). However, the ten year old had a wide edge margin and since it was sampled in early spring, it is likely that an opaque layer would have been laid down soon after it was sampled and this individual may have been closer to eleven years. As the skate that were aged were smaller than the largest sizes observed (largest aged individuals being 90 and 94% of maximum sizes for males and females, respectively), it is probable that maximum longevity may be around 15+ years. Given size at maturity, it is likely that Maugean Skate mature somewhere between 5 and 8 years of age.

The lack of small and large individuals in the aging sample caused the von Bertalanffy model to take a linear form (Figure 33). In fact a simple linear model fitted the data better (AIC = 141.09) than the von Bertalanffy model (AIC = 144.89). Nevertheless, the difference was minimal and the von Bertalanffy parameters ($K = 0.16$, $t_0 = -2.78$, $L_{inf} = 916.39$) have biological meaning and can be used for various other fisheries related calculations (e.g. natural mortality estimation) so are presented here in preference to the linear model.

Three PIT tagged skate were recaptured after more than six months at liberty, two being at large for around two years (Table 9). Two were relatively large males when tagged and each had only grown one millimetre. The third was a female that would have been immature when tagged (601 mm). This individual had increased by 166 mm in length in around two years. The von Bertalanffy growth model suggests that on average it would take between 4 and 5 years for a skate of this size to have grown that much.

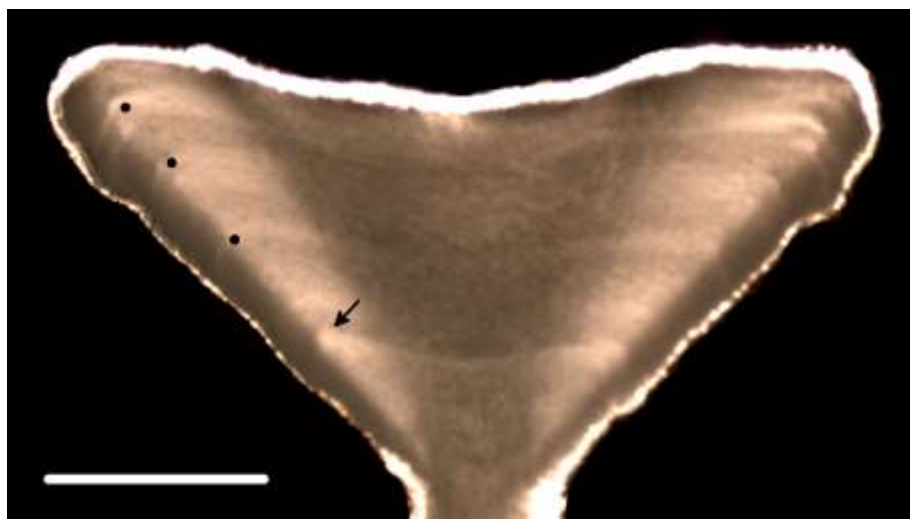


Figure 32: Longitudinal section of a Maugean Skate vertebrae. Arrow indicates the birth band, and dots indicate what is assumed to be annual growth increments. Scale bar is 1 mm.

Table 8: Age estimates derived from Maugean Skate vertebrae.

Readability ranking and edge characteristics (w = wide, n = narrow). Readability is based on the scheme in Morison *et al.*, (1998).

Total length	Sex	Age	Readability	Edge
551	Female	3	3	n
740	Female	5	3	n
816	Female	9	3	n
719	Female	7	4	n
574	Male	4	3	w
668	Male	4	3	n
661	Male	6	4	w
656	Male	6	3	n
616	Female	3	3	w
756	Female	8	3	n
672	Female	6	4	w
819	Female	10	4	w
450	Female	2	3	n

Table 9: Tag-recaptured individuals that had been at liberty for >6 months.

Length at release (mm)	Length at recapture (mm)	Sex	Time at Liberty (days)	Growth (mm)
661	662	Male	183	1
720	721	Male	732	1
601	767	Female	733	166

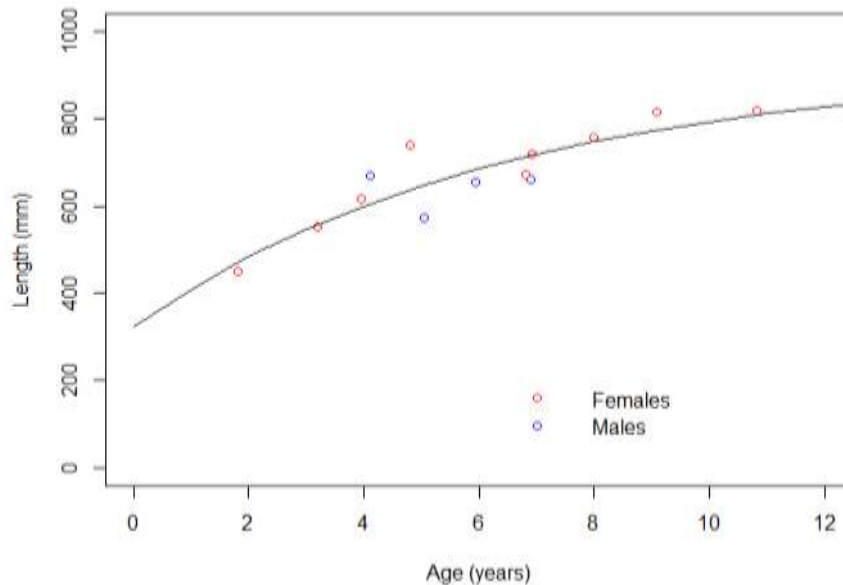


Figure 33: von Bertalanffy growth model of Maugean Skate length at age.

Population estimation

In total, 162 Maugean Skate were PIT tagged between November 2013 and November 2014. In addition, a further 113 were PIT tagged between April 2012 and February 2013 as part of a previous study (Lyle *et al.*, 2014). Nine PIT tagged skate were recaptured, all during the present study. The longest period of time at liberty was 733 days for a female skate that was tagged in November 2012. Due to the low number of recaptures, many closed population models failed to converge, and several that did had warnings suggesting they were unlikely to be accurate. Only models that ran successfully, without warning, are presented in Table 10.

The model that best fit the tag-recapture data was a Chao Lower Bound model in which capture probability varies through time and behavioural response to capture (Mth). This estimate was 3177 individuals with 95% confidence limits of 1827–6247. The second best model was again a Chao Lower Bound Model (Mh) in which capture probability varies due to a behavioural response to capture only. This model predicted a slightly greater abundance of 3254 (95%CL 1868–6403). Assumptions for both of these models are generally consistent with acoustic tagging information in that animals were shown to display unusual behaviour following release (detailed in movement section) and, with regard to the Mth model, we did see some individuals relocating at times. Given we repeatedly sampled the same locations, relocation would result in the probability of recapture changing through time.

Several Huggins models also ran successfully, the best of which was the Mt model in which capture probability varies through time alone. All of these models predicted a population estimate of 2800–2900, however, both AIC and BIC suggest these models were inferior to the Chao models.

The above population estimates need to be interpreted with caution. The acoustic telemetry suggested that most skate exhibit a high degree of site fidelity and, given we sampled in the same areas repeatedly, we were therefore more likely to recapture tagged skate thereby underestimating population size. Conversely, we believe that there may have been some tag loss which would potentially result in an overestimation of population size. Genetic analyses currently underway will provide an estimate of population size based on both tag-recapture and on genetic diversity (i.e. effective reproductive population) and may be more accurate than those provided herein.

Table 10: Maugean Skate population estimates using Chao Lower Bound and Huggins models of tag-recapture data.

Model	Abundance	St. error	Lower CL	Upper CL	AIC	BIC
Chao (LB) Mth	3177	987	1827	6247	169.9	238.1
Chao (LB) Mh	3254	1012	1868	6403	234.8	256.4
Huggins Mt	2824	786	1686	4874	1399.6	1472.4
Huggins Mth	2824	786	1686	4874	1399.6	1472.4
Huggins Mo	2895	807	1726	5000	1468.4	1474.5
Huggins Mh	2895	807	1726	5000	1470.4	1482.6

Salmonids

Movement

Atlantic Salmon

Four of the 30 Atlantic Salmon were not detected, presumably having died immediately following release – these were released at the aquaculture lease outside of detection range so their fate has to be assumed. A further two fish were judged to have died within two days of tagging. Of the remaining 24 salmon, most were highly active, displaying what appeared to be random dispersal and movement within the harbour immediately after the simulated escape event and for the remainder of the time they were detected (Figure 34A, B, C, E, F). As there was no clear immediate behavioural response to tagging we decided to include all of the post tagging data for the 24 individuals.

Atlantic Salmon were detected on all receivers throughout the harbour, with eight individuals detected on >40 receivers and most on >20. In most instances the UD's encompassed much of the harbour area reflecting the widespread movement throughout the system (Figure 34). There was, however, a tendency to follow the shoreline while traversing the length of harbour with individuals detected more often by those receivers nearest to the shore on the two main curtains (Figure 35). Many of the tagged individuals travelled the full length of the harbour in a single day and only a single fish (AS21) displayed any affinity for a particular region. Regional presence absence plots highlight their erratic behaviour (Figure 36) with fish being detected in multiple regions on any given day and most fish moving through all of the regions at some stage. Individual presence absence plots and movement summaries are presented in detail in the supplementary information.

The individual Atlantic Salmon that did display a degree of site fidelity (AS21) was regularly detected at receivers LP01 and SP03 in the central harbour (Figure 34D), which are on, and adjacent to, an aquaculture lease. It is feasible that this individual was attracted to the farms and was feeding on excess pellets, which is supported by the fact that it was detected for more than a month longer than any of the other Atlantic Salmon. This individual made a journey to Swan Basin in early January 2014 but returned to the lease area after about a week, remaining in that area until its last detection on the 7th February. None of the other Atlantic Salmon exhibited any obvious affinity for the aquaculture lease sites but several did spend periods of time around Double Cove (DC08), which is relatively close to several leases in the central south west of the harbour.

Six of the tagged Atlantic Salmon were last detected at Macquarie Heads (MH02) suggesting they exited the harbour. Although four individuals were detected at the Gordon River mouth, all were subsequently detected back in the harbour soon after indicating that none had spent much time in the river.

Recreational gillnet fishers reported the capture of six tagged Atlantic Salmon, most within three weeks of being released (range 6–31 days after tagging). Anecdotal reports from fishers suggest that there may also have been unreported recaptures.

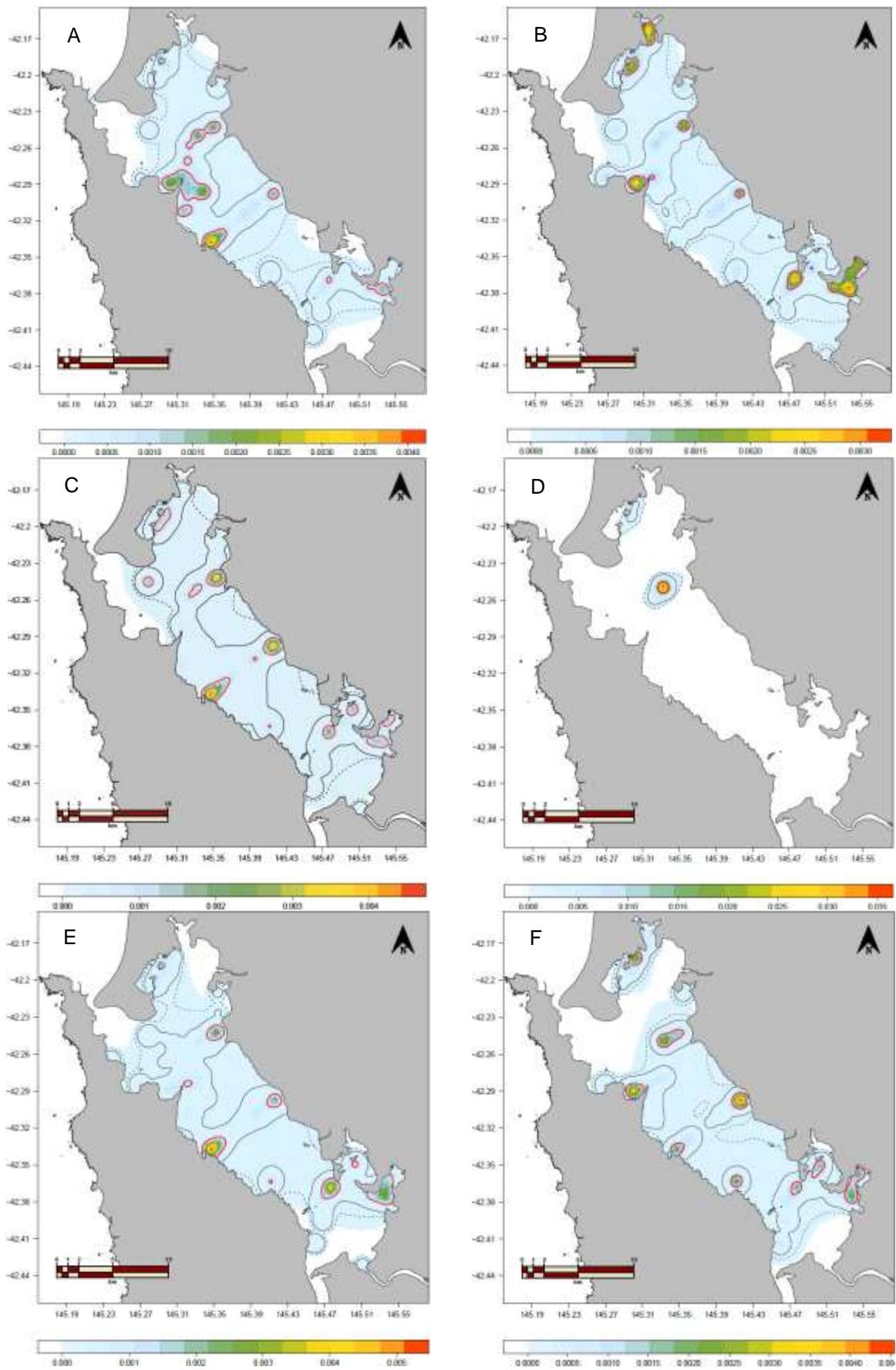


Figure 34: BBMMs for Atlantic Salmon that were at liberty for longer than one month. AS05 (A), AS16 (B), AS18 (C), AS21 (D), AS25 (D), AS26 (E) and AS28 (F).

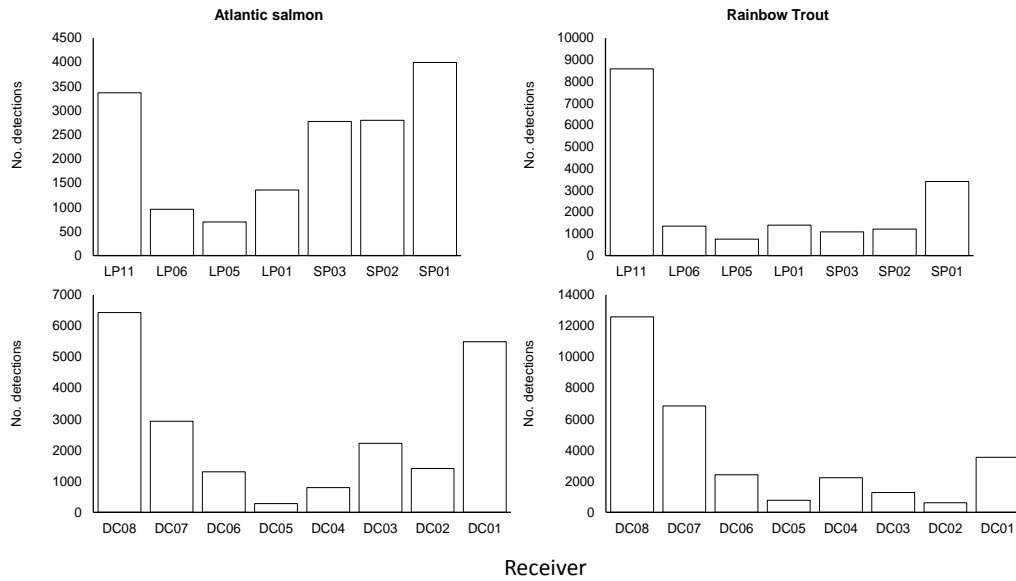


Figure 35: Frequency of salmonid detections by the two main curtains in Macquarie Harbour (refer to Figure 1 for receiver locations).

