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Vulnerability of the endangered Maugean Skate population to degraded environmental conditions in Macquarie Harbour

David Moreno, Jeremy Lyle, Jayson Semmens, Andrea Morash,
Kilian Stehfest, Jaime McAllister, Bailee Bowen and Neville Barrett

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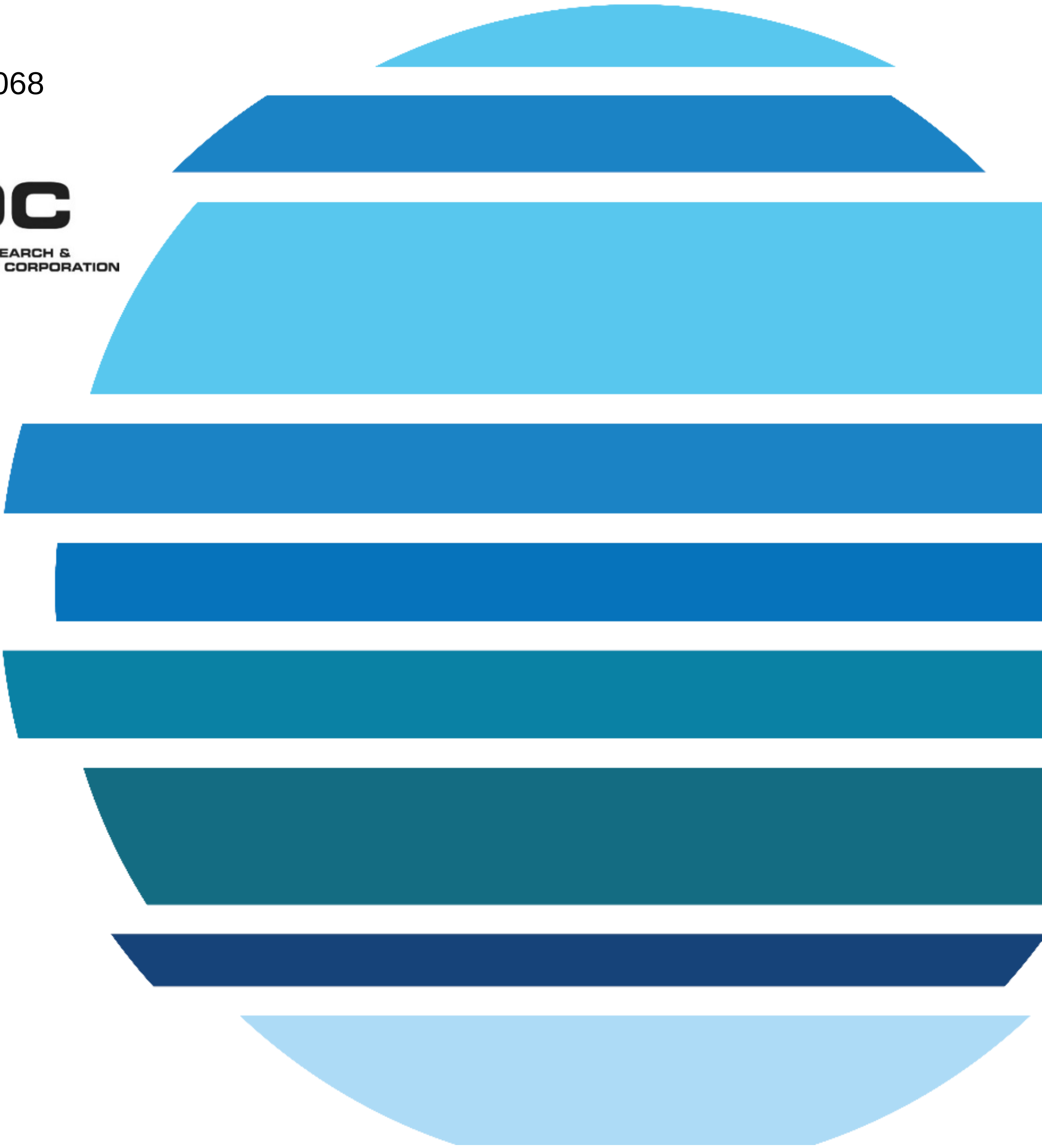

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Abbreviations

AIC	Akaike information criterion
AME	Assumed mortality event
ANOVA	Analysis of variance
COA	Centre of activity
DPIPWE	Department of Primary Industries, Parks, Water and Environment
DO	Dissolved oxygen
EPA	Environment Protection Authority (Tasmania)
EPBC Act	Environmental Protection and Biodiversity Conservation Act (Commonwealth)
Hb	Haemoglobin
Hct	Haematocrit
HIF	Hypoxia inducible factor
MO_2	Metabolic rate
KUD	Kernel utilisation distribution
N_2	Nitrogen
O_2	Oxygen
OU	Ornstein-Uhlenbeck process
OUF	Ornstein-Uhlenbeck Foraging process
PO_2	Partial pressure of oxygen
RBC	Red blood cells
TL	Total length
TW	Body weight
UD	Utilisation distribution

Executive Summary

Maugean Skate (*Zearaja maugeana*) are only known from two isolated estuarine systems located on the west coast of Tasmania, representing one of most restricted distributions of any elasmobranch. There is, however, uncertainty about the continued persistence of the species in one of these estuaries (Bathurst Harbour), suggesting that Macquarie Harbour may now represent the sole remaining habitat for the species. The species is listed as Endangered under the Threatened Species Protection Act (Tas) and the Environmental Protection and Biodiversity Conservation Act (Comm) and, apart from protected status, is without a recovery plan or management strategy.

The physicochemical conditions in Macquarie Harbour have changed markedly since European settlement, influenced by anthropogenic activities in and around the estuary (e.g. mining, forestry, hydro-electricity generation, and marine farming operations) as well as the more general effects of climate change. Of recent concern, has been a marked decline in dissolved oxygen (DO) conditions in Macquarie Harbour which are likely to have a significant impact on many resident species, including the Maugean Skate. In the absence of population dispersal or mixing outside of Macquarie Harbour, any longer-term changes to DO conditions in the harbour are likely to result in changes to ecological assemblages inhabiting this unique estuarine system. The current study describes the vulnerability of the Maugean Skate, across all its life history stages, to a range of environmental stressors. The ability to monitor population status and population responses of the Maugean Skate to any changes in environmental conditions will be critical in assessing the need for further conservation management action.

Objectives

The primary aim of this study is to establish the mechanistic links between the environmental conditions experienced by the Maugean Skate across multiple life stages and the implications of the changing environment in Macquarie Harbour. Specific objectives include:

1. Determine the distribution of Maugean Skate eggs within Macquarie Harbour, with particular reference to depth and bottom DO.
2. Examine the relationship between environmental conditions and survival and viability of Maugean Skate eggs.
3. Determine what DO levels Maugean Skate experience in Macquarie Harbour, particularly when in deeper waters.
4. Determine the physiological costs of the DO levels Maugean Skate are exposed to in Macquarie Harbour.
5. Assess the potential of using the size composition of Maugean Skate catches as an indicator of population change, in particular recruitment variability
6. Assess the implications of declining DO concentrations in Macquarie Harbour on the future viability of the Maugean Skate population.

Methodology

The study comprised five main components: (1) systematic field surveys of skate egg distribution, (2) laboratory study of embryo development; (3) field assessment of skate behaviour in relation to environmental conditions; (4) laboratory assessment of physiological responses to conditions experienced in the wild; and (5) fishery-independent surveys to examine the potential to inform on changes to population status.

A combination of beam trawl and dive surveys was employed to sample for Maugean Skate egg cases in areas of known high skate abundance. Egg capsules collected in the field were reared in the laboratory to describe embryonic development and gestation period. Acoustic telemetry was used to examine links between environmental conditions (i.e. dissolved oxygen and temperature) and the distribution, movement and habitat use of the Maugean Skate within Macquarie Harbour. An acoustic array comprising 52 acoustic receivers was deployed in the Table Head/Liberty Point region of Macquarie Harbour and 25 adult Maugean Skate were externally tagged with multi-sensor acoustic tags capable of continuously transmitting information on the depth, temperature and dissolved oxygen concentrations experienced by the individuals over a 12-month period. Physiological trials were conducted in a purpose-designed field laboratory to examine physiological responses to low DO and low salinity exposure, replicating environmental conditions experienced by the skate in Macquarie Harbour. Gillnet surveys were undertaken to extend the time series of Maugean Skate size composition data and evaluate its potential as an indicator of changing population status.

Key findings

Maugean Skate eggs were found across a wide range of depths (2.5 - 33 m) but appear to be most abundant in relatively shallow sites (< 10 m). Based on an analysis of the condition of egg-capsules, hatching success was estimated to be about 40%, which is comparable to success rates reported for other temperate skate species. An egg reared under laboratory conditions hatched 31 weeks (~ seven months) after oviposition. Respiratory channels opened after 15 weeks, exposing the embryo to external environmental conditions for over half of the gestation period. These findings indicate that, depending on the depths at which eggs are deposited in the wild, embryos will experience a range of dissolved oxygen, salinity and temperature conditions, reflective of localised fluctuations in environmental conditions. The implications of these varying conditions for development and survival are unknown.

This study represents the first use of animal-borne acoustic sensors to monitor long-term dissolved oxygen conditions experienced by a coastal elasmobranch and the links with behaviour. The results showed that skate are subject to wide ranging fluctuations in water chemistry. For instance oxygen levels experienced ranged from normoxic (50-100%), hypoxic (20-50%) and to near anoxic (~0-20%), with individuals often experiencing substantial variation (as high as > 90%) in the range of DO levels within the same day.