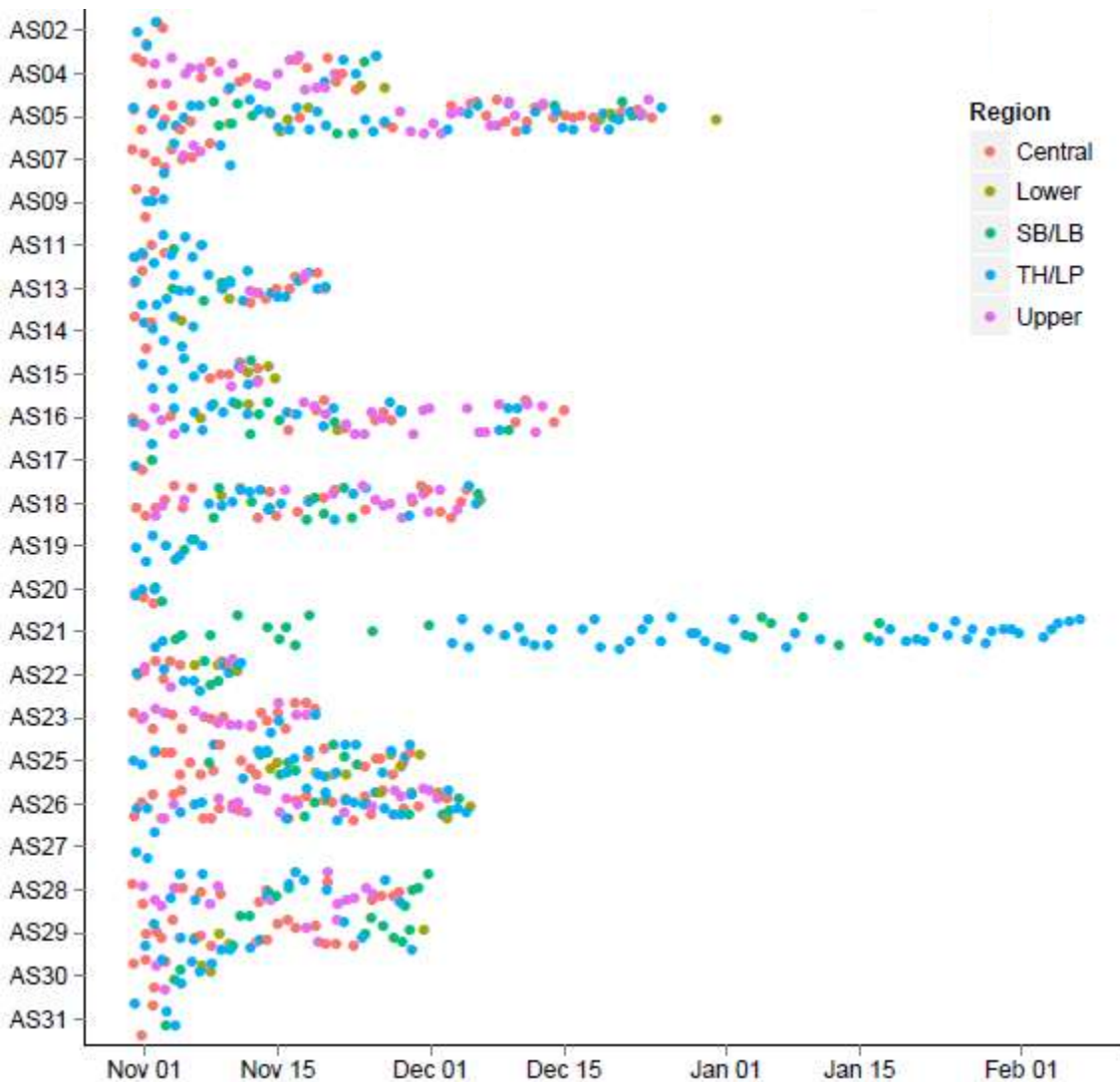


Figure 36: Regional utilisation of Macquarie Harbour by Atlantic Salmon.

Rainbow Trout

Although all of the tagged Rainbow Trout were detected, one fish was judged to have died within three days of tagging and three others were not detected after 3–5 days suggesting that they too may have also died. The remainder were highly active and were detected throughout the harbour (Figure 37), with seven individuals detected on >40 receivers and most detected on >20 receivers. It was not unusual for individual fish to have travelled the length of Macquarie Harbour within a day. The UD estimated by BBMM indicates that Rainbow Trout utilised much of the harbour area but in particular the central region (Figure 37). Presence absence plots in Figure 38, and in individual plots in the supplementary information, probably best represent individual movement, which was slightly less erratic than for the Atlantic Salmon, with many individuals remaining in specific areas for short periods (up to a week or so) before relocating. This behaviour resulted in discrete UDs in a number of areas (Figure 37). For instance, several individuals appeared to show preferences for the eastern Liberty Point to Double Cove region (Figure 37 A, C, F), which was in the general vicinity of active aquaculture leases. It is feasible that this behaviour may have been associated with feeding on excess pellets.

When moving throughout the harbour the Rainbow Trout tended to be detected more frequently on the inshore receivers of the two major curtains indicating that they tended to follow the shoreline, in particular the south-western shore (Figure 35).

Seven Rainbow Trout were detected at the Gordon River mouth, three of which were last detected by this receiver suggesting that these individuals may have travelled upstream and remained in the river system. In one instance, the individual (RT08) was detected at the Gordon River mouth in mid-January 2014 and not redetected until 209 days later in early August, again at the Gordon River mouth. On that occasion it was detected by the river mouth receiver over a period of about 14 h. The timing of the redetection was around 11 days after the recharge event and during a period of relatively strong river flows. It is possible that this fish had remained in the Gordon River during the intervening period, moved downstream for a short time and then moved upriver again. This hypothesis is supported by the absence of any subsequent detections by other receivers within Macquarie Harbour. Alternatively, it is also possible that the fish had died somewhere in the vicinity of GR01 and the high winter flows washed the tag past the receiver. Both explanations are plausible but if the first is true it would imply that this individual had taken up residence in the river and was presumably feeding on native fauna.

Four Rainbow Trout were detected at Macquarie Heads (MH02), three of which were detected there for brief periods of time before moving back into the harbour. Only one individual appeared to have permanently exited the harbour. A total of eight Rainbow Trout were captured by recreational fishers, most within a month (range 8–65 days) after tagging.

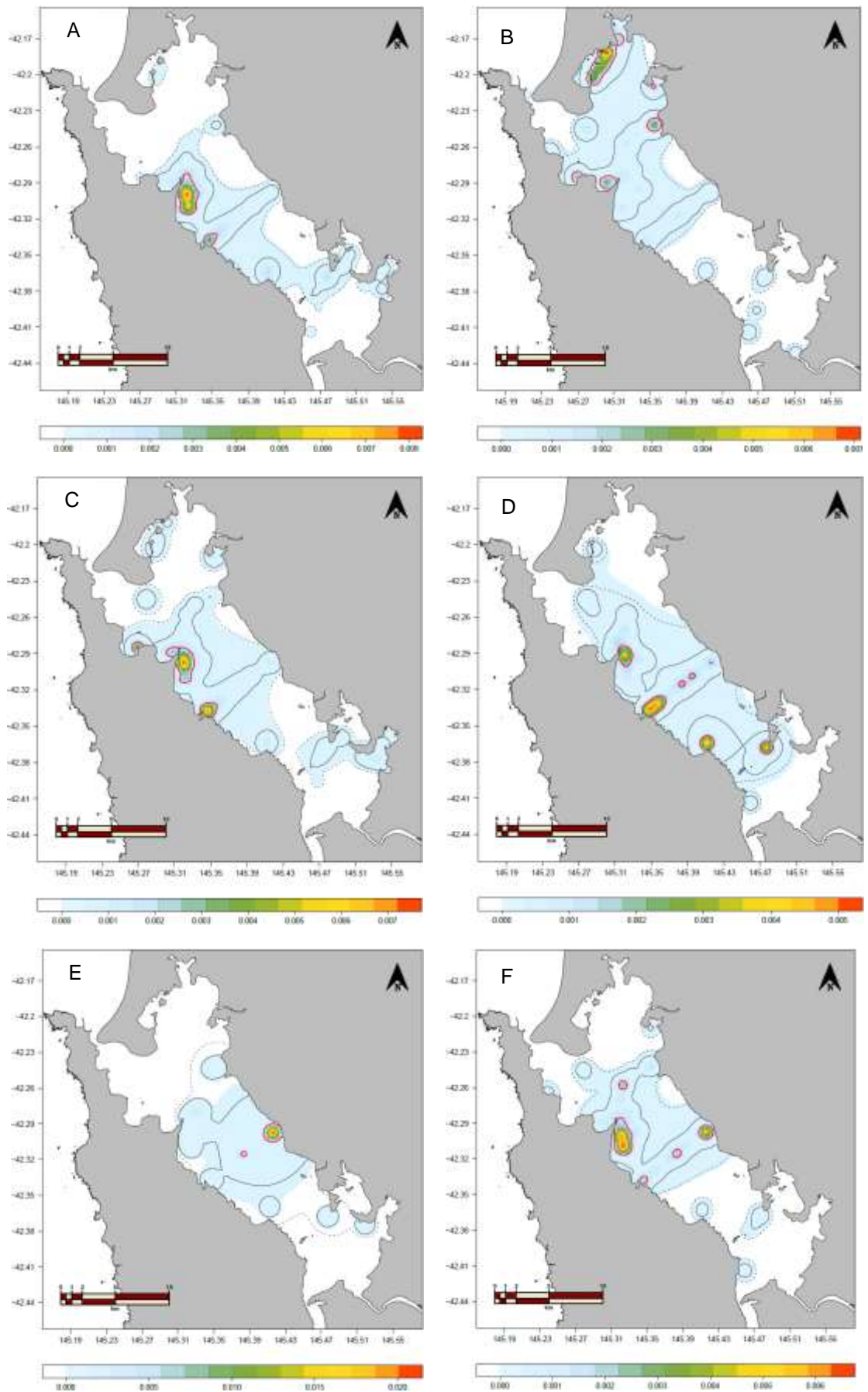


Figure 37: BBMMs for Rainbow Trout that were at liberty for the longest periods of time. RT03 (A), RT14 (B), RT18 (C), RT22 (D), RT25 (E) and RT28 (F).

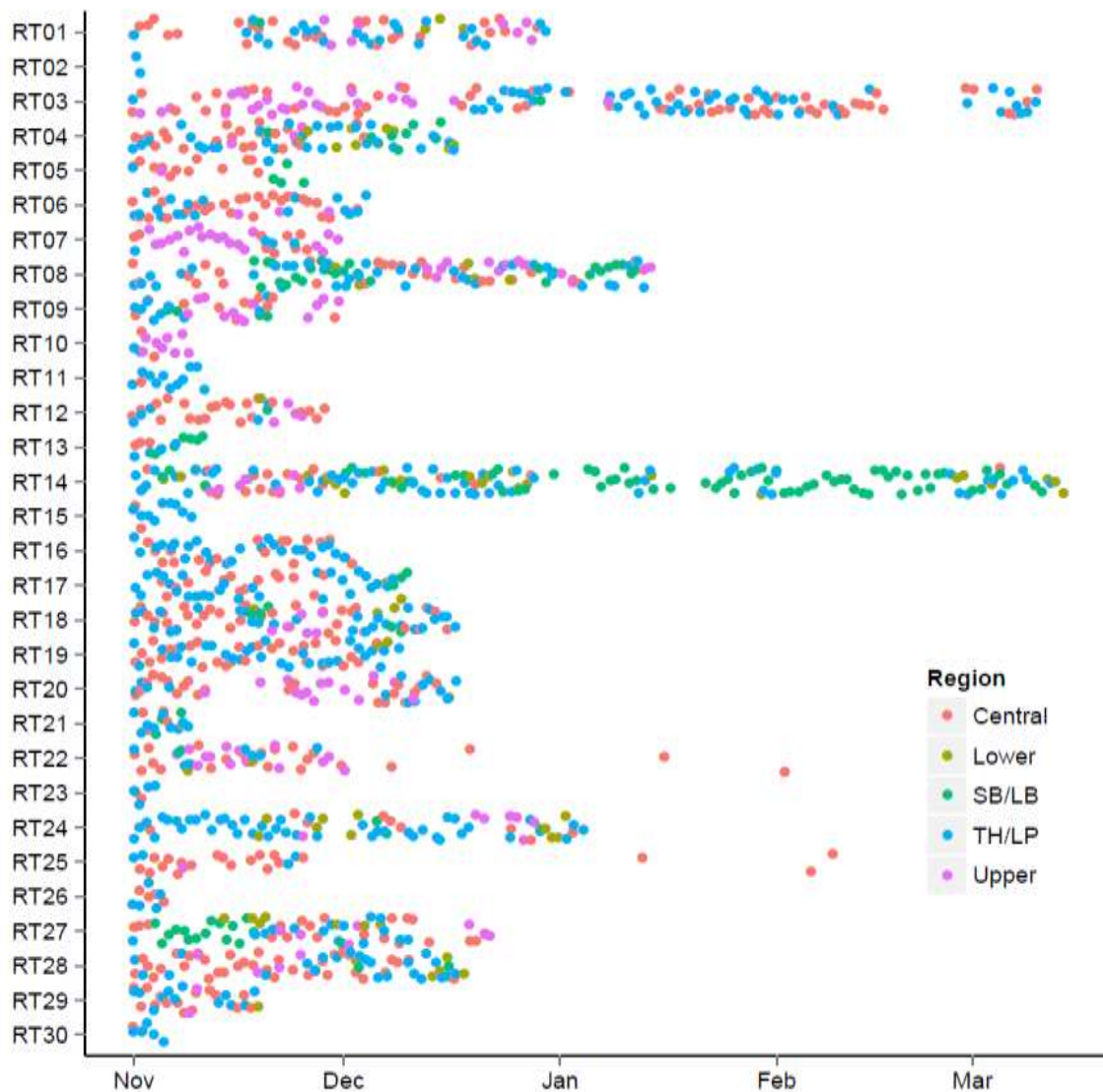


Figure 38: Regional utilisation of Macquarie Harbour by Rainbow Trout.

Survival

The ‘survival’ period for each tagged salmonid was assessed as the number of days between tagging and either its last detection whilst still obviously active within Macquarie Harbour or the reported date of capture by recreational fishers. Final detections for most recaptures corresponded to the day they were reported as recaptures, and in some instances we were able to detect the fish as they ceased movement while caught in the gillnet. Individuals last detected at the entrance to the harbour or at the entrance to the Gordon River were assumed to have left Macquarie Harbour at that point in time. As no inferences can be made about their survival outside of the harbour, the assigned survival period in such instances will be conservative.