Adult Maugean Skate exhibited a high level of site selectivity and site fidelity across a relatively small spatial extent and with a strong preference to depths between 7.5 and 12.5 m. Despite individual variation in long-term residency, noting that several individuals appeared to permanently exit the study area, the tagged skate showed a clear preference towards the Table Head and Liberty Point areas. Furthermore, home ranges calculated using continuous time movement models showed a high degree of spatial overlap in the core home ranges (50% utilisation distribution, UD) of each of the individuals. Their extended activity areas (95% UD) did, however, change throughout the year corresponding to variation in environmental conditions. For instance, a sharp increase in surface water temperature and declines in mid- and deep water dissolved oxygen levels during summer resulted in all detected individuals spending time at shallow depths before returning to their preferred depths. While the shallow sites provided access to improved oxygen conditions, higher temperatures and exposure to hyposaline conditions will place an increased metabolic demand on individuals, potentially driving them to continuously move back into deeper waters where temperature and salinity conditions are more stable. A large storm surge and subsequent oxygen recharge in March/April 2019 resulted in all tracked individuals expanding their extended activity areas considerably, including movement into deeper waters, while still maintaining a strong attachment to their core areas.

The present study shows that there is an intricate link between movement of the Maugean Skate and environmental conditions in Macquarie Harbour. The species appear to behaviourally modulate environmental stressors through movement, likely balancing optimal habitat requirements against the energetic cost of chronic hypoxia in deeper waters and increased thermal and osmoregulatory stress at shallow sites. Such behavioural plasticity may constitute a key adaptation that to date has enabled the species to tolerate such a challenging environment that Macquarie Harbour represents. However, since individuals continued to utilise the same core UD and preferential depth range, despite these areas being subject to the largest daily fluctuations in water chemistry conditions experienced by the skate, this behavioural plasticity appears to be limited to their extended use areas. Therefore, the site attached behaviour of the skate suggest that these core sites likely provide an advantage that outweighs the cost of exposure to unfavourable environmental conditions, such as access to prey that may not be as readily available elsewhere within the estuary.

Physiological experiments demonstrated that adult Maugean Skate are quite capable of surviving chronic exposure to hypoxic conditions (< 20% DO) by using metabolic depression as a survival strategy. Metabolic depression does, however, occur at the cost of other energy intensive life history processes, such as growth, foraging and reproduction. As such, metabolic depression cannot be sustained long-term, and as a result skate may seek oxygen in the shallower waters of the harbour where they will encounter hyposaline conditions. Maugean Skate are unusual in that they are the only known species of skate to live exclusively in a euryhaline environment (areas with a wide range of salinities) and, based on the range of depths utilised, are constantly exposed to a wide range of salinities. Routine metabolic rate appeared to be higher following acclimation to hyposaline conditions, although the corresponding statistical test only showed near-significance. Since individuals appear to utilise shallow areas as a DO refuge during the summer months, they will also be exposed to higher temperatures which in turn will result in further metabolic stress.

Physiological data based on a single individual suggests that neonates are oxyconformers (rate of oxygen consumption dependent on environmental DO), implying that even at an early age, Maugean Skate may be able to tolerate short-term hypoxic events through an increased reliance on anaerobic metabolism. The point below which oxygen uptake became negligible in the neonate was higher (~ 66% DO) compared with adults (10-25% DO), implying a lower tolerance threshold which could have important implications for survivorship of early life stages, especially in relation to the range of environmental conditions experienced in Macquarie Harbour.

During this study, an unexpectedly high proportion of tagged individuals died. Although causality cannot be established, the timing of these events suggest that the mortalities were unlikely to have been a direct consequence of tagging. Most mortalities (8 out of 11) were clustered in two discrete periods that corresponded with marked changes in environmental conditions within the core habitat used by the skate. In addition, behavioural changes (based on change point analysis) were observed at the same time in virtually all surviving individuals. It is feasible, therefore, that the deaths may have been related to stress caused (directly or indirectly) by the significant changes in the environmental conditions of the harbour. If this is the case, then recent changes in the environmental health of the harbour (especially dissolved oxygen levels), coupled with the consequences of climate change (including occurrence of extreme weather events), may already be challenging the skate’s capacity to cope with the environmental conditions in Macquarie Harbour.

Deriving an estimate of the effective population size of the Maugean Skate in Macquarie Harbour has proven challenging and there is a high degree of uncertainty associated with current estimates that limit their applicability in monitoring the continuing status of the population. An analysis of research gillnet data collected since 2012 suggests a reduction in the relative abundance of juvenile and sub-adult individuals and as well as an increase in the size of the larger individuals (adults) in the catches through time. Despite uncertainty as to the significance of the size composition data in terms of representing trends in population status, a recent decline in recruitment, possibly due lower hatching success or juvenile survival, coupled with adult growth represent plausible scenarios that could produce the changes observed.

Implications

This study provides a greater understanding of the ecology and life history of the Maugean Skate and describes for the first time some of the behavioural and physiological adaptations that have enabled the species to survive in such a unique and challenging environment as Macquarie Harbour. Our results suggest that the species can mitigate some environment variability by a combination of behavioural (movement) responses and physiological capability. However, the vulnerability of early life stages to the changing environmental conditions, long-term changes in the size structure of the population, and the mortality of some tagged individuals following significant environmental events

collectively highlight the vulnerability of the Maugean Skate in Macquarie Harbour and the need to consider further conservation action to support the persistence of this unique micro-endemic skate.

In addition to addressing uncertainties around the apparent changes in the size (and age) structure of the population through further research fishing, there is an urgent need to investigate cost-effective and non-invasive approaches to estimate population size and mortality rates, which can be applied to track population status and health over time. Importantly, managing the known anthropogenic impacts on the environmental health of Macquarie Harbour, both in terms water column and benthic conditions, will ultimately prove crucial to the success of any conservation strategy for the Maugean Skate. A multi-stakeholder and holistic environmental management approach for Macquarie Harbour should be considered as part of this strategy.

Keywords

Maugean Skate, *Zearaja maugeana*, Macquarie Harbour, telemetry, physiology, hypoxia, environmental tolerance, embryo development

Introduction

Maugean Skate (*Zearaja maugeana*) are only known from Macquarie and Bathurst Harbours (western Tasmania), suggesting that the species has one of most restricted distributions of any elasmobranch. Based on its limited geographic range the species has been listed as endangered under the Threatened Species Protection Act (Tas), the EPBC Act (Comm) and is listed on the IUCN Red List (Last and Gledhill, 2007). According to the listings, the main potential threats to the species are heavy metal pollution from historic mining operations, incidental capture in fishing activities and the introduction of non-native marine species. More recently, however, the increased input of organic wastes associated with the major expansion of marine farming operations has been implicated as contributing to a general reduction in dissolved oxygen (DO) levels within Macquarie Harbour (MHDOWG, 2014). Apart from its protected status, the species lacks a recovery plan or management strategy.

The first comprehensive study of the Maugean Skate highlighted that the environmental health of Macquarie Harbour, in particular levels of dissolved oxygen (DO) in the bottom waters, is likely to represent a crucial factor in the future well-being of the skate population (Bell et al., 2016, FRDC Project 2013-008). Maugean Skate exhibit a strong preference for benthic habitats in the 5–15 m depth range, occasionally venturing into depths of greater than 40 m for short periods. Within their preferred depth range salinities typically range between 18–27 ppt and DO levels generally remain above 30%. Preliminary physiology experiments showed that the species has little ability to tolerate low DO, the skate’s only mechanism for coping with low DO (< 25%) is to change from an aerobic to an anaerobic metabolic pathway (Morash et al., 2020). 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 and hence a higher environmental DO.

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 (Bell et al., 2016). While no skate laying egg capsules have been observed to date, the only documented living skate egg capsule was found entangled in a gillnet set in ~30 m (Treloar et al., 2017). This observation and the incidental capture of empty egg capsules in depths of > 20 m (Bell et al., 2016) suggest that Maugean Skate lay eggs at depths outside of their preferred depth range. Treloar et al. (2017) hypothesised that the deposition of eggs in deeper water may be a strategy to reduce predation (outside the depth range of many potential predators) and provide more stable environmental conditions (especially temperature and salinity). However, the developing embryos could be exposed to low DO concentrations at these depths and being unable to move away from unfavourable conditions, would be forced to rely on coping mechanisms rather than avoidance. The environmental conditions experienced by the eggs will play a major role in influencing recruitment success and population viability.

The physicochemical conditions in Macquarie Harbour have changed markedly since European settlement and the general decline in DO since 2009 (MHDOWG 2014), which occurred at the same time as the rapid expansion of marine farming operations, is likely to have had an impact on many resident species, including the endangered Maugean Skate. Given the species limited ability to cope with long-term exposure to low environmental DO, any reductions in bottom DO concentrations are expected to directly influence the distribution of the skate, highlighting the need to better understand the oxygen demands of the species. Current generation acoustic tags capable of measuring DO and activity in the field, which when combined with laboratory studies, represent methods to examine tolerance and responses to varying levels of DO and other environmental conditions.

Benthic environmental conditions also have implications for the development and survival of skate eggs. Limited available information suggests that eggs are deposited at depths of > 20 m where they are likely to be increasingly exposed to low DO concentrations. An understanding of the relationships between environmental conditions and the development and survival of embryos, coupled with the depths in which eggs are deposited, has particular relevance to assessing the implications of changing environmental conditions on future recruitment and productivity of the Maugean Skate population.

Ultimately, the ability to monitor the status of this population, especially given the rate and extent of recent environmental changes in Macquarie Harbour, will be critical to assess its future viability and the potential need for further conservation management. Tracking size composition, including data from previous studies, has the potential to provide a baseline against which reduced recruitment (if linked to the changed environmental conditions) could be detected.

Objectives

The primary aim of this study is to establish the mechanistic links between the environmental conditions experienced by the Maugean Skate across multiple life stages and the implications of the changing environment in Macquarie Harbour. Specific objectives included:

1. Determine the distribution of Maugean Skate eggs within Macquarie Harbour, with particular reference to depth and bottom DO.
2. Examine the relationship between environmental conditions and survival and viability of Maugean Skate eggs.
3. Determine what DO levels Maugean Skate experience in Macquarie Harbour, particularly when in deeper waters.
4. Determine the physiological costs of the DO levels Maugean Skate are exposed to in Macquarie Harbour.
5. Assess the potential of using the size composition of Maugean Skate catches as an indicator of population change, in particular recruitment variability
6. Assess the implications of declining DO concentrations in Macquarie Harbour on the future viability of the Maugean Skate population.

To address these objectives a multidisciplinary approach comprising four major components was used:

- *Early life history*: Egg distribution and development (Objectives 1, 2 & 6)
- *Spatial ecology*: Environmental drivers for space use and movement (Objectives 3 & 6)
- *Eco-physiology*: Physiological responses to chronic exposure to hypoxia and hyposalinity (Objectives 4 & 6)
- *Population status*: Recent trends in population size structure (Objectives 5 & 6)

Methods

Early life history

Egg surveys

Beam trawl surveys

Benthic surveys were conducted using a 1.5 x 0.5 m research beam trawl fitted with a single ground chain and net with a 10 mm mesh liner. The gear was deployed from the side gantry of the vessel and towed at a speed of 1-2 knots using a warp length of approximately three times the water depth. Tow duration and direction were site dependent, but in general were intended to follow a line parallel to the bathymetry contours. Start and finish locations, depths and times were recorded for each tow. To ensure appropriate contact between the sled and the substrate, initial tows had a video camera (Hero 3, GoPro) and external light sources affixed to the frame of the beam trawl.

On recovery, the catch from each haul was removed from the net, sorted and egg capsules set aside. Empty egg capsules were retained and stored for transport back to the laboratory and subsequent examination. Live eggs were immediately placed in seawater and retained for the captive rearing experiments (see below). Other fauna present in the trawls was identified (to high level taxa classification) and recorded prior to release, the presence of substrate (mud, gravel, rocks) in the net was also noted.

Beam trawl surveys were conducted at roughly four-monthly intervals between February 2017 and July 2019. To maximise the likelihood of encountering eggs, sampling effort was largely focused around Table Head/Liberty Point and Swan Basin, two areas where adults are known to be abundant (Lyle et al., 2014, Bell et al., 2016, Treloar et al., 2017). However, an initial exploratory trawl survey in February 2017 also included sites within the World Heritage Area (upper reaches of the estuary).

Dive surveys

Three dive surveys for eggs were conducted using SCUBA between May 2018 and July 2019. The first dive survey was undertaken near the track of successful trawls that produced relatively high numbers of (empty) egg capsules in the Liberty Point area. Divers swam along a total of 24 parallel transects (100 m each placed perpendicular to the trawl tracks) conducting a visual check for eggs. Underwater visibility in Macquarie Harbour is limited by low-light conditions caused by the heavy tannin content in the surface water. This resulted in a low likelihood of divers spotting any eggs that did not precisely overlap with the transect. In subsequent dive surveys the use of fixed transects was relaxed in favour of longer dives following the natural topography, resulting in a broader search area, and allowing the divers to investigate features of interest. The primary goal of the dive surveys was to undertake targeted searches for live eggs.

Diver pairs were dropped at the beginning of a specific feature (e.g. a channel or a drop-off), where they descended to the bottom and started the search in tandem. Each diver carried a collection bag where egg capsules could be safely kept prior to being returned to the surface. When an egg was found, the diver deployed an inflatable marker and the surface vessel recorded the depth and location.

Egg case morphology, predation rates and hatching success

Egg case length (ECL, measured as the distance between the anterior and posterior aprons) and egg case width (ECW, measured along the lateral plane and including the keels) were measured with callipers to the nearest mm.

The condition of the hatching slit and damage to the egg cases was examined to determine hatching success in the empty egg capsules. Eggs were considered to have successfully hatched when the case had a large slit along the anterior apron and there were no clear signs of predation damage.

Empty egg cases with an un-opened hatching slit were assessed for damage signalling predation. For example, small gastropods that prey on skate eggs elsewhere have been shown to produce distinctive boreholes (Lucifora and Garcia, 2004). It is unclear what species may prey on Maugean Skate eggs in Macquarie Harbour, so each egg was categorised based on the type of damage to help identify minimum predation rates and potential predator types. Empty egg capsules with no visible signs of external damage (predation) were classified as non-predated. Closed and empty egg cases occur when non-developing embryos (either unfertilised or due to an early death) get washed out after the respiratory slits in the egg become unblocked (Smith and Griffiths, 1997).

Egg cases too heavily eroded due to prolonged exposure to the environment were counted but excluded from the viability and predation assessment.

Captive egg rearing

Live Maugean Skate eggs collected in Macquarie Harbour were retained and transported to the laboratory for captive rearing. An egg was considered alive when it had an intact yolk mass in the capsule or if a developing embryo was visible.

Finding live eggs proved to be a greater logistical challenge than anticipated, therefore, in order to maximise the use of the small number of live egg samples collected, it was decided to forego the environmental manipulation experiments in favour of a simpler controlled rearing program to help fill in critical knowledge gaps including gestation period and embryonic development of the species.

Live egg samples were collected from November 2017 to March 2019 during beam trawl and dive surveys, and opportunistically from wild caught females that laid eggs while held captive during the adult physiology trials (May 2019, see below). Eggs found during the field surveys were placed in seawater in an aerated watertight container on the vessel for transport back to land. Once on land the container was kept in a climate-controlled room with continuous aeration and daily water exchanges until the end of the sampling trip. The eggs were then transported under these conditions to the aquarium facility at the Institute for Marine and Antarctic Studies (IMAS), Taroona. Seawater used in the field, initial holding period and during transport was drawn from Macquarie Harbour at a depth of approximately 10 m.

In the laboratory, eggs were placed in a 25 L tank fed by a flow-through system providing continuous filtration and temperature control. Temperature and salinity in the tanks were maintained at levels approximating the average environmental conditions in Macquarie Harbour at depths ~10 m. Rearing conditions were: normoxic oxygen concentrations (80-100% DO), salinity of 31-34 ppt and water temperatures between 12-14 °C (except for the first egg collected, which was kept in a system that utilised ambient temperature water). The tanks were covered to limit light exposure of the embryos to simulate the limited photoperiod in the harbour. Conditions were monitored three times a week using submersible conductivity and optical DO probes connected to an HQD meter (HQ40D, Hach Company, Loveland, CO).

For the first egg collected, macrophotography of the developing embryo was undertaken weekly using transmitted light (candling). Following the death this embryo due to unknown causes after 10 weeks, it was decided to limit handling of the eggs to a minimum. Subsequently, eggs were examined at three-week intervals for viability via candling using a submersible light. The developmental stage of the embryos was broadly classified using the seven stages of oviparous elasmobranch development proposed by Musa et al. (2018).

Spatial ecology

Acoustic telemetry was used to examine the mid and long-term links between environmental conditions (i.e. dissolved oxygen and temperature) and the distribution, movement and habitat use of the Maugean Skate within Macquarie Harbour. This form of passive tracking involved an array of omnidirectional hydrophones (VR2W, Innovasea, Canada) arranged in a grid-like pattern at approximately equidistant intervals (~700 m apart), which detect and record data transmitted from nearby individually coded tags. The acoustic array comprising 52 receivers was centred on the Table Head/ Liberty Point area, where Maugean Skate are known to be abundant (Bell et al., 2016), and encompassed several marine farm lease sites (Fig. 1). The study area covered a range of depths from 0-55 m and included the deepest areas in the harbour (central basin) as well as several active salmonid aquaculture leases. This design was chosen to maximise detectability of the skate based on data from previous tracking experiments, which suggests that individuals in this area show a high degree of site fidelity and occupy relatively small home ranges (Bell et al. 2016). One additional receiver was placed in the narrow channel at the ocean entrance to Macquarie Harbour to monitor potential departures from the estuary of any tagged individuals. Deployment of the acoustic array was completed in August 2017.

Each station consisted of a mooring block connected to a sub-surface buoy by a length of rope of ~2 m to which the receiver was attached. This ensured that the receivers maintained an upright orientation ~1 m above the seabed. To facilitate retrieval of the stations, a 100 m snag line with a weighted end was connected to each mooring block and lightly tensioned. Downloads of receiver data occurred twice a year for a small subset of receivers to help inform other components of this study (i.e. tag attachment pilot and adult physiology experiments). All stations were retrieved between October and November 2019 and subsequently data from all receivers was downloaded.