The surviving proportion of both salmonid species within Macquarie Harbour declined rapidly after release (Figure 39; Table 11), with only 50% of the Atlantic Salmon present after 24 days and 50% of the Rainbow Trout still present after 35 days. Within two months there was only one Atlantic Salmon remaining alive in the harbour, this individual was last detected alive 99 days after release, 38 days longer than any other of the Atlantic Salmon (Figure 39). This particular individual had been detected regularly at, and adjacent to, a large aquaculture lease in the central harbour region and may have been

feeding on the spill-over of feed pellets. As noted above, six Atlantic Salmon appeared to have emigrated to sea from Macquarie Harbour and at least six of the 24 successfully tagged individuals (25%) were captured by recreational gillnet fishers.

In the short term Rainbow Trout appeared to fare better following release (Figure 39; Table 11), with more than half of individuals still detected after a month and four individuals still active within the harbour after 100 days (Figure 39). The last confirmed mortality occurred on the 24th April 2014 when caught by a recreational fisher after having survived for 176 days. Only a single Rainbow Trout emigrated from Macquarie Harbour and eight individuals (27%) were reported as captures in recreational gillnets. Three Rainbow Trout were last detected at the Gordon River mouth but their fate within the river system is unknown.

Interestingly, no dietary items were found inside the stomachs of 15 Atlantic Salmon and three Rainbow Trout aquaculture escapees that were captured during biological sampling, providing no evidence for feeding on native fauna or pellets.

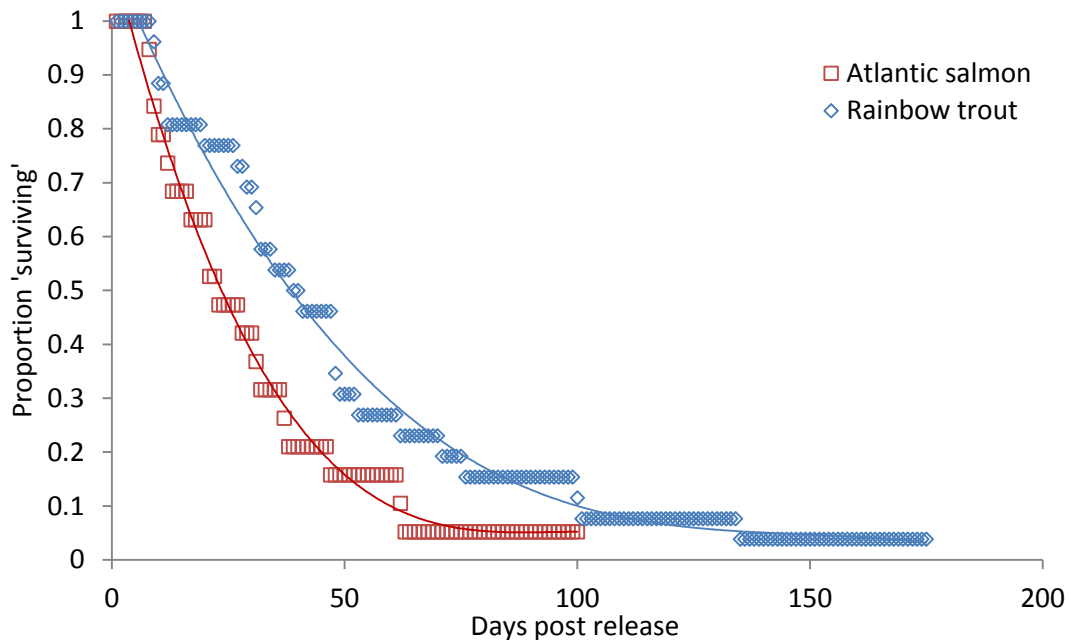


Figure 39: Survival rate and residency within Macquarie Harbour of salmonids after the simulated release event. Mortalities include fish captured in recreational gillnets.

Table 11: The number of days that 90, 75, 50, 25 and 10% of salmonids 'survived' following release.

Percent 'surviving'	Days post release	
	Rainbow Trout	Atlantic Salmon
90	10.9	7.0
75	19.1	12.4
50	34.5	23.6
25	52.5	40.2
10	64.7	59.8

Discussion

Distribution, habitat utilisation and movement of Maugean Skate

While there was no difference in the overall size composition of Maugean Skate collected in a previous gillnet study (November 2011–February 2013) and the present study (November 2013–February 2015), the size composition of males did differ between studies. The implications of this difference for the population are not, however, clear. Regionally there was some evidence for population structuring, with smaller individuals relatively more common in samples from the upper reaches of Macquarie Harbour than elsewhere. Although skate smaller than about 500 mm TL were not well represented, presumably due to the selectivity characteristics of the gillnets, our data do imply that juveniles may be more abundant in the upper reaches of the harbour. Further work using gear capable of sampling these smaller individuals is required to determine whether there are preferred juvenile habitats within Macquarie Harbour.

Maugean Skate occur throughout Macquarie Harbour in depths from <1 to >50 m but display a strong preference for benthic habitats in the 5–15 m depth range. In the context of this preferred habitat, their effective distributional range within Macquarie Harbour is in the order of just 70 km².

Within their preferred depth range environmental conditions are relatively consistent throughout Macquarie Harbour. Salinities generally ranged between 18–27 ppt and water temperatures were typically 12–15 °C throughout the study period. Dissolved oxygen levels tended to be more variable but generally remained above 30%. At depths >15 m temperature variation was minimal but salinity gradually increased with depth to almost marine conditions. Dissolved oxygen, however, declined rapidly and at depths >20 m tended to be below about 20%. The fact that Maugean Skate leave their preferred depth range at times suggests they are able to cope with decreased DO at depth and also the low salinities (above the halocline) in the shallows, at least for short periods of time. The physiology experiments tend to support these observations, with the skate's main mechanism for coping with low DO being to change from an aerobic to an anaerobic metabolic pathway. However, this is not a long term strategy as the lactate build up from glycolysis will eventually cause damage if not removed and its removal requires aerobic metabolism. While this strategy would allow them to move through areas of low DO over relatively short time periods, it would not allow them to be site attached in low DO water for long periods and as such it would make sense that they would shift to shallower, higher DO water.

There was no evidence that Maugean Skate moved into the Gordon River or out of the harbour itself, even though two individuals were recorded at the harbour entrance. These observations support the assertion that the species is estuarine dependent and that the population in Macquarie Harbour is likely to represent a discrete and isolated population.

Maugean skate exhibited a diurnal pattern in depth utilisation and activity, tending to become more active, utilise a broader range of depths and generally shallower waters during the night. Other skate species also display very clear diurnal patterns of depth utilisation and similar to the Maugean Skate, activity and depth variation increases during the night (e.g. common skate *Dipturus batis* (Wearmouth and Sims, 2009)). Comparable behaviour has also been observed in a variety of other elasmobranchs (Weng and Block, 2004; Weng *et al.*, 2007; Andrews *et al.*, 2009; Hoffmayer *et al.*, 2013; Carlson *et al.*, 2014) and has been linked to feeding, temperature and oxygen preferences (Sims *et al.*, 2005; Nasby-Lucas *et al.*, 2009). All three factors are possible for the Maugean Skate, although it is most likely that they are entering shallower waters at night to forage and returning to the more environmentally stable (salinity and temperature) deeper water during the day where they tend to be less active.

Overall, Maugean Skate displayed a high degree of site fidelity for most of the study period with most individuals remaining in the general area where they were originally tagged. That is, most skate

had specific home ranges and only occasionally left these. For example, within the Table Head Liberty Point region, a variety of individual home ranges were identified. These included; Table Head, the bay between Table Head and Liberty Point, western Liberty Point, northern Liberty Point, and, eastern Liberty Point. Other telemetry, tracking and conventional tagging studies of skates have found that most species have relatively small home ranges and limited dispersal even in oceanic habitats (Wearmouth and Sims, 2009; King and McFarlane, 2010; Peklova *et al.*, 2014).

It was notable from both the telemetry analyses and biological sampling that Table Head represented a particularly important area for the species. This area is characterised by a channel which is approximately 100 m wide and up to 13 m deep that runs around the headland and is flanked by a large, shallow, expanse to the north and east. The highest catch rates of all areas in Macquarie Harbour were achieved in this channel, with skate typically captured at the bottom of the drop-off on either side of the channel and within their preferred depth range. Similar channel systems exist in the Swan Basin and Rum Point areas and moderately high catch rates were also achieved in these regions.

Associated with site fidelity, many of the Maugean Skate did leave their home range area at some stage during the study period, sometimes for several weeks, before returning to their home area. This behaviour suggests that the species may have relatively advanced spatial memory and comparably advanced cognitive capabilities that have been found in a variety of chondrichthyans (Hueter *et al.*, 2005; Schluessel and Bleckmann, 2005; Papastamatiou *et al.*, 2011; Davy *et al.*, 2015).

There was no obvious pattern associated with these temporary excursions with the timing and behaviour varying between individuals. Several skate did, however, relocate and take up new home ranges. Of particular note, five individuals relocated to the lower reaches of the harbour, being detected regularly in the main shipping channel that leads to Macquarie Heads. Some of these individuals were also occasionally detected in a channel that runs along the south east shoreline. Given the bathymetry of the area, it is likely that they would have had to cross several kilometres of shallow (<5 m) sand flats to travel between these areas. As we only had three receivers in the lower estuary, each in channels, our coverage of the general area and especially the shallows was low. While these channels were utilised regularly the skate also spent a large amount of time outside of detection range and almost certainly in shallower waters. Recreational gillnet fishers report that they occasionally encounter Maugean Skate while targeting flounder on the shallow sand flats in the area which tends to support this hypothesis. Although it was logistically impractical to place receivers in such shallow waters, it is clear that these habitats are utilised by the species and that the lower estuary also represents a relatively important habitat for the skate.

Biology and population size of Maugean Skate

Diet

As a group, skate play an important ecological role in many communities due to their abundance and trophic position, which is typically similar to that of marine birds, mammals and sharks (Ebert and Bizzarro, 2007). Larger species tend to occupy higher trophic levels with their diet often primarily comprised of fish, whereas benthic crustaceans such as crabs are more important in smaller species (Bizzarro *et al.*, 2007; Ebert and Bizzarro, 2007).

The Maugean Skate diet is restricted in terms of diversity, suggesting a highly specialised epibenthic feeding mode. Two groups of crustaceans, namely crabs and shrimps dominated, with the crab *Paragrapsus gaimardii* followed by the carid shrimp *Palaemon* spp of particular significance (58 and 26% IRI, respectively) in the diet. Despite contributing relatively little in terms of weight, mysid shrimps were of secondary importance, being consumed in relatively large numbers when encountered (10% IRI). Fish represented the only other taxon of importance and, with the exception of a single Jack Mackerel, included relatively small species such as Australian Anchovy (*Engraulis australis*) and

possibly hardyhead (Atherinidae) or whitebait (*Galaxias* spp.) species. As it is unlikely that skate would be able to capture a highly active pelagic species such as Jack Mackerel it is possible that it was consumed as a result of scavenging on discards or represented an opportunistic predation event. Although excluded from the dietary analyses, we did encounter one individual skate that had consumed the discarded skin of a recently filleted Atlantic Salmon. In addition, Maugean Skate were occasionally captured on hooks baited with pieces of squid. These observations tend to confirm that the species will scavenge on dead food items when available. Other taxa, including annelids, cephalopods and tunicates were rare prey items. Plant and algae material was also present in the stomachs of small number of individuals, presumably taken incidentally whilst feeding and unlikely to represent a source of nutrition.

Crabs have been shown to be particularly important for other small, shallow water, skate species (Bizzarro *et al.*, 2007) and appear to be particularly important for the lineage as a whole. Interestingly, the diet of the close relative of the Maugean Skate, *Z. chilensis*, is mainly comprised of fish (Lucifora *et al.*, 2000); however, this species inhabits deeper waters and other studies have also found that the diet of large, deep sea skates, tends to be predominantly comprised of fish (Bizzarro *et al.*, 2007).

Ontogenetic shifts in diet are common in skate (Bizzarro *et al.*, 2007; Barbibi *et al.*, 2009). Very small individuals were not sampled due to the risk of causing damage to the oesophagus or stomach while carrying out the lavage. Nevertheless, a relatively large range of sizes (470–827 mm TL) were sampled and there was no evidence of an ontogenetic shift, with the largest and smallest individuals all containing crabs of similar size. Nor was there any obvious spatial variability in diet, with crabs a common prey item in skate sampled from Table Head/Liberty Point, Swan Basin and Kelly's Basin regions. Shrimp did, however, appear to be more common in the upper reaches of the system (Kelly's Basin) but this observation was based on only five individuals and is too few to draw firm conclusions.

No aquaculture feed was found in any of the stomachs examined suggesting that feed pellets do not comprise a major component, if at all, of the skate diet. However, since the majority of the skate were captured >2 km away from the leases it is possible that if pellet feeding did occur that the individuals were not vulnerable to the sampling gear shortly after feeding due to limited short-term movement behaviour. The lack of pellets in the diet may thus be more a result of spatial separation between the sampling sites and the marine farm leases, coupled with the skate's preference for shallower depths that lie outside of the main areas of aquaculture operations. By contrast, pellet feeding was observed in the Whitespotted Dogfish, another elasmobranch common in the harbour. In fact that species was found to feed almost exclusively on aquaculture pellets (D. Moreno, unpubl. data).

Reproduction

Female Maugean Skate attain maturity at larger sizes (between 660 and 680 mm TL, with the smallest mature individual recorded being 650 mm TL) than males (about 620 mm TL), equivalent to at least 76 and 86% of the maximum recorded lengths for females and males, respectively. This would suggest that, unlike some skate species (see Ebert (2005)), Maugean Skate are potentially reproductively active for up to ~10 years given they are likely to mature at 5–8 years of age.

Maugean Skate are oviparous, with an asynchronous discontinuous reproductive cycle in which a proportion of the adult females are reproductively active at any given time. For instance, in a sample of six adult females examined during spring, only one individual had vitellogenic follicles present, the remaining five individuals were in a resting phase with small un-yolked follicles. Although there was no strong seasonal pattern in spawning activity there was some evidence to suggest that the species may be least active during summer. Males are likely to produce sperm in most seasons and an increase in testosterone levels between spring and autumn may be associated with mating activity over a protracted period.

Skate are believed to be secondarily oviparous; that is, they evolved from a viviparous ancestor they share with the Rhinobatoid lineage. This has been proposed as a strategy to increase fecundity as body size does not limit the fecundity of oviparous species as it does viviparous and oviviparous species

(Lucifora and Garcia, 2004). In fact many skate species have a continuous reproductive cycle that maximises fecundity (Hamlett and Koob, 1999; Henderson *et al.*, 2004; Colonello *et al.*, 2012). Within the genus *Zearaja*, *Z. nasutus* has a distinct seasonal cycle in which eggs are laid in spring and summer (Francis, 1997), whereas *Z. chilensis* has a continuous reproductive cycle (Bustamante *et al.*, 2012). By contrast, the discontinuous reproductive cycle utilised by the Maugean Skate probably represents a less productive strategy, however, as the frequency of egg laying and duration of the active phase are unknown it is not possible to estimate fecundity.

No skate were observed to be laying egg capsules when captured, nor were any *in utero* egg capsules observed by ultrasound. However, while grappling an acoustic receiver in ~20 m of water to the east of Swan Basin a number of hatched Maugean Skate egg capsules were collected. Additionally, while retrieving a longline set at >20 m depth in the channel adjacent to this receiver, we retrieved a lost gillnet that had several dozen hatched skate egg capsules entangled in it. Whether these were intentionally laid among the gillnet meshes is uncertain. The only documented living Maugean Skate egg capsules were found entangled in a gillnet that was set in 20–30 m (Treloar *et al.*, 2013). Collectively these observations suggest that Maugean Skate lay some eggs at depths outside of their preferred depth range. Salinity and temperature tend to be quite stable in deeper waters and this stability may increase survival of developing embryos, although DO tends to be low in these deeper waters. Conversely, egg laying may occur in the shallower low salinity waters of the harbour as even the developing embryos of cartilaginous fish that have low tolerance for low salinities (e.g. Elephant Fish (Hyodo *et al.*, 2007)) have the ability to osmoregulate with their yolk sac membrane (Takagi *et al.*, 2014) and as such can tolerate lower salinities than adults. Maugean Skate have a far greater osmoregulatory ability than do Elephant Fish so it is possible that their embryos would be quite tolerant of lower salinities. Also, given the relatively stable environment below about 5 m, suitable egg laying habitat may extend into shallower water than has been proposed previously (Treloar *et al.*, 2013). However, if skate do select deeper waters in which to lay their eggs, the low and declining trend in bottom DO concentrations observed in recent years may present a threat to their development and survival. There is presently insufficient information to ascertain the preferred egg laying habitat or depth but given the small home range of the females tagged in the present study it appears likely that at least some egg laying does occur within the depth range that adults spend most of their time.

Respirometry

This is the first study to examine respiratory physiology in this genus. It has shown that the Maugean Skate's only mechanism for coping with low DO is to switch to anaerobic metabolism. However, this is not a long term strategy as the lactate build up that results from this metabolic pathway will eventually cause cell damage and subsequently death if aerobic metabolism cannot be resumed. This suggests that these skate are poorly adapted to very low DO levels and they essentially can only “hold their breath” and move through the low DO water until they reach more suitable habitat. As such, any long term changes to DO levels in Macquarie Harbour are likely to restrict their habitat.

Age and growth

We were successfully able to obtain preliminary age and growth estimates from longitudinal vertebral sections of Maugean Skate. While un-validated, this technique is widely used in other skate species and has been validated for or verified for several species including the related *Z. chilensis* (Holden and Vince, 1973; Sulikowski *et al.*, 2002; Licandeo *et al.*, 2006).

Based on the assumption that the growth increments are deposited annually, and have been correctly interpreted, our data suggest that Maugean Skate are a relatively short lived species in comparison with many other elasmobranchs. Given individuals close to the maximum observed sizes were not aged, and due to the small sample size, it is probable that maximum longevity is in the mid to late teens. The von Bertalanffy model derived from the ageing data was more linear than typically expected, probably due to the limited numbers of small and large individuals aged, although a study that involved ageing a large number of the related *Z. chilensis* found relatively linear growth throughout their lifetime (Licandeo *et al.*, 2006). Further sampling of the Maugean Skate would be

required to confirm whether this was a characteristic of the lineage or, in this case, an artefact of sampling and/or aging error.

Both other members of the *Zearaja* genus have been aged. Francis *et al.* (2001) found that *Z. nasutus* had a maximum age of nine years but conceded that they did not age animals close to the maximum recorded size. In addition, the population has been heavily fished for many years and thus older fish may be underrepresented. The most thorough investigation into age and growth of a related species is that for *Z. chilensis* (Licandeo *et al.*, 2006). This study verified their age estimates using marginal increment analysis and back-calculated early year classes, providing a very thorough analysis in which longevity was 21 and 18 years, and age at 50% maturity was 14 and 11 years, for females and males respectively. *Zearaja chilensis* is considerably larger than the Maugean Skate and *Z. nasutus* and this may explain its greater longevity.

Maximum age (and size) is a useful proxy for productivity and this has proved true for other species of skate; populations of larger species have tended to decline due to fishing whereas smaller species have tended to flourish (reviewed by Dulvy and Forest (2010)). Our preliminary findings suggest that in terms of growth rates Maugean Skate may be relatively productive like many other small skate species.

Population size

The Maugean Skate population in Macquarie Harbour was estimated to be approximately 3200 individuals, although this estimate has considerable uncertainty associated with it. The low number of recaptures in conjunction with following issues contribute to this uncertainty:

- Most skate have relatively small home ranges and show a high degree of site fidelity. As a result, the population did not mix randomly between each sampling event and, since fishing occurred in the same regions throughout the study period, there was an increased likelihood of recapturing tagged individuals. These factors combine to negatively bias the estimates of population size.
- Notwithstanding above, there were some individuals that did move away from areas that were sampled routinely, such as into the lower reaches of the harbour, and remained in these areas for most, if not all, of the study period. This small-scale 'emigration' would tend to represent a positive bias in the population estimate.
- Several acoustically tagged skate were determined to have died during the study period, a combination of natural mortality and tag induced mortality. Those individuals that died as a direct result of tagging (i.e. within two weeks) were identified and have been removed from the analysis. Any additional tag induced mortality, or natural mortality, will have resulted in a positive bias when estimating population size. While it was not possible to determine whether there were unaccounted mortalities associated with PIT tagging, any such effect is likely to have been minor since the tagging and handling process was less invasive than the acoustic tagging.
- PIT tag loss is feasible and on at least one occasion a PIT tag was lost within 24 hours of the skate being tagged (one of the skate retained for respirometry). The effect of unaccounted tag loss would be to positively bias the population estimate.
- Finally, small individuals were underrepresented in the sampling (due to gillnet selectivity) and thus this segment of the population was excluded, resulting in a negatively biased population estimate.

Although the biases identified above act in opposing directions, limited mixing within Macquarie Harbour and the concentration of sampling in three main areas, coupled with size selectivity of the gillnets, suggests that the reported population size represents an underestimate. An associated genetic study is underway and is expected to provide an independent estimate of effective population size,

which will provide greater confidence in assessing the status of the Maugean skate population in Macquarie Harbour.

Despite the possibility that the population may be larger than the data suggest, the fact that the species is restricted to Macquarie Harbour and Bathurst Harbour means that the Maugean Skate has one of the smallest distributional ranges of any chondrichthyan. This, in conjunction with the pronounced depth preference, means that their core habitat area is even smaller (~70 km²) in Macquarie Harbour. Limited habitat and low productivity of both estuarine systems are likely to be key factors in determining population sizes; however, within Macquarie Harbour Maugean skate are relatively abundant and are probably one of the most abundant large vertebrates in the system.

Spatial and temporal dispersal pattern of salmonid escapees

The tagged Atlantic Salmon and Rainbow Trout dispersed more or less immediately upon release, generally moving with little pattern throughout Macquarie Harbour and often travelling the full length of the system in a given day. Both species did, however, tend to be detected more frequently by those receivers closest to the shoreline, implying that much of this movement involved the fish following the shore. The rapid dispersal of fish away from the release site suggests that attempts to recapture escapees close to the farm from which the escape event occurred is likely to have limited success.

Key uncertainties about the environmental impacts of escapees relate to their potential to compete with endemic species for resources, including feeding on native fauna, and whether they can establish self-sustaining populations. How long escapees survive in the wild is thus a critical factor in understanding such impacts. Through the use of acoustic tags we have been able to demonstrate that escapees did not survive/persist in Macquarie Harbour for a long period of time following release. It is possible that the low visibility of the tannin stained waters may impede feeding as salmonids are visual predators and Atlantic Salmon have been shown to feed less efficiently at low light levels (Valdimarsson and Metcalfe, 1999). However, wild Brown Trout (*Salmo trutta*) populations do inhabit the harbour and salmonids inhabit tannin stained waterways throughout the world. It is, therefore, more likely that the escapees have not developed the hunting skills required to survive in the wild, a situation possibly exacerbated by the low productivity of Macquarie Harbour. Escapees are presumed to have died of natural causes (possibly starvation or predation, potentially by fur seals) or due to fishing pressure (reported recaptures accounted for about 25% of the fish successfully tagged and there were anecdotal reports of unrecorded captures). In addition, there was evidence of some emigration out of the harbour, either to sea (mainly Atlantic Salmon) or into the tributary rivers (Rainbow Trout). Our data indicated that about half of the escapees had either died or moved out of Macquarie Harbour within 24 days for Atlantic Salmon and 35 days for Rainbow Trout. The maximum duration that any Atlantic Salmon survived in Macquarie Harbour was just over three months, although the vast majority survived for less than two months. On average Rainbow Trout survived longer than Atlantic Salmon, with one individual detected sporadically over nine months, apparently spending an extended period (undetected) upstream in the Gordon River. In the main, however, the majority of the Rainbow Trout survived for less than three months.

Both salmonid species were detected at the Gordon River mouth, although there was no evidence that Atlantic Salmon actually spent an extended time in the river and most Rainbow Trout were detected back within the harbour shortly after being detected at the river mouth. Anecdotal reports from recreational fishers indicate that both species are captured in the Gordon River and there are reports of Atlantic Salmon being captured in the rivers to the north of Macquarie Harbour, supporting the observation that some fish leave the harbour. While escapee Atlantic Salmon look very similar to sea run Brown Trout, locals on the west coast are very familiar with both species, suggesting that identifications are reliable. The survivorship and behaviour of individuals that left the harbour (to sea or up the Gordon River) is unknown; this group represented almost 20% of the tagged salmonids.

The fact that Rainbow Trout tended to survive longer than Atlantic Salmon could indicate that the former does have some limited success in foraging on native fauna and/or feeding on uneaten pellets from farming operations. Consistent with this hypothesis we did observe that several Rainbow Trout exhibited some affinity for the eastern Liberty Point and Double Cove regions where many of the

marine farms are located. By contrast, only a single Atlantic Salmon showed behaviour suggestive of an affinity to marine farming operations and this individual survived for a month longer than any of the other tagged Atlantic Salmon. Although none of the salmonids captured by the research team had any dietary items in their stomachs, previous studies have found a higher, albeit low, incidence of feeding on aquaculture pellets, and to a lesser extent on native fauna, in Rainbow Trout compared with Atlantic Salmon (Steer and Lyle, 2003; Abrantes *et al.*, 2010; Abrantes *et al.*, 2011).

Between the mid-1800s and the early 1990s there were several unsuccessful attempts to introduce Atlantic Salmon into Tasmania. The only instances of Atlantic Salmon successfully being introduced outside of their natural range is in a few landlocked areas of New Zealand and Argentina. As such, there would appear to be a low probability that escapees could establish self-sustaining populations in Tasmania. On the other hand, Rainbow Trout were introduced into Tasmania in 1898 and are found throughout the state. Self-sustaining populations are common, although they are not as successful a coloniser as Brown Trout. To the best of our knowledge, Rainbow Trout populations have not established in any of the lower west coast rivers and although it is possible that escapees could colonise, they do not appear to have done so to date.

Potential impacts of marine farming operations on the Maugean Skate population

Maugean Skate display a clear depth preference for waters of 5–15 m depth, which tends to limit overlap with marine farming as most leases are located in areas of deeper waters (>20 m). Furthermore, as noted above we found no evidence of pellet feeding suggesting that direct interactions with marine farming operations are likely to be limited. However, there may be indirect or secondary interactions or consequences of the farming operations. For instance, it is possible that uneaten food pellets are consumed by benthic crustaceans (or their prey), which in turn become prey of the skate. In addition, the introduction of nutrients due to fish farming operations can affect water quality through the production of organic wastes that increase biological oxygen demand, reducing DO levels as well as enriching the pelagic environment through the excretion of dissolved nutrients (*e.g.* ammonium and nitrate). Between 2009 and mid-2014 there was a clear downward trend in the dissolved oxygen levels of the deep-waters (>15 m) of Macquarie Harbour (MHDOWG, 2014), which corresponded with the major expansion of salmonid aquaculture in the harbour as well as a period of historically low river flow. As such causal attribution for this decline is uncertain. The significant recharge event in late July 2014 did, however, result in an increase in bottom DO to 2009–2011 levels (Aquadynamic Solutions, 2015; DPIPWE, 2015a). Low DO concentrations in the deeper waters of Macquarie Harbour are a natural phenomenon due to the high level of stratification, whereby the heavier marine layer does not come into contact with the surface and circulation or exchange of the deep marine waters is limited. Ultimately factors such as river flow, storm events and nutrient enrichment from human activities play a role in influencing trends in DO concentrations both temporally and spatially.

The influence of DO on effective habitat range is unknown, but it is reasonable to assume that any significant declines in DO will reduce the area of suitable habitat available to the species. Linked to this, there is also uncertainty regarding the osmoregulatory capabilities of Maugean Skate. Their ability to tolerate low salinities could be very important should DO decline in their preferred depths. Evidence herein suggests they tend to spend relatively short amounts of time in depths above the halocline, possibly indicating a limit to their osmoregulatory ability and long periods in fresh or low salinity waters may result in significant metabolic stress.

The environmental health of Macquarie Harbour, in particular DO levels of the bottom waters, is likely to represent a crucial factor in the future well-being of the Maugean Skate population. The aquaculture industry along with other human activities impacting on Macquarie Harbour (mining, hydro-electricity generation [river flows], coastal development) all play a role in shaping the environmental conditions of this unique system.

Strategies to reduce Maugean Skate interactions with gillnetting

Anecdotal reports from fishers with a long history of fishing in Macquarie Harbour confirm that although not targeted, Maugean Skate have been caught quite regularly in gillnets and occasionally by line fishing activities since at least the early 1900s. On occasions skate have been retained for human consumption. Despite the unknown magnitude and impacts of this historic by-catch on the skate population, the species remains relatively abundant in Macquarie Harbour, implying that previous fishing mortality did not significantly impact population viability. This conclusion is, however, made in the absence of any indicators of current population size relative to historic population levels.

Although there are several management options that could be implemented, singularly or in combination, to reduce the likelihood of fishery interactions with Maugean Skate, only a prohibition on gillnetting and line fishing will completely eliminate interactions. Given that such a ban is not likely in the short-term, the challenge for management is, therefore, to balance the risk of interactions and their consequences against the size and productivity of the population to ensure that any incidental fishing mortality does not exceed the rate at which the population is able to regenerate.

The present and previous study (Lyle *et al.*, 2014), demonstrate that Maugean Skate can be caught readily in gillnets. A survey of recreational gillnet fishers also indicated that ‘skate/rays’, almost certainly Maugean Skate, were an occasional by-catch of netting in Macquarie Harbour (Lyle and Tracey, 2012). It should be noted, however, that the relatively high catch rates achieved by Lyle *et al.*, (2014) and in the present study are largely the result of targeted effort in the 5–15 m depth range in where Maugean Skate are most common. In this regard our studies do not represent normal recreational fishing practices. For example, recreational fishers tend to set nets close to shore in relatively shallow water in the belief that salmonids tend to hug the shoreline (confirmed by the tracking in this study). Similarly, when targeting flounder, gillnets are set on shallow sand flats. In general, experienced fishers will avoid deeper waters to avoid catching Whitespotted Dogfish, which are occasionally encountered in large numbers and they roll up the net, reducing its efficiency and sometimes damaging it. Nonetheless, we are aware of firsthand reports from recreational fishers involving the by-catch of Maugean Skate, which are occasionally captured in large numbers (>10 in a single net). Furthermore, we have observed recreational gillnets set in areas where Maugean Skate are abundant, particularly in the Table Head and Liberty Point regions. In some of these areas it is impossible to avoid waters >5 m with a 50 m gillnet. Recreational gillnets are also often set overnight, increasing the potential to catch skate in shallow water.