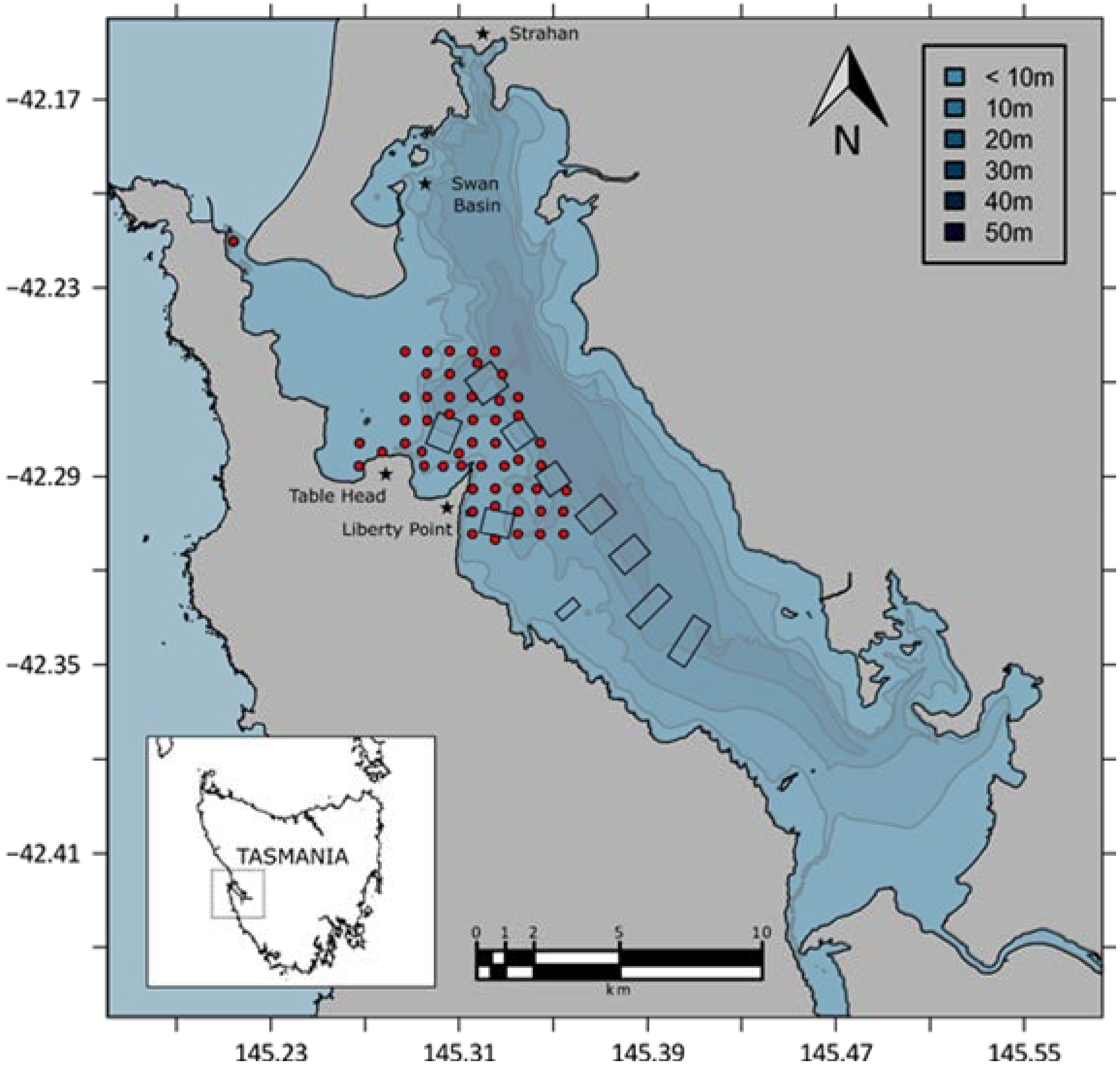

Fig. 1. Map of Macquarie Harbour showing the location of VR2 acoustic receivers (●), names of key areas and boundary lines of all salmonid aquaculture leases (□). Bathymetry contours show approximate depth at 10 m increments.

Multi-sensor acoustic tags

To investigate environmental conditions experienced by Maugean Skate in Macquarie Harbour, animals were tagged using a newly developed acoustic telemetry tag (Innovasea, Canada) able to record depth (0- 68 m ± 3.4 m), temperature (-5-35 °C ± 0.15 °C), and dissolved oxygen concentrations (Optical sensor 0-140% sat ± 2%). Tag dimensions were 105 mm length, 18 mm diameter, and 20 g weight in water. Transmitters pulsed over a pseudo-random delay of 160-200 s and had preprogramed battery cut-off of 365 days. The three sensors had unique identification codes which were emitted on a cycle along with the measured value.

Capture and tag attachment

Maugean Skate were captured using standard monofilament graball nets (50 m long by 33 mesh drop, 114 mm stretched mesh). Nets were set during daytime and soak times were restricted to under 2 hrs to ensure maximum fitness of individuals for tagging. Upon capture, the skate were carefully removed from the net and placed in a 250 L recovery tank of oxygenated seawater drawn from ~ 10 m depth. Following a 10-20 min period of recovery, the skate were measured and sexed. Individuals were then placed in a shallow tray filled with oxygenated water to ensure continued gill ventilation during the tagging procedure. Duration of the tag attachment surgery (see below) was approximately 5-10 minutes, after which individuals were administered an intramuscular antibiotic injection (Oxytetracycline @ 50 mg/ kg). The skate were placed back in the recovery tank and closely monitored for signs of distress or agitation for a further 10 - 15 minutes. Individuals were then released close to the capture point and near an acoustic receiver to ensure the tag was operational.

The multi-sensor acoustic telemetry tags were fitted externally to the skate using the attachment method developed by Le Port et al. (2008) for short-tail stingrays *(Bathytoshia brevicaudata*). A strand of 200 kg Nylon monofilament line was inserted through the muscle dorso-laterally approximately 10 cm down from the base of the tail, taking great care to avoid the vertebrae and caudal artery. The line exited ventrally and was reinserted on the opposite side. The points of contact between the monofilament and the skin were protected using a silicon disc to minimise rubbing and irritation. The loose monofilament strands were joined using a metal crimp and leaving a tail ~5 cm in length, to which the acoustic tag was secured using a second crimp (Fig. 2). The tag was oriented with the DO sensor pointing towards the animal and a small PVC gill net float was affixed to the opposite end. The float made the tag neutrally buoyant to help minimise drag from the attachment assembly on the animal. The attachment method was designed with the expectations that following the conclusion of this study (~15 months) the crimps would corrode, allowing the assembly to become detached from the animal.

A pilot trial was conducted in the field to validate the use of the proposed attachment method. On the 28th of July 2018, five male Maugean Skate with total lengths of 665-701 mm were externally fitted with acoustic tags. Because this was a trial, V16-TP depth tags (Innovasea, Canada) were used in place of the DO tags. These had similar dimensions to the DO tags but were less expensive and had a shorter battery life. After a month, a subset of five receivers was downloaded to assess the success of the technique. All five individual skate were successfully detected throughout the pilot period and each appeared to resume normal behaviour shortly after initial release. Following the successful completion of the pilot, 25 adult Maugean Skate, 14 males and 11 females were tagged with acoustic DO/depth/temperature tags between the 12th and 16th of November 2018.

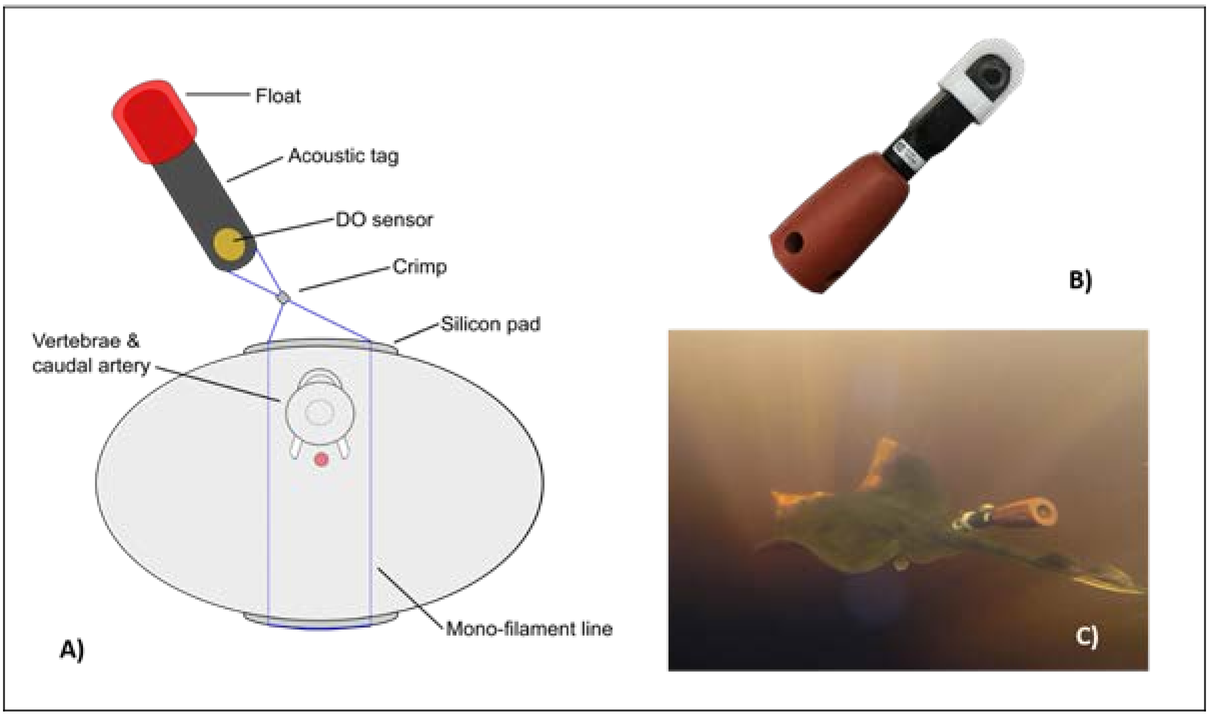

Fig. 2. Acoustic tags and attachment method. A) Transversal diagram showing the attachment method for the dissolved oxygen tag to the base of the tail of the Maugean Skate. B) Photograph of the sensor tag fitted with a float and attachment harness. C) Post-release photograph of a female Maugean Skate fitted with an acoustic tag.

Range testing

The efficacy with which acoustic signals travel underwater can be affected by environmental conditions (e.g. salinity, bathymetry, etc.) as well as human activity (e.g. boat traffic, industrial noise levels, etc.). These conditions can vary through time and space, affecting the range and detection efficacy of acoustic telemetry. If not accounted for in the analysis of the data, this variability can severely compromise interpretation (Huveneers et al., 2016, Kessel et al., 2014). Rigorous range testing was conducted during a previous tracking study in Macquarie Harbour that utilised the same equipment deployed in a different configuration throughout the harbour. A combination of fixed sentinel-tags and recurrent tests on key receivers (i.e. increasing distance drift tests) were utilised over a 1.5-year period. The areas targeted in the present study (Table Head/Liberty Point) showed little spatio-temporal variation in detection efficiency regardless of deployment depth (Bell et al., 2016).

For the current study, an additional five sensor tags that were identical to the ones attached to the skate, were fixed to a subset of listening stations to act as sentinels. These sentinels remained active throughout the full deployment time of the acoustic array.