As a general rule, gillnet captures involve the skate’s long snout and associated spines becoming lightly entangled in the gillnet meshes, with the vast majority of individuals still alive and healthy, even after an overnight deployment. Most mortalities that the research team have experienced appear to be the result of the restrained individual being unable to escape predators rather than from suffocation or net damage. For instance, in the present study a number of the skate captured at Table Head showed evidence of sea lice damage, despite very short soak times (~2 h). The injuries sustained included tissue damage on anal fins and cloacal region and included the claspers of males with some minor damage to other regions of the dorsal surface. All of the individuals were still relatively lively and deemed to have a relatively high likelihood of survival so were released. In a previous study (Lyle *et al.*, 2014), again at Table Head but in an overnight deployment, several skate had been attacked by lice, one of which was clearly moribund, the lice having breached the body cavity. Lyle *et al.* (2014) also reported a skate mortality attributed to predation by Whitespotted Dogfish. It is almost certain, therefore, that there will be some level of incidental mortality associated with recreational gillnetting, especially given the long soak durations in this fishery.

Assuming that recreational gillnetting will continue in Tasmania, there are a number of options to minimise the likelihood of capturing Maugean Skate and to maximise post release survival. These include:

- *Spatial management*; given Maugean Skate have relatively small home ranges, spatial closures will afford a proportion of the population reasonable protection. An obvious area to consider spatial management measures is in the upper reaches of the harbour, in the World Heritage

Area, where gillnetting is less common. However, Maugean Skate do not appear to be particularly abundant in this part of the harbour and thus such a measure is likely to protect only a small proportion of the population. Possibly greatest protection would be afforded if areas within the lower reaches of the harbour, in particular the Table Head/Liberty Point, were closed to gillnetting. This is an area where recreational gillnetting is common due to its relatively close proximity to access points and because it is sheltered from the prevailing south-westerly winds. It is also close to aquaculture farms where fishers believe they have a higher likelihood of catching escapees.

- *Soak times*; in all other areas of Tasmania a maximum soak time of six hours applies to gillnets, with no provision for overnight sets for recreational fishers. In this regard Macquarie Harbour is treated differently and overnight netting is permitted. Given that day length varies so greatly in Tasmania, gillnets can legally be left unattended for up to 17 hours during winter. Previous research (Lyle *et al.*, 2014) has demonstrated that such long soak times can impact the potential survival of Maugean Skate (and other by-catch). Implementation of reduced maximum soak time for gillnets has the potential to increase the survival of any skate by-catch, although as demonstrated in this study predation (lice) can occur in sets involving short soak times.
- *Depth controls*; as Maugean Skate have a clear preference for waters deeper than ~5 m it would be possible to minimise interactions by preventing or reducing fishing effort in the deeper waters of the harbour. This would certainly reduce interactions and have a secondary benefit of minimising the likelihood of sea lice damage as it is unlikely that the lice would be able to tolerate the lower salinities in the shallows. However, any skate caught in shallow waters will be subject to the prevailing environmental conditions and the physiological implications of extended exposure to low salinities and increased variability in temperatures are currently unknown.
- *Prohibition on overnight netting*; given Maugean Skate move into shallower water and are more active at night, an obvious way to reduce interactions would be to prevent overnight netting. This would, as occurred elsewhere in the state, considerably decrease fishing effort and therefore reduce the number of interactions. It would also mean that soak durations would be shorter and therefore increase potential for post release survival. Set lines were reduced to daylight sets only in the 2015 review of the Tasmanian Scalefish Fishery Management Plan, which may have some benefit to Maugean Skate, although we did not observe this fishing method being used while sampling indicating it is comparatively rarer than gillnetting.

Recognising the need to minimise the by-catch of Maugean Skate, gillnet regulations for Macquarie Harbour were revised in late 2015 as part of a broader review of the Tasmanian Scalefish Fishery Management Plan. Various options relevant to gillnetting in Macquarie Harbour were canvassed by DPIPW as part of the management review, these included a prohibition on overnight netting during winter (due to excessively long soak times) and an extension of the no-netting area in the south-east of the harbour to encompass all of the World Heritage Area. The West Coast Recreation Association (WCRA) provided an alternative proposal (Figure 40) informed by the current and previous study. Their proposal involved the closure of a larger area of the harbour that included areas of high skate abundance (Table Head/Liberty Point), while allowing gillnetting to continue in the shallow lower reaches where most fishing effort is focussed. The proposal also enabled gillnetting to continue in areas of the upper harbour where locals have established fishing camps. The DPIPW considered this proposal in light of the potential impact that it would have on the Maugean skate and subsequently recommended to the Minister for Primary Industries and Water that the WCRA's proposal would deliver significant conservation outcomes for the species. The Minister accepted the advice and new restrictions. Although the winter overnight netting ban was not implemented, overnight gillnet soak times have been reduced. These nets can now only be set one hour before sunset and must be removed one hour after sunrise (previously overnight nets could be set two hours before sunset and left until two hours after sunrise before being retrieved). These changes are anticipated to reduce the likelihood of Maugean Skate interactions with gillnets whilst enabling the targeting of escapees with the benefit of removing them from the environment. It was considered beneficial by DPIPW that the major

management strategy to be implemented was suggested by a stakeholder group. This means that there has been far greater acceptance of the changes that include the closure of gillnetting in the areas where Maugean Skate appear to be most abundant (Table Head/Liberty Point).

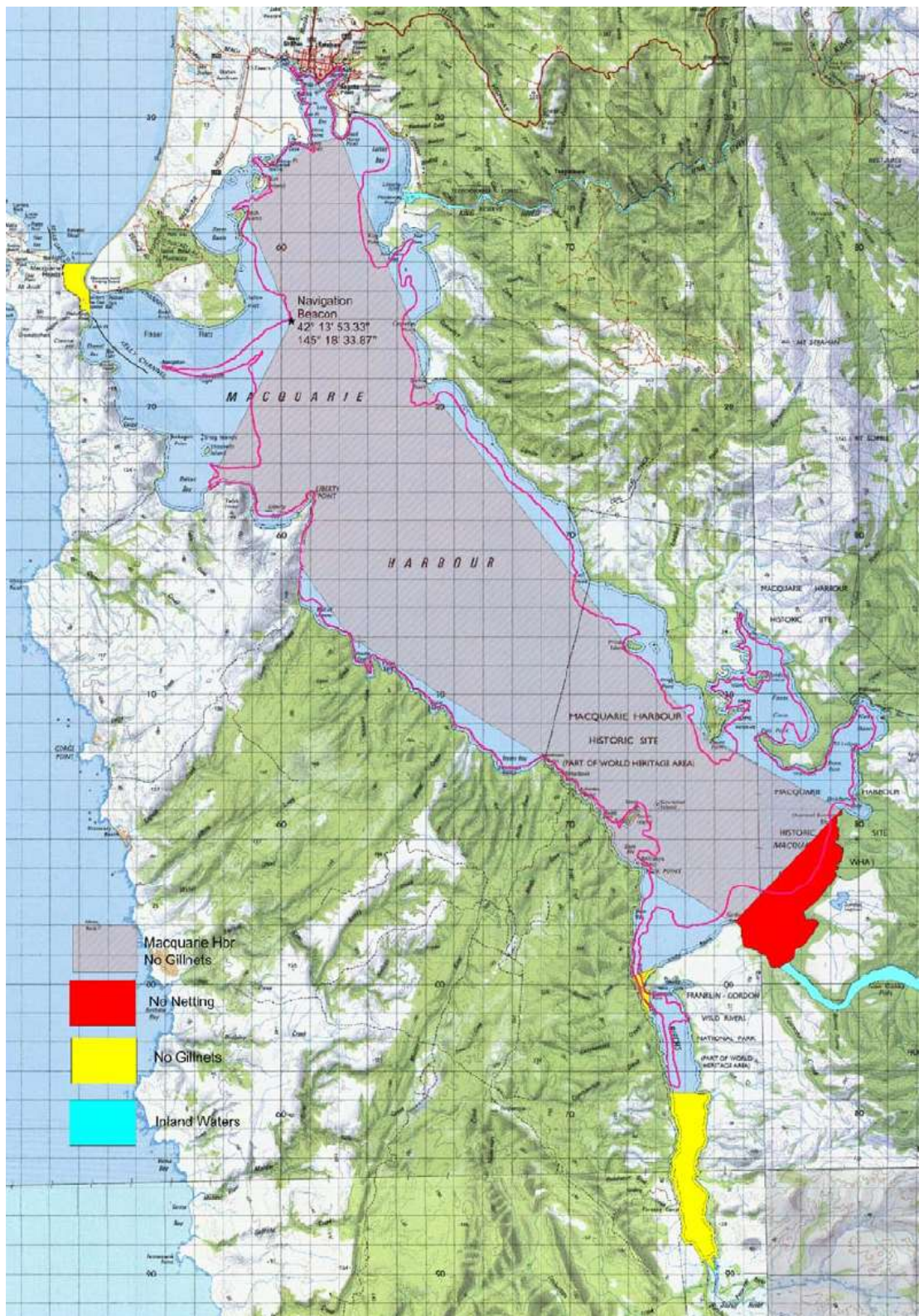


Figure 40: Management changes (indicated in grey and yellow) to recreational gillnet usage implemented in November 2015.

The 5 m contour is depicted in red. Source: DPIPWE (2015b).

Conclusion

The Maugean Skate population in Macquarie Harbour is probably one of the smallest of any chondrichthyan species. The species is only known from Macquarie Harbour and Bathurst Harbour, western Tasmania. Their preferred habitat within Macquarie Harbour and the small size of Bathurst Harbour means they probably have one of the smallest distributions of any chondrichthyan. The best estimate of the population size in Macquarie Harbour was in the order of 3200 individuals. There are, however, potential biases in this assessment that suggest it may be an underestimate and thus a feasible minimum population size.

Although Maugean Skate were detected in depths ranging from 0.6–55 m, 95% of detections were in <15 m and thus in depths and habitats shallower than most of the marine farm leases in Macquarie Harbour. The species displayed a high degree of site fidelity with most individuals remaining within the general region that they were tagged for most of the study period. Individual skate tended to have specific home ranges which they only occasionally left, often returning to the same general area after absences of a few days or weeks. This behaviour suggests that the species may have advanced spatial memory and cognitive capabilities. There was, however, no obvious pattern to these temporary excursions with the timing and behaviour varying between individuals. A number of individuals did relocate to alternative areas during the study, and in particular to the lower reaches of the harbour, an area with very sparse receiver coverage. Although several individuals were detected at Macquarie Heads all were re-detected inside the harbour shortly afterwards, providing no evidence to support long-term movement out of the harbour. None of the skate were detected at the Gordon River mouth suggesting they do not venture into the main tributary feeding into the harbour.

Most of the Maugean Skate exhibited diurnal patterns in depth utilisation and activity levels, being more active and moving into shallower water at night. This behaviour was assumed to be linked to nocturnal foraging.

Maturation occurred at larger sizes in females (between about 660 and 680 mm TL) than males (~620 mm TL). Maugean Skate are oviparous, with an asynchronous discontinuous reproductive cycle in which a proportion of the adult females are reproductively active at a given time while the remainder are inactive. The frequency of egg laying and duration of the active phase are unknown so it has not been possible to estimate fecundity. No females with *in utero* egg capsules were observed.

A number of hatched Maugean Skate egg capsules were collected incidentally, all taken from relatively deep water (>20 m). It is possible, therefore, that Maugean Skate lay at least some eggs in the deeper areas of the harbour, possibly seeking stable physio-chemical conditions. Due to various anthropogenic influences, DO tends to be very low in deeper waters and the impact on embryo development and survival is unknown.

Preliminary estimates of age and growth from sectioned vertebrae for thirteen Maugean Skate suggest that the species is relatively short lived (maximum age observed of 11 years) and probably lives for 15+ years. Maximum age (and size) is a useful proxy for productivity and our results suggest that Maugean Skate are probably relatively productive.

Maugean Skate have a restricted diet dominated by three groups of epibenthic crustaceans, namely crabs, carid shrimp and mysids. Fish represented a minor prey item. While there was no evidence of pellet feeding, this cannot be ruled out since sampling was conducted some distance away from the farm lease sites and skate tend to have small home ranges.

Atlantic Salmon and Rainbow Trout dispersed rapidly upon release, moving widely and generally randomly throughout Macquarie Harbour. Several Rainbow Trout and a single Atlantic Salmon exhibited affinities for regions near aquaculture leases suggesting at least some of these individuals were feeding on aquaculture overfeed. The vast majority did not, however, survive or remain in the harbour for longer than about two months following release. As a general observation, Rainbow Trout

tended to survive slightly longer than Atlantic Salmon. While about a quarter of the salmonids were recaptured by recreational fishers (in gillnets), most are assumed to have died of natural causes (starvation and possibly predation). Some fish did, however, leave the harbour moving into the sea (mainly Atlantic Salmon) or into the Gordon River (exclusively Rainbow Trout).

Maugean Skate display a clear depth preference for waters of 5–15 m depth, which tends to limit overlap with marine farming as most leases are located in areas of deeper waters (>20 m). Furthermore, the present study found no evidence of pellet feeding suggesting that direct interactions with marine farming operations are likely to be limited. However, there may be indirect or secondary interactions or consequences of farming operations. For instance, it is possible that some uneaten food pellets are consumed by benthic crustaceans (or their prey), which in turn become prey of the skate. In addition, the introduction of nutrients due to fish farming operations can affect water quality through the production of organic wastes that increase biological oxygen demand, reducing DO levels as well as enrichment of the pelagic environment through the excretion of dissolved nutrients (e.g. ammonium and nitrate). In fact between 2009 and mid-2014 there was a clear downward trend in the dissolved oxygen levels of the deep-waters (>15m) of Macquarie Harbour. This period of decline corresponded with a major expansion of salmon aquaculture in the harbour as well as historically low river flow and thus causal attribution for this decline is uncertain. The significant recharge event in late July 2014 did, however, result in an increase in bottom DO to 2009–2011 levels.

Despite low oxygen levels in deeper waters, Maugean Skate do occasionally spend time in depths >20 m, suggesting an ability to cope with DO concentrations lower than those typically recorded in their preferred habitat (i.e. > ~30%). The influence of DO on effective habitat range is unknown, but it is reasonable to assume that the species is sensitive to reduced DO and any significant declines in DO will reduce the area of suitable habitat available them.

The osmoregulatory capabilities of Maugean Skate are also unknown. Their ability to tolerate low salinities could be very important should DO decline dramatically in their preferred depths. Evidence herein suggests they tend to spend relatively short amounts of time in depths above the halocline, indicating that they are probably not truly euryhaline. Longer periods in fresh or low salinity waters may cause metabolic stress and potentially mortality.

Maugean Skate are a relatively common by-catch in gillnets, although recreational gillnet catch rates are expected to be substantially lower than those experienced in the present study. Recreational fishers tend to set their nets close to shore in relatively shallow water to target salmonids or on the shallow sand flats in the lower reaches of the estuary to target flounder. The majority of the Maugean Skate captured in gillnets are likely to be alive and thus can be released in healthy condition. Occasionally, however, mortalities occur, mainly due to predation, but possibly also due to prolonged exposure to unfavourable environmental conditions (low salinity and/or variable temperatures) whilst restrained in the net.