Environmental data

Conditions in the water column corresponding to the tracking period for the skate were recorded using a real-time environmental monitoring sensor string located near the centre of the acoustic array (42°15'34.6"S, 145°18'55.5"E). The string consists of a series of salinity and DO/temperature sensors (Innovasea, Canada) placed at 2.5-5 m intervals that are connected to a surface buoy which is kept static by two large mooring blocks. The sensors continuously record and broadcast data wirelessly to an acoustic receiver in the surface buoy, where it is relayed in real time via iridium satellite connection. In March 2019, the buoy was also outfitted with a weather station (200WX-IPX7, Airmar), providing information on air temperature, wind speed, and wind direction. The sensor string is part of an FRDC funded project looking at real-time environmental monitoring that was conducted concurrently with this study (FRDC 2017-067). Environmental depth profiles for DO, salinity and water temperature were calculated through kriging interpolation using the gstat library (Pebesma, 2004) in R.

Data analysis

Tracking data was collated into a database using the Innovasea user environment software (VUE). The tags had three distinct identities, one for each of the sensors (depth, temperature, DO), which were combined for each skate. Data was exported and all subsequent manipulation and analysis was conducted using the R statistical environment. Detections within the first three days after tagging were removed to exclude non-natural behaviours caused by the tagging procedure. To minimise false positives caused by signal collision, a cleaning algorithm was applied to the data, where any instances with fewer than three unique detections within 1 hr of each other were removed.

Latitude and Longitude data were reprojected using universal transverse Mercator to ensure that all three spatial dimensions were on the same unit (m). Position was estimated using a weighted mean location similar to the centre-of-activity approach (COA) (Simpfendorfer et al., 2002, Udyawer et al., 2018). However, instead of using a fixed time interval, calculations were performed on a sliding window based on time (± 15 minutes). This approach acts as a high-pass filter and unlike the traditional COA, data density is not lost. Since the sensors had asynchronous broadcast cycles, the sliding window calculation was also applied to the depth, DO and temperature to create a mean interpolated value at each timestep. For analysis that required further smoothing (i.e. Behavioural change-point analysis) or a regularised sampling period (e.g. spectral deconstruction), a regular COA calculation was performed using a 6 hr timestep.

Behavioural changepoint analysis

Long term changes in movement behaviour were investigated using changepoint analysis. Previous implementations of changepoint analysis to animal movement data require that location be converted to a univariate response. However, when multidimensional data is available, a multivariate approach provides additional information (i.e. cross-correlation and internal structure) that can improve change point detection (Matteson and James, 2014). Accordingly, Maugean Skate behaviour was investigated using multivariate, non-parametric change point analysis based on a divisive algorithm using the ecp package in R (James and Matteson, 2013). Hierarchical divisive estimation is a bisection algorithm that detects change points sequentially using a permutational analysis based on distributional changes, including changes in the variance/covariance of multivariate data. The statistical significance of the change points is determined using the energy statistic introduced by Rizzo and Székely (2010) to measure the divergence between the distribution of two random vectors. A value of sigma 0.05 was chosen as the cut-off for significance.

Environmental and vertical activity spaces

Vertical space use (depth), experienced temperature and experienced DO levels were separated into discreet one-month intervals. A kernel gaussian estimator weighted by detection frequency was used to calculate the frequency distribution of all three variables for the combined sample of tagged individuals. Additionally, the one-dimensional kernel for the daily range of DO values experienced for each tagged individual was also calculated. The relationship between the DO and depth was explored using two-dimensional cumulative bivariate normal kernels with automated bandwidth selection.

In order to determine if DO levels recorded in the water column by the real-time environmental monitoring sensor string corresponded with those experienced by the skate at equivalent depths (on the seafloor), mean DO values at 15 minute intervals were calculated from the tag DO sensors and the interpolated water column data and compared using a linear regression.

Diel patterns of vertical space use

Diel patterns in vertical movement were investigated using harmonic analysis. Because the data were an irregularly sampled time series with missing observations, a discrete Fourier transform was used to calculate the Lomb-Scargle periodogram (Dilmaghani et al., 2007). Peak significance was estimated through an iterative process using an alpha level of 0.05.

State transition models of vertical movement behaviour

Depth kernels provide an estimation of which depths are most commonly being used by individuals based on the amount of time they are recorded there. To investigate movement between multiple depths, mean depth at 30 min intervals was calculated for all individuals and classified into one of three categories: preferred (7.5-12.5 m), shallow (<7.5 m), and deep (>12.5 m). The sequence of locations (depth) for all individuals was calculated. A chain of locations was considered to end when more than 2 hrs lapsed since the last detection. This data was used to build an adjacency matrix where the nodes represent the three depth states, and the edges represent the movement of individuals between them. The process was assumed to be Markovian and a discreet time Markov chain was used to calculate the stochastic transition probability matrices (Jacoby and Freeman, 2016). Confidence intervals were estimated using a bootstrapping procedure. To account for potential changes in behaviour over time (a violation of the assumptions of a Markovian process), transition matrices were calculated for each month. The dominant eigenvector of the transpose matrix was calculated for each monthly matrix. This common network metric is an indicator of centrality (steady states) and can be generally understood as the probability of an individual ending in each state (depth) regardless of their initial location. Other useful metrics were also calculated including mean reoccurrence time (average time it will take for an individual to return to each depth once they depart) and betweenness (number of times a node acts as a bridge between two others in a path). Model fitting and matrix calculations were conducted using the MarkovChain library (Spedicato, 2017)

Home range analysis

Data from individuals which were detected throughout the entire study period were sufficient to allow analysis of movement as a continuous time stochastic process. Movement diffusion was modelled using a modified version of the Ornstein-Uhlenbeck (OU) process, the OU Foraging process (OUF), which accounts for autocorrelated velocities and restricted space use (Fleming et al., 2014). The OUF processes improves on commonly used endlessly diffusing models (i.e. Brownian motion and integrated OU) as it assumes a tendency to remain in a particular area and is, therefore, better suited for home range estimation (Uhlenbeck and Ornstein, 1930). The OUF model was compared to these alternate models using the corrected Akaike's information criterion (AICc) and the OUF model was the most parsimonious for all individuals. The predicted model fit for each individual was overlaid with the movement variograms to provide a visual diagnosis of performance. The variograms showed a clear asymptote suggesting that all the individuals in the analysis exhibited true home ranging behaviour, validating the adequacy of modelling movement as a continuous time stochastic process. The movement models were used to calculate individual home ranges using the weighted autocorrelated kernel density estimator to help account for temporal sampling bias. Model fitting and distribution calculations were performed using the ctmm package (Calabrese et al., 2016).

Environmental drivers of horizontal space use

To investigate temporal changes in the extent of horizontal activity spaces across the tagged population, daily, weekly, and cumulative bivariate normal kernel utilisation distributions were calculated for each individual. Kernel bandwidth was set to be equivalent to the mean location error (250 m). Portions of the utilisation distribution (UD) that overlapped with land were clipped using a polygonal mask and the probabilities rescaled to 1. The isolines corresponding to the 50% (core area) and 95% (extended activity area) were produced and used to calculate activity areas in km^2. To avoid underestimating the size of the activity areas, instances (day or week) with less than 30 unique detections were excluded (days with few data points had large detection gaps which generated unrepresentative UDs). The effect of time (seasonality) on cumulative area (week) for the 50% and 95% kernels were tested using a one-way ANOVA. The data were log transformed to ensure normality and homogeneity of variance.

To investigate the link between environmental conditions and activity area size of the skate, a series of linear mixed effects models were fitted to the daily 50% and 95% activity areas. Given that data were skewed, a series of transformations was tested to ensure heteroskedasticity and a log transform was found to have the best result across all response variables (i.e. 50% and 95% area from daily and weekly estimates). Individuals were included as a random effect on all models to account for the repeated measures nature of the data. Validity of individual as a random term was verified by calculating the random residual plot. Since DO, water temperature and salinity are dependent on depth, the mean daily values at 10 m (apparent preferred depth of the skate) were used as the environmental variables in the model. In addition to the environmental variables, sex, and size of the individual (TL) were also included as model terms. A series of candidate models was constructed using different permutations of these variables. Each model was compared against the null model (i.e. model that included only a random term for individuals) using a likelihood ratio test. Model fit was examined using Akaike's information criterion (AIC), where the candidate models showing the lowest AIC scores are considered to be the best representation of which factors drive space use patterns in the species. Due to the complexity of mixed model designs, *t* values were not used to evaluate term significance as this generally increases the probability of type-1 errors. Instead, pseudo R^2 values (Nakagawa and Schielzeth, 2013) and summary tables were calculated for the leading candidate models using the Kenward-Roger d.f. approximation (Kenward and Roger, 1997). Models were fitted using the lm4 (Bates et al., 2014) and merMod libraries (Long, 2018).

Eco-physiology

Adults

Animal care and husbandry setup

During May 2019, skates (n=16) were captured from Macquarie Harbour using the same methodology and equipment as described above to capture individuals for electronic tagging. Upon capture, the skate were carefully removed from the net and placed in a 250 L recovery tank with seawater drawn from ~ 10 m and an air stone connected to an O_2 supply. Individuals were transported to an onshore facility in Strahan, where a mobile husbandry setup was constructed (Chapman and Chapman, 2017).

Skate were housed in one of three holding tanks (i.e. round vinyl-lined, above-ground pools with a 2.5 m diameter and a 1200 L capacity) filled with water pumped from ~10 m to approximate capture conditions (Fig. 3). Each tank was covered to limit light exposure, approximating the conditions usually encountered by the skate in the harbour (heavy tannin content in the water limits light penetration past 3 m). A maximum of six skate were housed in each tank at any one time. Each tank was an independent closed system providing water filtration, temperature control and oxygen manipulation capabilities (Fig. 3). Filtration was provided by a 3-step canister filter (UV, mechanical and bio filtration) with a capacity of 10000 L/h. Water temperature was kept constant throughout at ~14 ± 1 C° using heater/chiller units. Dissolved oxygen levels were monitored using optical fibre dipping probes and a Witrox-4 unit (Loligo System, Tjele, Denmark). Ammonia was tested daily and remained low (<0.1 mM) throughout the duration of the experiment. Each tank had two gas bubblers providing O_2 and N_2 and the supply was regulated by solenoid valves connected to a DAQ-M controller unit (Loligo System, Tjele, Denmark). Gas control was automated using the WitroxCTRL software (Loligo System, Tjele, Denmark) to activate the solenoids based on the readings from the optical sensors.

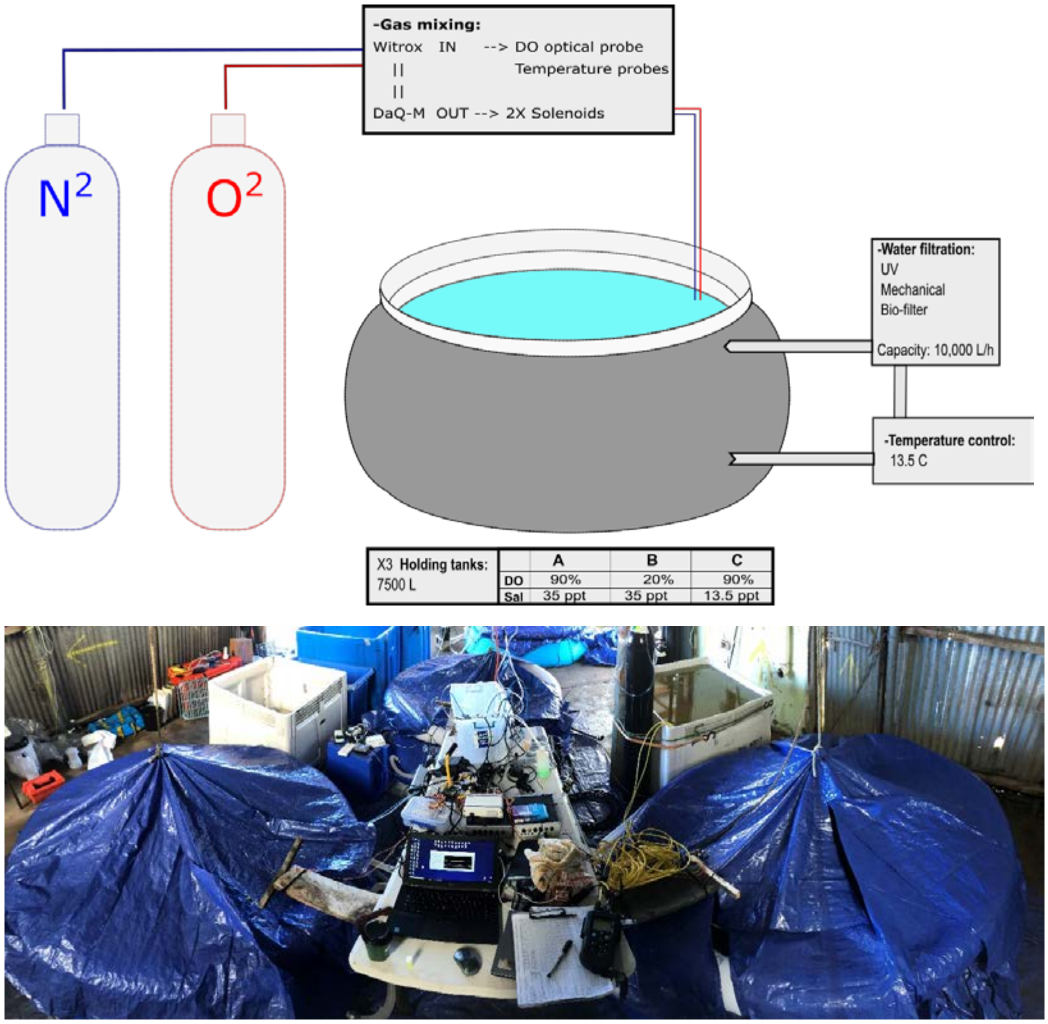

Fig. 3. Eco-physiology experimental holding tank system (top) and photograph of field laboratory set-up (bottom).

Experimental protocol

Upon arrival individuals underwent a 24-hr holding acclimation at ~14 ± 1°C, salinity of 27 ppt and 90% DO saturation. A control blood sample was then taken (0.5-1 mL) through caudal ventral venipuncture in the region immediately posterior to the cloaca using a heparinised syringe and a 22-gauge needle. After the 24-hr holding acclimation and blood collection, an initial respirometry measurement was also taken using a static chamber as per Morash et al. (2020) (see below) and then the skate was returned to its holding tank. Afterwards conditions in each of the tanks were changed to match one of three experimental treatments mimicking conditions experienced by the skate (DO levels estimated using a preliminary partial download of the acoustic tags in March 2019):

1—Control: 27 ppt salinity and 90% DO. Conditions in this treatment were unchanged from the initial acclimation period. DO was continuously monitored and kept within 5% of the treatment level.

2-- Hypoxia exposure: 27 ppt salinity and 20% DO. Dissolved oxygen levels were lowered to 20% at a rate of 5% per 30 min by bubbling N_2 into the water. DO was continuously monitored and kept within 5% of the treatment level.

3-- Hyposalinity exposure: 13.5 ppt salinity and 90% DO. Freshwater from the surface layer of the harbour was added to dilute the salinity level at a rate of 5 ppt per 30 min until 13.5 ppt was reached. DO was continuously monitored and kept within 5% of the treatment level.

Individuals were kept under treatment conditions for at least 96 hours after which a second respirometry trial was conducted (lasting only one hour in water matching treatment conditions) and a second blood sample was taken immediately afterwards. Skate were then euthanised by blunt force trauma to the skull, followed immediately by brain pithing.

Tissue samples and measurements were taken from the heart, liver, brain, rectal gland, gills, and white muscle and stored in liquid nitrogen for transport and later analysis or fixed in Bouin's solution. The remaining carcases were frozen and stored for later biological assessment and collection of additional tissue (e.g. ageing samples). All individuals were fasted throughout the full captivity period.

Respirometry

The respirometry chamber consisted of a tray slightly larger than the animal containing a small submersible pump for circulation as well as DO and temperature sensors. The chamber was filled with water from the holding tank to a level that covered the animal. After a 1 hr acclimation the chamber was sealed using gas impermeable translucent plastic sheets. Oxygen levels were allowed to drop as the skate consumed oxygen from 100% to 10% after which oxygen consumption was negligible and the trials were concluded (this process lasted 6 to 12 hours). Background consumption of O_2 due to microbial activity was measured by running multiple trials with an empty chamber.

The average slope of O_2 consumption from each 10% increment was used to calculate routine aerobic metabolic rate (MO_2; $mgO_2\ kg^{-1}\ h^{-1}$), considering background O_2 consumption, the volume of the tray, skate mass, temperature, and barometric pressure. The values for each individual at each increment were then averaged.

Haematological analysis

Immediately following collection of blood samples, haematocrit, haemoglobin, glucose, and lactate concentrations were measured. Haematocrit was measured in duplicate in a SpinCrit Microhaematocrit centrifuge (SpinCrit). Haemoglobin was measured using HaemoCue Hb210$^+$ system in accordance with the manufacturer's instructions and values were corrected as per Clark et al. (2013). Whole blood glucose and lactate were measured with the hand-held OneTouch Ultra2 glucometer (LifeScan, Milpitas, California) and a Lactate Pro lactate meter (Arkray Global Business, Inc.), respectively. Red blood cells were separated from plasma by spinning the remaining blood samples at 13000 rpm for 4 min. The plasma was transferred into a clean microfuge tube and the buffy coat was removed from the red blood cells. Both were stored in liquid nitrogen until further analysis.

Plasma assays

Lactate. To corroborate the validity of the lactate measurements obtained in the field, plasma lactate was extracted and quantified using a modified version of the protocol proposed by Tunnah et al. (2017). The samples were thawed on ice and diluted 1:2 (v:v) in 8% perchloric acid (PCA). They were briefly vortexed and centrifuged in an at 16438 x g for 4 minutes at 4 °C and neutralised the supernatant with 2 M KOH$^+$ 0.4 M Imidazole before centrifuging again at 16438 *x g* for 2 minutes at 4°C. A known volume of supernatant was collected and stored at -80 ˚C until further use. The extracted lactate was thawed on ice and assayed in triplicate with a standard curve of L-lactic acid. Final in-well concentrations of assay components were: 0.16 M glycine, 0.13 M hydrazine, 1.9 mM NAD$^+$, and lactate dehydrogenase (10 units/well). The reaction was incubated at room temperature until completion (~45 min) and absorbance of the generated NADH was read at 340 nm using a SpectraMax 190 Plate Reader and SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

Ketone bodies. Total ketone body concentrations (acetoacetic acid (AcAc) + β-hydroxybutyrate (BOH)) were quantified using the EnzyChromTM Ketone Body Assay Kit (EKBD-100) (BioAssay

Systems). The samples were assayed in duplicate with 0 mM and 8 mM standard curve. The reaction was run until completion (5 min for AcAc, 15 min for BOH) and NADH absorbance was read at 340 nm using a SpectraMax 190 Plate Reader and SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

Osmolality. Osmolarity was measured using a Wescor vapour pressure osmometer (Wescor Inc, Logan, UT, USA) according to the manufacturer's instructions. Samples were read in triplicate.

Tissue assays

Frozen tissues were powdered under liquid nitrogen a mortar and pestle and kept at −80°C until further analysis. All assays were performed in triplicate in a 96 well plate at room temperature using a SpectraMax 190 Plate Reader and SoftMax Pro software (Molecular Devices, Sunnyvale, CA).

Lactate. Powdered tissues were homogenised in 1:4 (w:v) 8% PCA containing 1 mM EDTA and frozen in liquid nitrogen. The solution was sonicated using a Fisher F60 Sonic Dismembrator (Fisher Scientific, Ottawa, Canada) and centrifuged in an Eppendorf Centrifuge 5430 R at 16438 x g for 4 minutes at 4 ˚C. Samples were neutralised with 2 mM KOH and centrifuged again at 16438 x g for 1 minute at 4 ˚C. Final supernatants were flash frozen in liquid nitrogen and placed back into -80 ˚C until further use. Lactate assays were performed according to the procedure outlined for plasma (above).