Although there are several management options that could be implemented, singularly or in combination, to reduce the likelihood of fishery interactions with Maugean Skate, only a prohibition on gillnetting will effectively eliminate interactions. Given that such a ban is not likely in the short-term, management needs to balance the risk of interactions and their consequences against the size and productivity of the population in order to ensure that incidental fishing mortality does not exceed the rate at which the population is able to regenerate. From November 2015, a variety of changes specifically aimed at reducing the impacts of fishing on Maugean Skate populations were implemented. These include a closed area to gillnet use that encompasses most of the deeper waters (>5 m) of Macquarie Harbour in addition to Table Head and Liberty Point, areas the this study identified as having high abundances of Maugean Skate. Furthermore maximum soak durations for overnight sets have been reduced. Overall these management changes are anticipated to reduce (but not eliminate) the likelihood of Maugean Skate interactions with gillnets whilst enabling fishers to continue to target escapees and flounder.

Implications

Management of the aquaculture industry

Direct interactions between Maugean Skate and aquaculture operations appear to be limited. The aquaculture industry expansion strategy in Macquarie Harbour involves the location of new lease sites into the deeper regions of Macquarie Harbour, which given the Maugean Skate's preference for shallower depths means that there is minimal overlap between core skate habitat and the marine farm lease sites. Furthermore, this study provided no evidence of feeding on fish pellet overfeed by the skate. It is not possible, however, to completely discount this occurring, noting that skate are capable of moving throughout the entire harbour, including into the deepest areas.

There may, however, be indirect interactions, for instance the production of organic wastes associated with marine farming operations increase biological oxygen demand and act to reduce DO as well as enriching of the pelagic environment through the excretion of dissolved nutrients (*e.g.* ammonium and nitrate). The influence of bottom DO in determining suitable skate habitat is uncertain but it is highly likely that any reductions in bottom DO, regardless of cause, will negatively influence the area of core habitat (preferred depths). Conversely, increases in DO at depth may support an expansion of available habitat. While the short-term movement of skate into deeper water immediately following a major recharge event tends to support this hypothesis, it was not the only explanation. It is possible that the movement was a response to the physical disturbance in the shallower waters as a result of the storm or salinity declined to intolerable levels in their preferred depth range. Although elevated levels of bottom DO persisted for many months following the recharge event many of the skate moved back into the shallower depths within a couple of weeks of the recharge event.

The environmental health of Macquarie Harbour, in particular levels of DO in the bottom waters, is likely to represent a crucial factor in the future well-being of the Maugean Skate population. The aquaculture industry along with other human activities impacting on Macquarie Harbour (mining, hydro-electricity generation [river flows], coastal development) all play a role in shaping the environmental conditions of this unique system. The maintenance of best environmental practices by the aquaculture industry supported by effective monitoring and environmental management policies represent essential requirements if industry and Maugean Skate populations are to coexist in perpetuity.

Management of the recreational gillnet fishery

As a listed endangered species Maugean Skate are protected under both State and Commonwealth legislation and thus there is a statutory obligation to minimise negative impacts on the species. Maugean Skate are a relatively common by-catch in gillnets and although the majority of those captured are likely to survive when released there is, nonetheless, a risk of mortalities or sub-lethal impacts arising from capture (for example capture stress can have long term effects on reproduction, growth etc.).

There are several management options that could be implemented to reduce the likelihood of fishery interactions with Maugean Skate but only a prohibition on gillnetting will completely eliminate these interactions. Given that a ban is unlikely in the short to medium term, management must balance the risk of interactions occurring, and their consequences, against the size and productivity of the population to ensure that fishing induced mortality does not exceed the rate at which the population is able to regenerate. A number of changes specifically aimed at reducing the impacts of fishing on Maugean Skate populations were implemented in late 2015. Of these, limiting gillnetting mainly to waters shallower than 5 m and closure of areas around Table Head and Liberty Point are anticipated to reduce (but not eliminate) the likelihood of Maugean Skate interactions with gillnets.

Management of escapees

Salmonids that escape from the aquaculture pens do not survive/remain in Macquarie Harbour for long periods, with no evidence to suggest that escapees are effective as predators of native fauna or to establish wild populations.

The rapid dispersal of fish away from the release site suggests that attempts to recapture escapees in large numbers close to the farm from which the escape event occurred is likely to have limited success. However, recreational gillnet fishing pressure appears to be relatively high and this study found that about one in four escapees were recaptured by recreational fishers within about two months of escape. Thus, while commercial fish-downs may be of some value immediately following a major escape event, recreational gillnet activity is likely to play an important role thereafter in reducing escapee numbers.

Recommendations

The present study has substantially enhanced our understanding of the life history, behaviour and status of the Maugean Skate population in Macquarie Harbour. It has also highlighted a number of potential threats and areas requiring further research to more fully assess and manage these threats.

Environmental conditions, in particular DO but perhaps also salinity, along with the availability of key prey species, are likely to play crucial roles in determining habitat suitability for Maugean Skate. The physio-chemical conditions in Macquarie Harbour have changed markedly since European settlement, influenced by effluent from mine tailings, changed river flows as a result of damming the tributaries for hydro-electric production, and increased nutrient load arising from marine farming operations. The gradual decline in DO experienced within Macquarie Harbour between 2009 and 2014 is likely to have had a significant impact on the ecology of many resident species, including the Maugean Skate. As the consequences of these environmental changes on the availability (and extent) of suitable habitat is unknown it is not possible to determine whether the current depth distribution favoured by Maugean Skate is representative of that prior to human disturbance. Changes in bottom DO are highly likely to influence the distribution of the skate given its poor ability to cope with low DO and thus better understanding the oxygen demands of the Maugean Skate is of particular interest, as is the distribution of their prey. A directed study of these factors would provide essential insights into how the species is likely to be impacted by any changes in DO. The physiological work contained in this report while preliminary in nature, represents a major step forward in our understanding of the physiology of this unique species. There is, therefore, a need to further this understanding, including the use of more complex and longer term aquaria/laboratory studies, combined with field studies employing current generation acoustic tags capable of measuring *in situ* oxygen levels and activity at these levels. The distribution of key prey, particularly crabs, could be assessed using field surveys (i.e. traps, remotely operated underwater vehicles along with *in situ* DO measurement) and tolerance to varying levels of DO and salinity determined by laboratory experimentation.

The depths at which Maugean Skate deposit eggs is uncertain, however, the observation that at least some skate eggs are deposited in depths greater than about 20 m suggests they could be exposed to low DO concentrations. Developing embryos are unable to move away from unfavourable conditions and are therefore forced to rely on coping mechanisms rather than avoidance. An understanding of the relationships between environmental conditions and development and survival of embryos, coupled with the depths in which eggs are deposited has relevance in assessing the implications of environmental changes on the productivity of the population and should represent a priority in terms of further research.

The osmoregulatory capabilities of Maugean Skate are unknown but have relevance since the species does enter low salinity (fresh) waters in the shallows. Chondrichthyans osmoregulate by adjusting blood urea concentration in order to prevent excessive loss, or uptake, of ions (Hyodo *et al.* 2007). This strategy is predominantly designed to prevent hyper-osmotic stress (Hyodo *et al.* 2007). Whether Maugean Skate are able to physiologically adapt to low salinity environments or whether they begin losing ions (sodium and chloride) to the environment when in low salinity conditions has not been studied. In itself, this is not necessarily problematic, however, low DO in the deeper waters mean that Maugean Skate could be increasingly exposed to lower salinities. The ability of the species to tolerate low salinities also has implications for skate captured in gillnets set in shallow waters. Once entangled in the net, individuals could be held in low salinity conditions for extended periods, which may affect survival.

The present study and the recently completed gillnetting study (Lyle *et al.*, 2014) provide valuable baseline information about the population structure (size and sex composition, catch rates) of the Maugean Skate. Periodic gillnet surveys, utilising the same sampling strategy, should be considered as an option to monitor the future status of this species, especially given the rate and extent of environmental change in Macquarie Harbour.

Extension and Adoption

The current project has been disseminated to resource managers, industry recreational fishers and the broader public in the following ways:

- An article was published in the December 2013 Australian Society for Fish Biology Newsletter, volume 43(2), pages 44–45. This article briefly described the reasons the project is being undertaken and the methods to be employed to achieve the project objectives.
- An article was published in the December 2013 TARFish Bulletin. This article briefly outlined the project, methods and objectives.
- An article was published in the February/March 2014 edition of Fishing Today: Tasmanian Fishing and Boating News volume 27 (1), page 25. The article outlined the project objectives and detailed what we have learned about Maugean skate from previous studies (i.e. FRDC 2010/016) and progress to date with the current project.
- The PI has presented preliminary results of the project to staff of the Marine Farming, Wild Fisheries and Biodiversity Conservation Branches of DPIPWE (27 August 2014), Tassal environmental division (29 August 2014), and the University of Tasmania's Animal Ethics Committee (10 October 2014).
- The PI presented preliminary findings at the West Coast Community forum on 25 February 2015.
- Key results were presented to the FRDC Board on 14 April 2015 at its meeting in Hobart.
- Key results were presented to aquaculture industry representatives and the CEO of the Tasmanian Salmonid Growers Association on the 9 July 2015.
- Progress has been discussed with the project steering committee on three separate occasions, the final Steering Committee was held on 6 August 2015.
- The PI presented the key results of the study to the National Estuaries Network symposium in Hobart on 19 November 2015.

The results have informed the 2015 review of the Scalefish Fishery Management Plan and have particular relevance to on-going issues surrounding DO in Macquarie Harbour.

Project coverage

On the 28/08/2013, Hobart newspaper 'The Mercury' reported that the present study was to take place and described the key aims and objectives (Figure 41).



Figure 41: Newspaper article describing the present study.

Appendices 1: Range testing

The present study scored a range testing ranking of 40 out of a possible 49 on the scale proposed by Kessel *et al.*, (2014) indicating that we can be confident of the results found herein. Detection range was reasonably good throughout the harbour with 50 and 90% detection probability occurring at 405 m (SE = 22 m) and 262 m (SE = 38 m) respectively (Figure A1.1). Detections were still being received at >1000 m at times and some of the sentinel tags were detected almost 2000 m from a receiver; however, at these distances, the probability of detection was low. There was some spatial and temporal variation in detection range; however, there was no significant trend with any variable other than distance (Table A1.1). Sentinel tags further support this with detection fluctuating temporally but not noticeably seasonally (Figure A1.2). As such, all analyses were completed using a non-variable detection range and using a detection probability of 405 m. The distance at 50% detection range was selected as skate are a fairly immobile animal and therefore unlikely to move quickly in an out of detection range as could fast moving species.

Several sentinel tags did have relatively poor detection efficiency (Figure A1.2). In particular, the sentinel associated with the receiver at the Gordon River mouth (GR01) had a low frequency of detection, probably because high river flow prevented the sound waves travelling against the current despite the receiver and tag being separated by only 200 m. The detection efficiency of the sentinels at LP01 and LP07 followed similar trends and were particularly variable from when they were put in place until around April 2014 and then again from October onward until the end of the study. These receivers, and sentinels, were in reasonably shallow water (LP01 hung from marine farm infrastructure and LP07 at 7 m). Water chemistry at these depths is highly variable and the sound may not penetrate the halocline efficiently. The LP01 sentinel was also located near a barge and other marine farming infrastructure, and was also located 360 m from the receiver. There was a brief decline in the detection efficiency at most sites in late July 2014, which was associated with the recharge event. There was increased river flow at this time but there was also very heavy rainfall, the noise from which may have been the causative factor.

On each drift undertaken at Macquarie Heads the test tag was detected by the receiver on the jetty, even from distances >400 m. This suggests that it was unlikely that any animals could have left Macquarie Harbour without being detected.

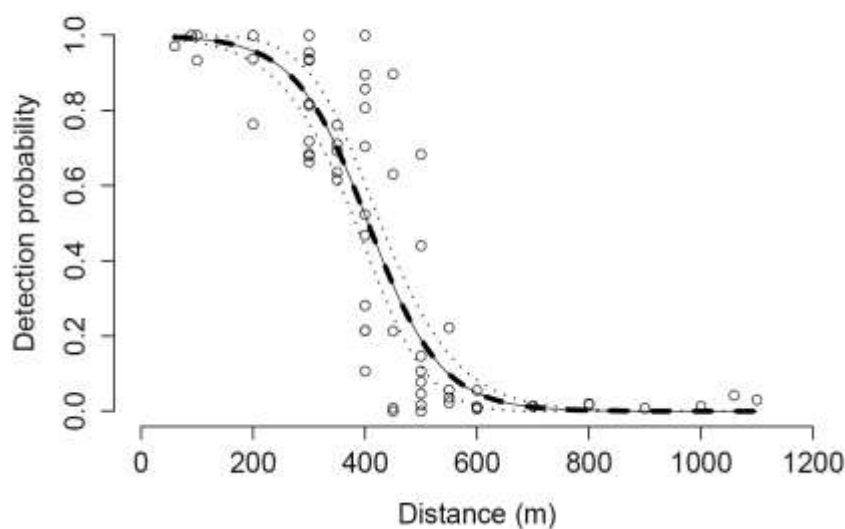


Figure A1.1: Detection probability with distance.

Table A1.1: Logistic regression of variation in acoustic receiver detection probability with distance, date and location within Macquarie Harbour.

All regions are compared to Kelly's Basin as this was the region that had the shortest detections range.

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	4.923	1.813	2.715	0.007**
Distance	-0.015	0.004	-3.395	0.001***
Date	0.006	0.004	1.734	0.083
Liberty Point	0.998	1.222	0.817	0.414
Long Bay	0.896	1.698	0.527	0.598
Pine Cove	2.440	1.318	1.851	0.064.
Swan Basin	1.200	0.996	1.205	0.229
Table Head	1.339	1.114	1.202	0.229

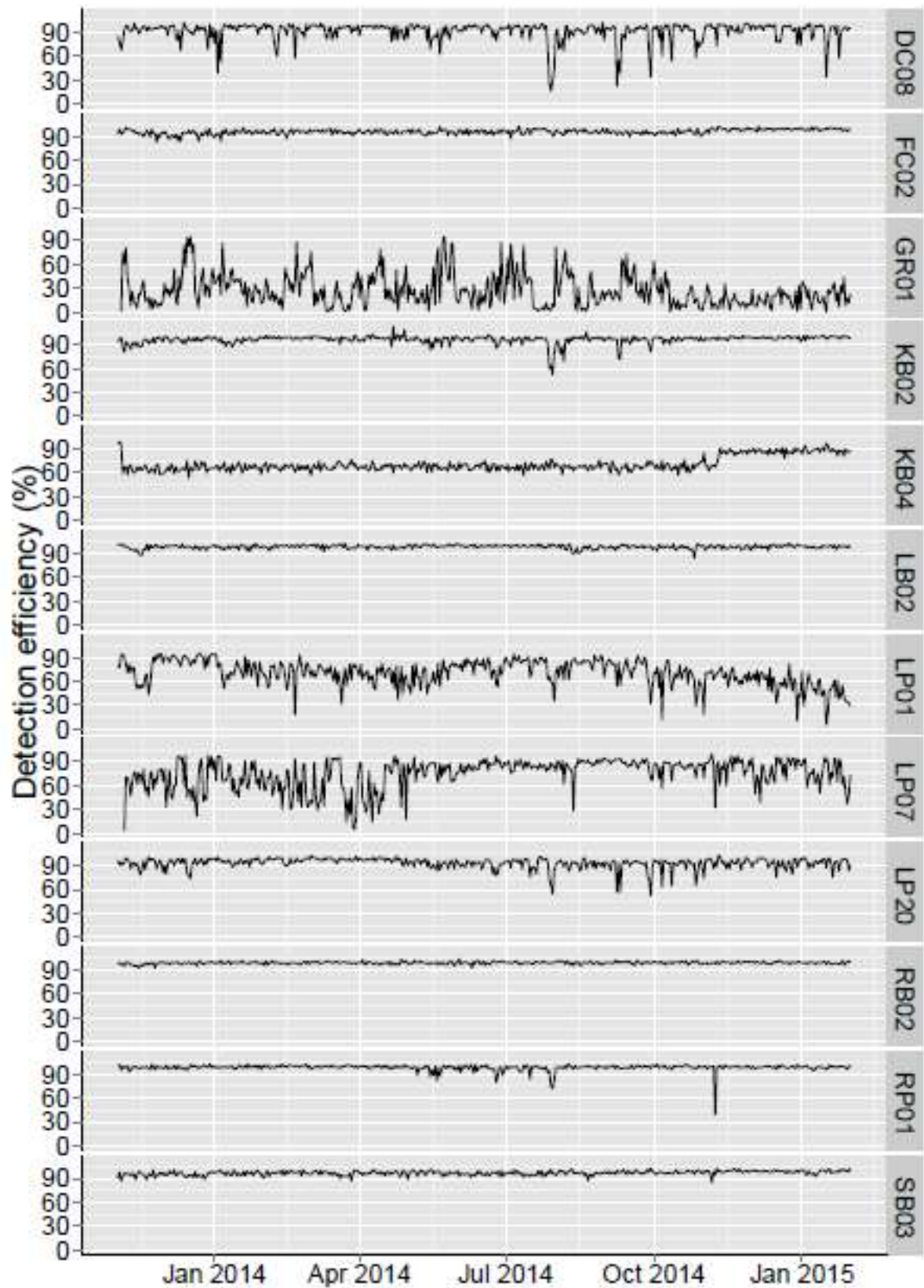


Figure A1.2: Detection efficiency throughout the study by the sentinel tags located throughout Macquarie Harbour.