Glycogen. Tissue glycogen was extracted and fully hydrolysed to glucose according to Clow et al. (2008) and the hydrolysates were kept at −80°C until further analysis. Glucose was measured according to Bergmeyer (1974) using a glucose standard curve. The assay medium contained 250 mM imidazole, 5 mM $MgSO_4$, 10 mM ATP, and 0.8 mM $NADP^+$. G6PDH (25 µL of 10 U/mL) was added to the wells and incubated at room temperature for 10 min to eliminate any endogenous glucose-6-phosphate. Background absorbance of NADH was established at 340 nm before 25 µL of hexokinase (10 units/mL) was added to each well. The reaction was incubated for a further 45 minutes before reading the plate again at 340 nm. Glucose concentration was calculated using the standard curve.

Data analysis

All analyses were conducted using R 4.0.0 (R development Core Team, 2010). Post exposure measures of metabolic rate, plasma lactate, osmolality and ketone body concentrations were compared to their respective controls using paired *t*-tests. Tissue lactate and glycogen concentrations were compared among treatment groups using one-way ANOVAs. Liver glycogen concentrations were log transformed to meet the assumption of normality.

Neonate

Due to the difficulties associated with the capture of live eggs, only a single individual was hatched and housed in captivity that could be used for experimental physiology trials. Given that nothing is currently known about the early life stages of the Maugean Skate, observations from even a single individual can be of great value. However, they should be interpreted with care.

To establish the routine aerobic metabolic rate of the individual soon after birth, intermittent respirometry trials were conducted one month after hatching. The newborn skate was placed in a submerged respirometry chamber which was sealed and flushed in an intermittent cycle as per Rosa et al. (2014). Oxygen consumption rates were estimated using the averaged slopes for the sealed periods.

To test the juvenile's hypoxia tolerance, the experimental protocol used for the adults (see above) was replicated. A pilot static respirometry trial was conducted to test the equipment and establish if the aerobic scope of the juvenile was similar to that of the adults. A low volume static respirometry chamber was constructed that included a low capacity submersible pump for water circulation.

Otherwise, the equipment used to measure, control, and regulate gas levels was the same as used for the adults. Prior to each trial the individual was starved for a day. Water used in the chamber was provided by the same temperature-controlled filtration system that fed the holding tank (see above). To help maintain a constant water temperature during respirometry trials, the whole apparatus was semi-submerged in a larger tray with a continuous flowthrough of water from the same supply as the holding tank. The individual was sealed in and oxygen levels (~100% DO) were allowed to decrease until the oxygen consumed by the skate became negligible (~4 hrs). MO_2 levels at 5% intervals were calculated as per the adults (see above) and the oxygen critical partial pressure (P_{crit}) was calculated using a segmented regression approach implemented using the respR library (Harianto et al., 2019).

Approximately one week after the initial trial the final experimental protocol was started. A blood sample was taken for haematological analysis of lactate, glucose, and haemoglobin using the same hand-held meters that were used in the field for adults. These meters require a very low volume of blood to work (<0.5 microliter), so the smallest possible amount of blood was collected using a 1 mm heparinised syringe and a 27 gauge needle. Immediately afterwards a respirometry trial was conducted as described above. At the conclusion of the trial the individual was transferred back to the holding tank and oxygen levels were slowly reduced (5% per 60 mins) until a saturation of 75% DO was reached. DO was continuously monitored and kept within 5% of the treatment level. Approximately 40 to 45 hours into the 96-hour exposure period, the individual perished. There is some uncertainty regarding the precise time of death because the event occurred overnight during a 6 hr gap when the individual went unobserved. Immediately after the individual was discovered, a second blood sample was taken, and tissue samples were collected.

Size composition

Gillnet surveys were conducted at roughly three-month intervals between February 2017 and December 2018 in the Table Head / Liberty Point area. Sampling was conducted using the same gear and methodology to that described above to capture individuals for electronic tagging. The ensuing data is an extension of the sampling effort that commenced in 2011 and was carried out through two earlier FRDC studies (Bell et al., 2016, Lyle et al., 2014). The sampling gear (gillnets) used throughout has not changed, allowing for the direct comparison of size frequency distributions of the catch across the different studies. To ensure valid comparison of size frequencies between studies, only gillnet data for the Table Head/Liberty Point region of Macquarie Harbour have been used in this analysis. There have, however, been some changes in sampling strategy over time that means that catch rate comparisons (as indicators of relative abundance) may not be valid. In the present study a maximum set duration of two hours was applied, with fishing limited to daytime sets only. By contrast, longer daytime set durations as well as overnight sets were applied in the earlier studies. Several skate mortalities were linked to these longer set durations, necessitating a more conservative approach to gear usage to reduce the likelihood of negative impacts on the skate (and other bycatch).

Relevant data for each study – FRDC2010-016 (April to November 2012), FRDC2013-008 (October 2013 to November 2014) and the current study (October 2017 to November 2018) was separated by sex and plotted alongside each other to explore possible changes in the size distribution (based on TL) of the population through time. Changes in the distributions were tested using bootstrapped Kolmogorov-Smirnov tests with a Bonferroni adjusted alpha value. Likewise, sex-specific median total lengths were compared using a Wilcoxon rank test.

Ethics and permits

All procedures were undertaken with University of Tasmania Animal Ethics Committee approval (permit A16526) and scientific permits 17083, 17197 and 19026 issued under Section 14 of the Living Marine Resources Management Act 1995 and permits TFA 17107 and 17359 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, tagging and biological sampling (including eggs) of an endangered species.

Results

Early life history

Egg distribution

A total of 110 Maugean Skate eggs and egg-cases were collected across all sampling methods from February 2017 to July 2019. The sample comprised a total of 102 empty egg capsules, seven live eggs and one capsule containing a near-term embryo (111 mm TL) in the early stages of decomposition. The cause of death in the latter case was not evident but appeared to have occurred shortly prior to collection. Despite considerable targeted sampling effort, live eggs were extremely difficult to find. In fact, only three of the seven live eggs were collected during the field surveys, the remaining four were deposited by two females whilst held in captivity for the adult physiology experiments (see below).

Beam trawl surveys comprised 275 individual deployments covering a total bottom area of ~58,768 m^2 (Fig. 4). Tows covered an average of 216 m, although individual distance and duration varied widely depending on depth, substrate type and weather conditions. The range of sampled depths varied from 2 to 51 m, but given that Table Head/Liberty Point (TH/LP) and Swan Basin (SB) regions are relatively shallow, sampling effort was predominantly focused in depths of between 5 and 20 m (Fig. 5). Trawl surveys yielded 99 Maugean Skate eggs (2 live eggs, one dead near-term embryo and 96 empty capsules) captured in 50 successful deployments (i.e. 18.2 % success rate). Apart from three hauls which yielded 6, 7 and 15 capsules, respectively, all successful hauls contained between 1 and 4 capsules ($\bar{X}$= 1.9).

Dive surveys comprised 24 transects and seven active searches (individual dives spaned from 15 to 60 minutes with two divers searching separate areas) and produced five egg capsules (one alive) and included the sighting of a small juvenile Maugean Skate (TL~200 mm based on video footage) in Swan Basin at a depth of around 4 m.

All egg samples were collected from the Swan Basin and Table Head/Liberty Point regions; clustered in the inner basin and outer channel of Swan Basin, around the western tip of Liberty Point and adjacent to Table Head (Fig. 4). Despite similar sampling effort being allocated to the two regions, Swan Basin accounted for the highest number of eggs (N=81) and density per trawl area (0.35 eggs / 100 m^2). Egg capsules were found in bottom depths ranging from 2.5 to 33 m, although the highest incidence occurred between 5 and 10 m in depth (N=40) (Fig. 5). However, since so few live eggs were collected and trawl effort was concentrated in the general localities where Maugean Skate eggs were caught, these results need to be treated with caution in terms of making inferences about spatial patterns of egg deposition.