Appendix 2: Individual depth utilisation

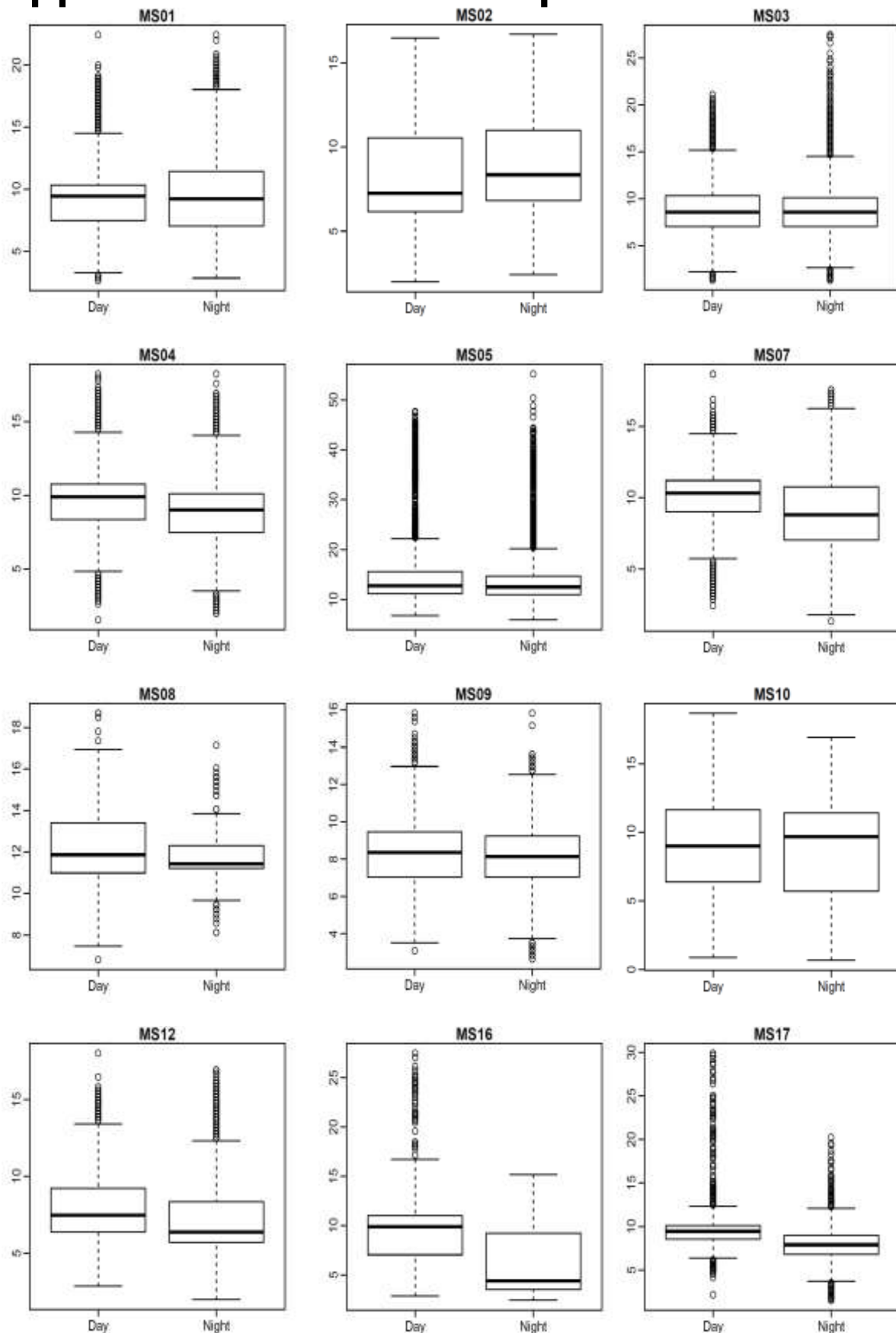


Figure A2.1: Diurnal depth utilisation of individual Maugean Skate throughout the duration of the study.

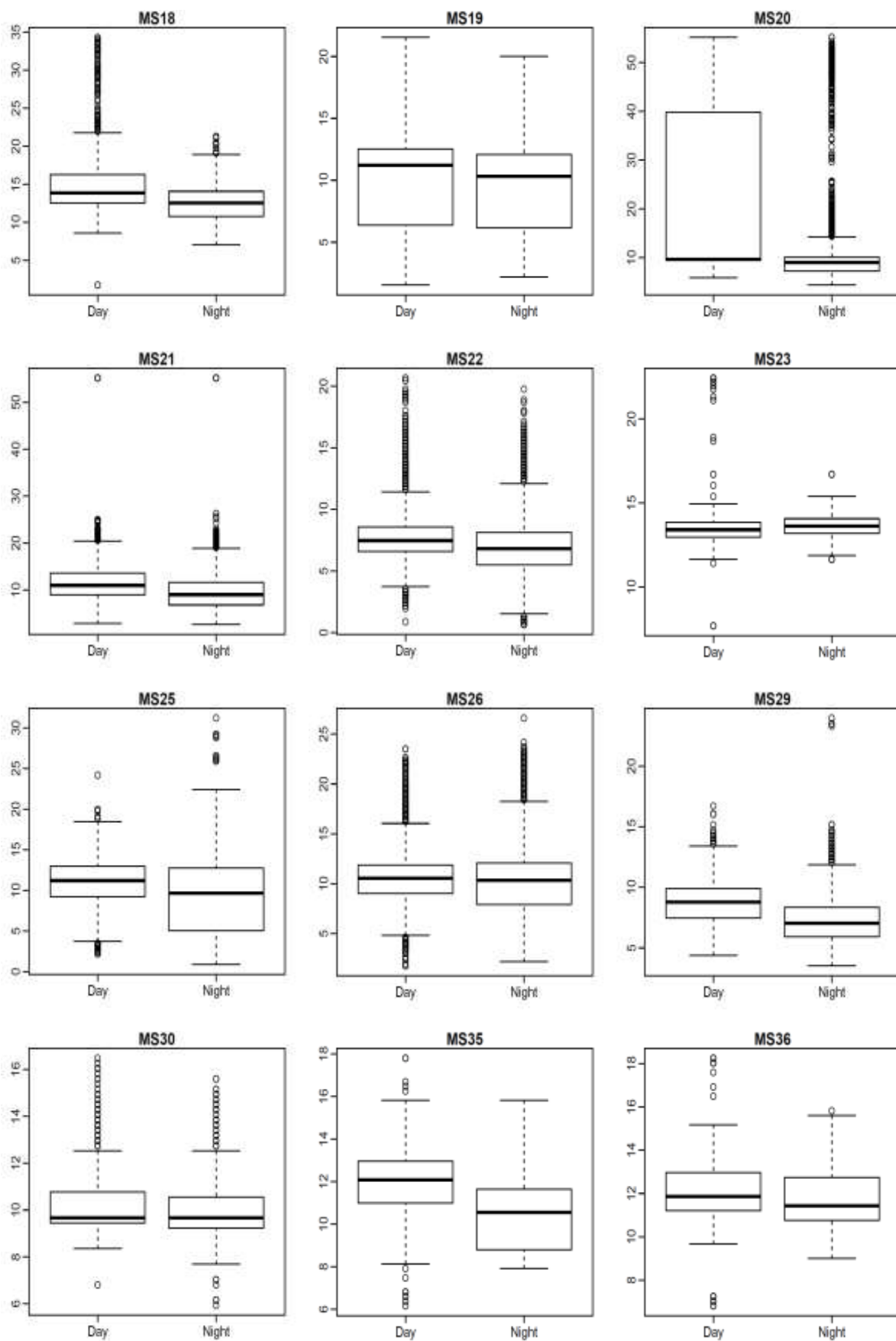


Figure A2.1 continued: Diurnal depth utilisation of individual Maugean Skate throughout the duration of the study.

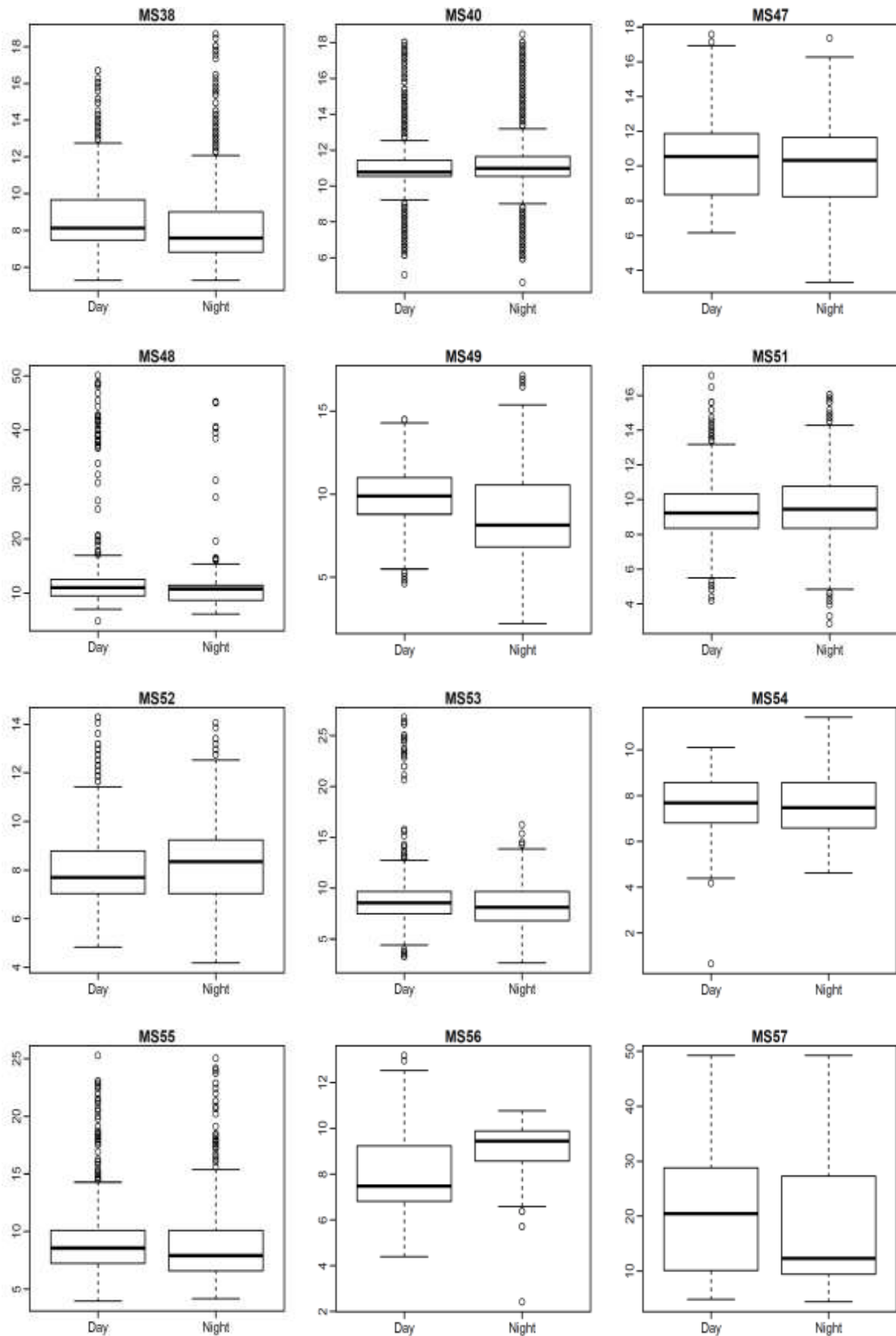


Figure A2.1 continued: Diurnal depth utilisation of individual Maugean Skate throughout the duration of the study.

Appendix 3: Project staff

University of Tasmania

Institute for Marine and Antarctic Studies

Dr Jeremy Lyle

Dr Justin Bell

Assoc Prof Jayson Semmens

Dr Jeff Ross

Dr Neville Barrett

David Moreno

Kay Weltz

Edward Forbes

Graeme Ewing

Jaime McAllister

School of Biological Sciences

Dr Cynthia Awruch

Mount Allison University, Canada

Suzie Currie

Dalhousie University, Canada

Andrea Morash

Appendix 4: References

- Abrantes, K.G., Semmens, J.M., Lyle, J.M., and Nichols, P.D. (2010) Can biochemical methods determine if salmonids feed and thrive after escaping from aquaculture cages? A pilot study. NRM Cradle Coast Project CCCPR24006. Report by the Tasmanian Aquaculture and Fisheries Institute.
- Abrantes, K.G., Lyle, J.M., Nichols, P.D., and Semmens, J.M. (2011) Do exotic salmonids feed on native fauna after escaping from aquaculture cages in Tasmania, Australia? *Canadian Journal of Fisheries and Aquatic Sciences* **68**(9), 1539-1551.
- Andrews, K.S., Williams, G.D., Farrer, D., Tolimieri, N., Harvey, C.J., Bargmann, G., and Levina, P.S. (2009) Diel activity patterns of sixgill sharks, *Hexanchus griseus*: the ups and downs of an apex predator. *Animal Behaviour* **78**, 525–536.
- Anon (2014) Australian fisheries and aquaculture statistics 2013. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra.
- Aquadynamic Solutions (2015) MHDOWG Update Report (Draft) April 2015. Aquadynamic Solutions SDN BHD.
- Baillargeon, S., and Rivest, L.P. (2007) Rcapture: loglinear models for capture-recapture in R. *Journal of Statistical Software* **19**(5), 1-31.
- Barbibi, S.A., Scenna, L.B., Figueroa, D.E., Cousseau, M.B., and Diaz de Astarloa, J.M. (2009) Feeding habits of the Magellan skate: effects of size, sex, maturity stage and body size on diet. *Hydrobiologia* **641**, 275-286.
- Bivand, R., and Lewin-Koh, N. (2015) mapproj: Tools for reading and handling spatial objects. R package version 0.8-30.
- Bizarro, J.J., Robinson, H.J., Rinewalt, C.S., and Ebert, D.A. (2007) Comparative feeding ecology of four sympatric skate species off central California, USA. *Environmental Biology of Fishes* **80**(2-3), 197–220.
- Burgman, M.A., and Fox, J.C. (2003) Bias in species range estimates from minimum convex polygons: implications for conservation and options for improved planning. *Animal Conservation* **6**(1), 19–28.
- Bustamante, C., Vargas-Carlo, C., Oddone, M.C., Concha, F., Flores, H., Lamilla, J., and Bennett, M.B. (2012) Reproductive biology of *Zearaja chilensis* (Chondrichthyes: Rajidae) in the south-east Pacific Ocean. *Journal of Fish Biology* **80**, 1213-1226.
- Callard, I.P., St. George, J., and Koob, T.J. (2005) Endocrine control of the female reproductive system. In *Reproductive Biology and Phylogeny of the Chondrichthyes*. (Ed. WC Hamlett) pp. 283-300. (Science Publishers, INC: Enfield, New Hampshire)
- Carlson, A.E., Hoffmayer, E.R., Tribuzio, C.A., and Sulikowski, J.A. (2014) The use of satellite tags to redefine movement patterns of spiny dogfish (*Squalus acanthias*) along the U.S. east coast: implications for fisheries management. *PLoS ONE* **9**(7).
- Carpenter, P., Butler, E., Higgins, H., Mackey, D., and Nichols, P. (1991) Chemistry of trace elements, humic substances and sedimentary organic matter in Macquarie Harbour, Tasmania. *Marine and Freshwater Research* **42**(6), 625-654.

- Colonello, J.C., García, M.L., Lasta, C.A., and Menni, R.C. (2012) Reproductive biology of the spotback skate *Atlantoraja castelnaui* in the south-west Atlantic Ocean. *Journal of Fish Biology* **80**(7), 2405-2419.
- Cox, D.L., and Koob, T.J. (1993) Predation on elasmobranch eggs. *Environmental Biology of Fishes* **38**, 117-125.
- Creswell, G.R., Edwards, R.J., and Barker, B.A. (1989) Macquarie Harbour, Tasmania - seasonal oceanographic surveys in 1985. *Papers and Proceedings of the Royal Society of Tasmania* **123**, 63-66.
- Davy, L.E., Simpfendorfer, C.A., and Heupel, M.R. (2015) Movement patterns and habitat use of juvenile mangrove whiprays (*Himantura granulata*). *Marine and Freshwater Research* **66**(6), 481-492.
- DPIPWE (2015a) Macquarie harbour status report. Internal report prepared by the Department of Primary Industries, Parks, Water and Environment, Tasmania.
- DPIPWE (2015b) Scalefish Fishery Management Plan: final report to the minister on the remake of the Scalefish Fishery Management Plan. Department of Primary Industries, Parks, Water and the Environment.
- Dulvy, N.K., and Forrest, R.E. (2010) Life histories, population dynamics, and extinction risks in chondrichthyans. In *Sharks and their relatives II: biodiversity, adaptive physiology, and conservation*. (Eds. JC Carrier, JA Musick and MR Heithaus) pp. 653-659. (CRC Press)
- Ebert, D.A. (2005) Reproductive biology of skates, *Bathyraja* (Ishiyama), along the eastern Bering Sea continental slope. *Journal of Fish Biology* **66**, 618-649.
- Ebert, D.A., and Bizzarro, J.J. (2007) Standardised diet and trophic level of skates (Chondrichthyes: Rajiformes: Rajoidei). *Environmental Biology of Fishes* **80**(2-3), 221-237.
- Edgar, G.J., Barrett, N.S., and Last, P.R. (1999) The distribution of macroinvertebrates and fishes in Tasmanian estuaries. *Journal of Biogeography* **26**(6), 1169-1189.
- Fenton, G.E. (1985) Ecology and taxonomy of Mysids. PhD Thesis, University of Tasmania,
- Francis, M.P. (1997) A summary of biology and commercial landings, and a stock assessment of rough and smooth skates (*Raja nasuta* and *R. innominata*). New Zealand Fisheries Assessment Research Document 1997/5, National Institute of Water and Atmospheric Research, Wellington, New Zealand.
- Francis, M.P., Ó Maolagáin, C., and Stevens, D. (2001) Age, growth, maturity, and mortality of rough and smooth skates (*Dipturus nasutus* and *D. innominatus*). New Zealand Fishery Assessment Report 2001/17, National Institute of Water and Atmospheric Research, Wellington, New Zealand.
- Francis, M.P., and Gallagher, M.J. (2009) Revised age and growth estimates for Antarctic starry skate (*Amblyraja georgiana*) from the Ross Sea. *CCAMLR Science* **16**, 211-220.
- Gledhill, D.C., and Last, P.R. (2005) *Zearaja maugeana*. The IUCN Red List of Threatened Species. Version 2015.2. www.iucnredlist.org. Downloaded on 26 June 2015.
- Hamlett, W.C., and Koob, T.J. (1999) Female reproductive system. In *Sharks, skates and rays: the biology of elasmobranch fishes*. (Ed. WC Hamlett) pp. 388-443. (Johns Hopkins University Press: Baltimore)

Henderson, A.C., Arkhipkin, A.I., and Chtcherbich, J.N. (2004) Distribution, growth and reproduction of the white-spotted skate *Bathyraja albomaculata* (Norman, 1937) around the Falkland Islands. *Journal of the Northwest Atlantic Fishery Science* **35**, 79-87.

Heupel, M.R., Semmens, J.M., and Hobday, A.J. (2006) Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. *Marine and Freshwater Research* **57**, 1-13.

Hoff, G.R. (2009) Skate *Bathyraja* spp. egg predation in the eastern Bering Sea. *Journal of Fish Biology* **74**, 250–269.

Hoffmayer, E.R., Franks, J.S., Driggers, W.B., and Howey, P.W. (2013) Diel vertical movements of a scalloped hammerhead, *Sphyrna lewini*, in the Northern Gulf of Mexico. *Bulleting of Marine Science* **89**(2), 551–557.

Holden, M.J., and Vince, M.R. (1973) Age validation studies on the centra of *Raja clavata* using tetracycline. *Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer* **35**(1), 13-17.

Holden, M.J. (1975) The fecundity of *Raja clavata* in British waters. *Journal du Conseil* **36**, 110-118.

Hueter, R.E., Heupel, M.R., Heist, E.J., and Keeney, D.B. (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of the Northwest Atlantic Fishery Science* **35**, 239–247.

Hyodo, S., Bell, J.D., Healy, J.M., Kaneko, T., Hasegawa, S., Takei, Y., Donald, J.A., and Toop, T. (2007) Osmoregulation in elephant fish *Callorhynchus milii* (Holocephali), with special reference to the rectal gland. *Journal of Experimental Biology* **210**, 1303-1310

Kessel, S.T., Cooke, S.J., Heupel, M.R., Hussey, N.E., Simpfendorfer, C.A., Vagle, S., and Fisk, A.T. (2014) A review of detection range testing in aquatic passive acoustic telemetry studies. *Reviews in Fish Biology and Fisheries* **24**, 199-218.

King, J.R., and McFarlane, G.A. (2010) Movement patterns and growth estimates of big skate (*Raja binoculata*) based on tag-recapture data. *Fisheries Research* **101**, 50-59.

Kranstauber, B., and Smolla, M. (2014) move: visualizing and analyzing animal track data. R package version 1.2.475.

Laake, J. (2013) RMark: an R interface for analysis of capture-recapture data with MARK. AFSC processed rep 2013-01, 25p. 2.1.13 edn. (Alaskan Fisheries Science Centre, NOAA, National Marine Fisheries Service: 7600 Sand Point Way NE, Seattle WA 98115)

Last, P.R., and Yearsley, G.K. (2002) Zoogeography and relationships of Australasian skates (Chondrichthyes: Rajidae). *Journal of Biogeography* **29**, 1627–1641.

Last, P.R., and Gledhill, D.C. (2007) The Maugean Skate, *Zearaja maugeana* sp. nov. (Rajiformes: Rajidae) — a micro-endemic, Gondwanan relict from Tasmanian estuaries. *Zootaxa* **1494**, 45-65.

Licandeo, R.R., Lamilla, J.G., Rubilar, P.G., and Vega, R.M. (2006) Age, growth, and sexual maturity of the yellownose skate *Dipturus chilensis* in the south-eastern Pacific. *Journal of Fish Biology* **68**, 488–506.

Lucifora, L.O., Valero, J.L., Bremec, C.S., and Lasta, M.L. (2000) Feeding habits and prey selection by the skate *Dipturus chilensis* (Elasmobranchii: Rajidae) from the south-western Atlantic. *Journal of the Marine Biological Association of the United Kingdom* **80**(5), 953-954.

- Lucifora, L.O., and Garcia, V.B. (2004) Gastropod predation on egg cases of skates (Chondrichthyes, Rajidae) in the southwestern Atlantic: quantification and life history implications. *Marine Biology* **145**, 917–922.
- Lyle, J.M., and Tracey, S.R. (2012) Recreational gillnetting in Tasmania - an evaluation of fishing practices and catch and effort. Institute for Marine and Antarctic Studies, University of Tasmania.
- Lyle, J.M., Bell, J.D., Chuwen, B.M., Barrett, N., Tracey, S.R., and Buxton, C. (2014) Assessing the impacts of gillnetting in Tasmania: implications for by-catch and biodiversity. Final Report for the Fisheries Research and Development Corporation, project 2010/16, FRDC, Canberra.
- Matta, M.E., and Gunderson, D.R. (2007) Age, growth, maturity, and mortality of the Alaska skate, *Bathyraja parmifera*, in the eastern Bering Sea. *Environmental Biology of Fishes* **80**(2), 309-323.
- MHDOWG (2014) Macquarie Harbour Dissolved Oxygen Working Group Report October 2014. 63 pp.
- Morison, A.K., Robertson, S.G., and Smith, D.G. (1998) An integrated system for production fish aging: image analysis and quality assurance. *North American Journal of Fisheries Management* **18**, 587-598.
- Nasby-Lucas, N., Dewar, H., Lam, C.H., Goldman, K.J., and Domeier, M.L. (2009) White shark offshore habitat: a behavioral and environmental characterization of the eastern Pacific shared offshore foraging area. *PLoS ONE* **4**(12), e8163.
- Nelson, G.A., and Ross, M.R. (1995) Gastric evacuation in little skate. *Journal of Fish Biology* **46**, 977–986.
- Nielson, R.M., Sawyer, H., and McDonald, T.L. (2013) BBMM: Brownian bridge movement model. R package version 3.0.
- Otis, D.L., Burnham, K.P., White, G.C., and Anderson, D.R. (1978) Statistical inference from capture data on closed animal populations. (Wiley Online on behalf of the Wildlife Society) 134 pp
- Papastamatiou, Y.P., Cartamil, D.P., Lowe, C.G., Meyer, C.G., Wetherbee, B.M., and Holland, K.M. (2011) Scales of orientation, directed walks and movement path structure in sharks. *Journal of Animal Ecology* **80**, 864-874.
- Parent, S., Pépin, S., Genet, J.-P., Misserey, L., and Rojas, S. (2008) Captive breeding of the barndoor skate (*Dipturus laevis*) at the Montreal Biodome, with comparison notes on two other captive-bred skate species. *Zoo Biology* **27**(2), 145-153.
- Peklova, I., E., H.N., Hedges, K.J., Treble, M.A., and Fisk, A.T. (2014) Movement, depth and temperature preferences of an important bycatch species, Arctic skate *Amblyraja hyperborea*, in Cumberland Sound, Canadian Arctic. *Endangered Species Research* **23**, 229-240.
- Pinkas, L. (1971) Food habits of albacore, bluefin tuna, and bonito in California waters. *Fish Bulletin* **152**, 5-10.
- Schluessel, V., and Bleckmann, H. (2005) Spatial memory and orientation strategies in the elasmobranch *Potamotrygon motoro*. *Journal of Comparative Physiology A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **191**, 695-706.
- Simpfendorfer, C.A., Heupel, M.R., and Hueter, R.E. (2002) Estimation of short-term centers of activity from an array of omnidirectional hydrophones and its use in studying animal movements. *Canadian Journal of Fisheries and Aquatic Sciences* **59**(1), 23-32.

- Sims, D.W., Southall, E.J., Tarling, G.A., and Metcalfe, J.D. (2005) Habitat-specific normal and reverse diel vertical migration in the plankton-feeding basking shark. *Journal of Animal Ecology* **74**, 755–761.
- Steer, M., and Lyle, J. (2003) Monitoring escapees in Macquarie harbour: a collaborative study between the salmon industry (TSGA) and the Tasmanian Aquaculture and Fisheries Institute (TAFI) Report by the Tasmanian Aquaculture and Fisheries Institute.
- Sulikowski, J.A., Morin, M.D., Suk, S.H., and Howell, W.H. (2002) Age and growth estimates of the winter skate (*Leucoraja ocellata*) in the western Gulf of Maine. *Fisheries Bulletin* **101**, 405-413.
- Takagi, T., Kajimura, M., Tanaka, H., Hasegawa, K., Bell, J.D., Toop, T., Donald, J.A., and Hyodo, S. (2014) Urea-based osmoregulation in the developing embryo of oviparous cartilaginous fish (*Callorhynchus milii*): contribution of the extraembryonic yolk sac during the early developmental period. *Journal of Experimental Biology* **217**, 1353-1362.
- Treloar, M., Barrett, N., and Edgar, G. (2013) Biology and ecology of the endangered Maugean skate (*Zearaja maugeana*). Report to the Winifred Violet Scott Charitable Trust, Institute for Marine and Antarctic Studies, Hobart.
- Valdimarsson, A.K., and Metcalfe, N.B. (1999) Effect of time of day, time of year, and life history strategy on time budgeting in juvenile Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 2397-2403.
- Walker, T.M., and Poore, G.C.B. (2003) Rediagnosis of Palaemon and differentiation of southern Australian species (Crustacea: Decapoda: Palaemonidae). *Memoirs of Museum Victoria* **60**(2), 243-256.
- Ware, J. (1908) *Tasmanias Eldorado: Strahan and Macquarie Harbour*. (John Ware: Strahan, Tasmania)
- Wearmouth, V.J., and Sims, D.W. (2009) Movement and behaviour patterns of the critically endangered common skate *Dipturus batis* revealed by electronic tagging. *Journal of Experimental Marine Biology and Ecology* **380**, 77-87.
- Weng, K., and Block, B.A. (2004) Diel vertical migration of the bigeye thresher shark (*Alopias superciliosus*), a species possessing orbital retia mirabilia. *Fisheries Bulletin* **102**, 221–229.
- Weng, K.C., Boustany, A.M., Pyle, P., Anderson, S.D., Brown, A., and Block, B.A. (2007) Migration and habitat of white sharks (*Carcharodon carcharias*) in the eastern Pacific Ocean. *Marine Biology* **152**(4), 877–894.
- White, G.C., and Burnham, K.P. (1999) Program MARK: survival estimates from populations of marked animals. *Bird Study* **46**(supp), S120-139.
- Young, N.D., Crosbie, P.B.B., Adams, M.B., Nowak, B.F., and Morrison, R.N. (2007) *Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*). *International Journal for Parasitology* **37**(13), 1469-1481.