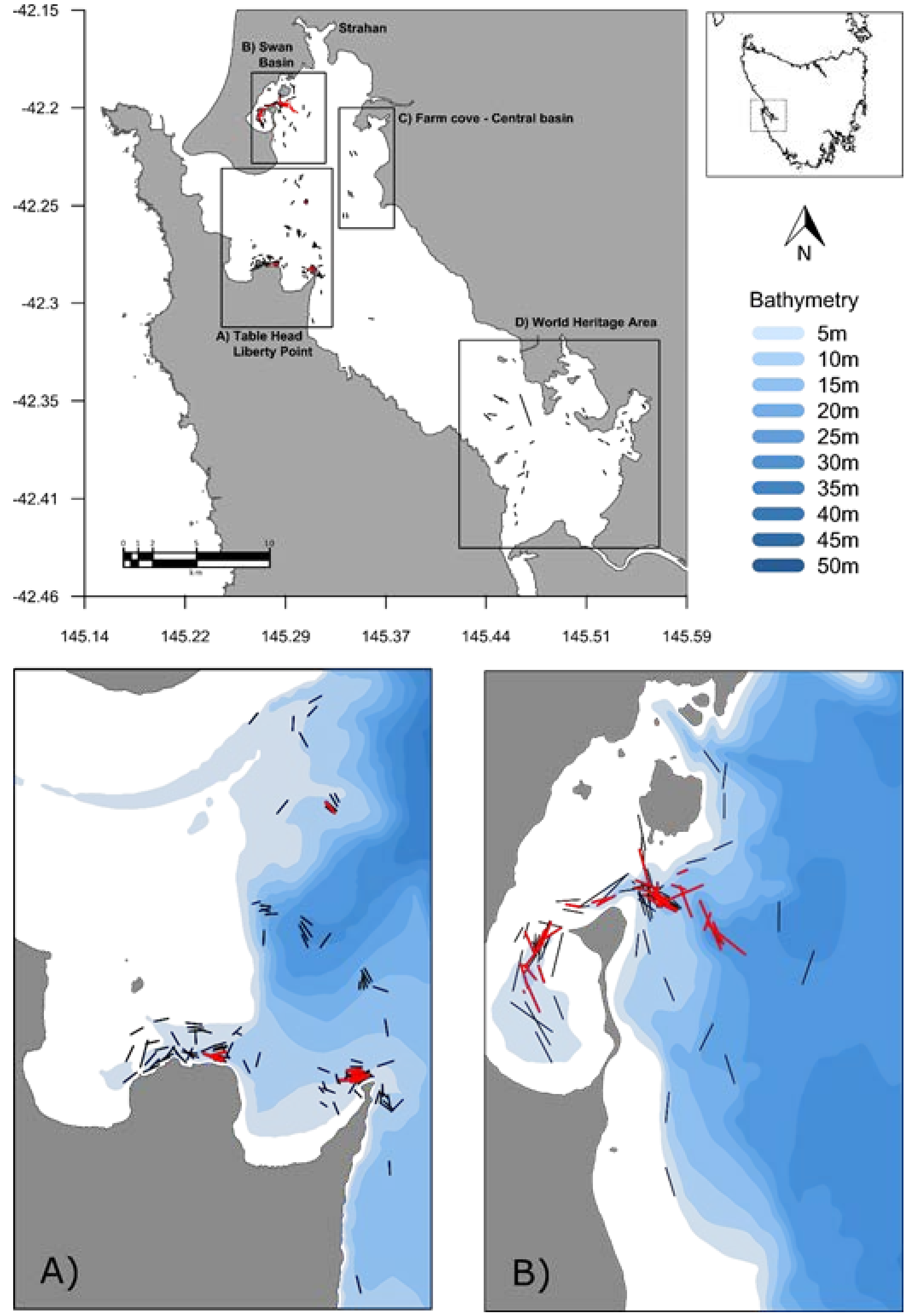

Fig 4. Map of Macquarie Harbour showing the location of beam trawl survey sites (top). A) Table Head/Liberty Point and B) Swan Basin areas showing beam trawl tracks, with tows yielding egg cases indicated in red.

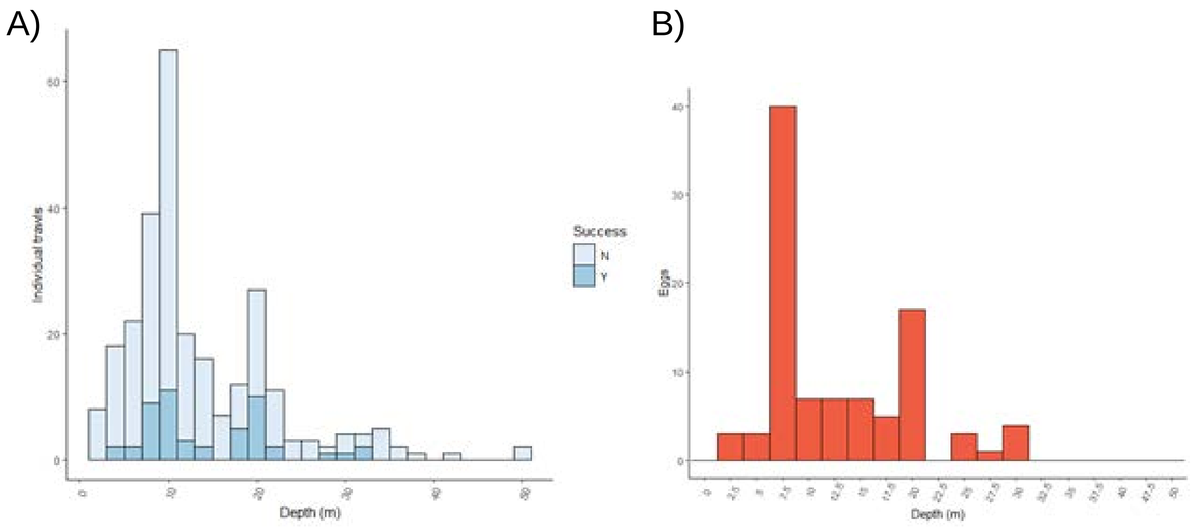

Fig. 5. A) Numbers of beam trawl tows by depth - tows in which egg cases were present are indicated (success Y), and B) number of egg cases collected by depth.

In addition to the Maugean Skate eggs, 178 thornback skate (*Dipturus lemprieri*) and three elephant fish (*Callorhinchus milii*) egg capsules were collected in the trawls, all of which were empty. Thornback eggs differ from those of the much larger Maugean Skate in shape, size, and coloration; and were easily distinguishable through visual examination. In general, thornback eggs are smaller in both length and width, lack a lateral keel and have attachment fibres along the lateral margin that are absent in Maugean Skate eggs. Thornback eggs were most abundant in Swan Basin, although a small number were also caught in Table Head.

Abundance and diversity of incidental catch was relatively low in the trawls, so it was not possible to define patterns of co-occurrence with Maugean Skate eggs. The most encountered fish species were Pacific seahorse (*Hippocampus ingens*)[1] and greenback flounder (*Rhombosolea tapering*). The most common invertebrates were caridean shrimp (*Palaemon* spp.), crabs (mainly *Paragrapsus graimandii*) and heart urchins (*Echinocardium cordatum*).

Egg viability

A total of 51 of the empty egg cases were analysed for signs of hatching success and predation. Maugean Skate egg-capsules displayed a 41% success hatch rate and a predation rate of 20% (Fig. 6). The remaining 39% of egg capsules in the sample were classified as non-predation damage.

Two distinct categories of predation damage were observed. Category A consisted of one or several rounded boreholes small in diameter (<2mm). Category B consisted of straight cuts or tears that resulted in a large slit. Category B was the most common (70%) form of predation damage.

Several egg cases were excluded from the analysis because they showed heavy signs of erosion, many of which had small sessile invertebrates growing on them. It could not be determined, however, if this had any impact on the viability of the egg.

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[1] Present in < 10 tows but in large numbers in one tow in Swan Basin.

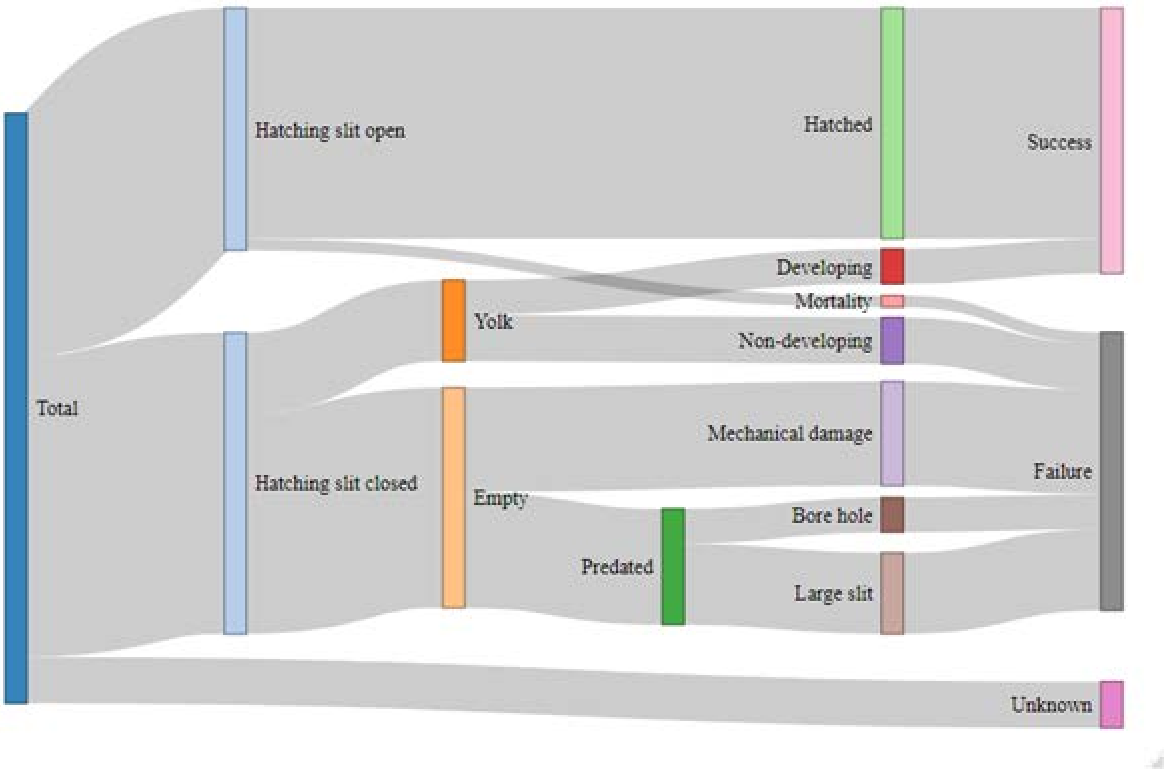

Fig. 6. Sankey diagram showing a breakdown of the classification of Maugean Skate egg capsules to determine hatching success and predation rates. The nodes are ordered to reflect the qualitative assessment flow and the width of the pathways is proportional to the number of eggs at each category.

Egg description and development

When freshly laid, Maugean Skate egg cases are bright golden-green in colour, becoming darker-brown with time. The main body of the capsule has an oblong shape and appears smooth to the touch (Fig. 7). A few fibroids running transversally are visible, but there are no attachment fibres or tendrils present. The lateral keel is not very pronounced (~12% of ECW) and the ventral side is convex, resulting in a curved lateral profile. The anterior horns are small and curve slightly inwards. By contrast, the posterior horns are longer and straight. The anterior apron is concave and strong, while the posterior is fringed, thin, and nearly translucent. Egg case length, excluding the horns averaged ~78.06 mm (SD=6.99) and case width averaged 50.74 mm (SD= 5.14). There is a weak linear relationship between ECL and ECW (ECL=0.553 * ECW +50.005, R^2=0.16).

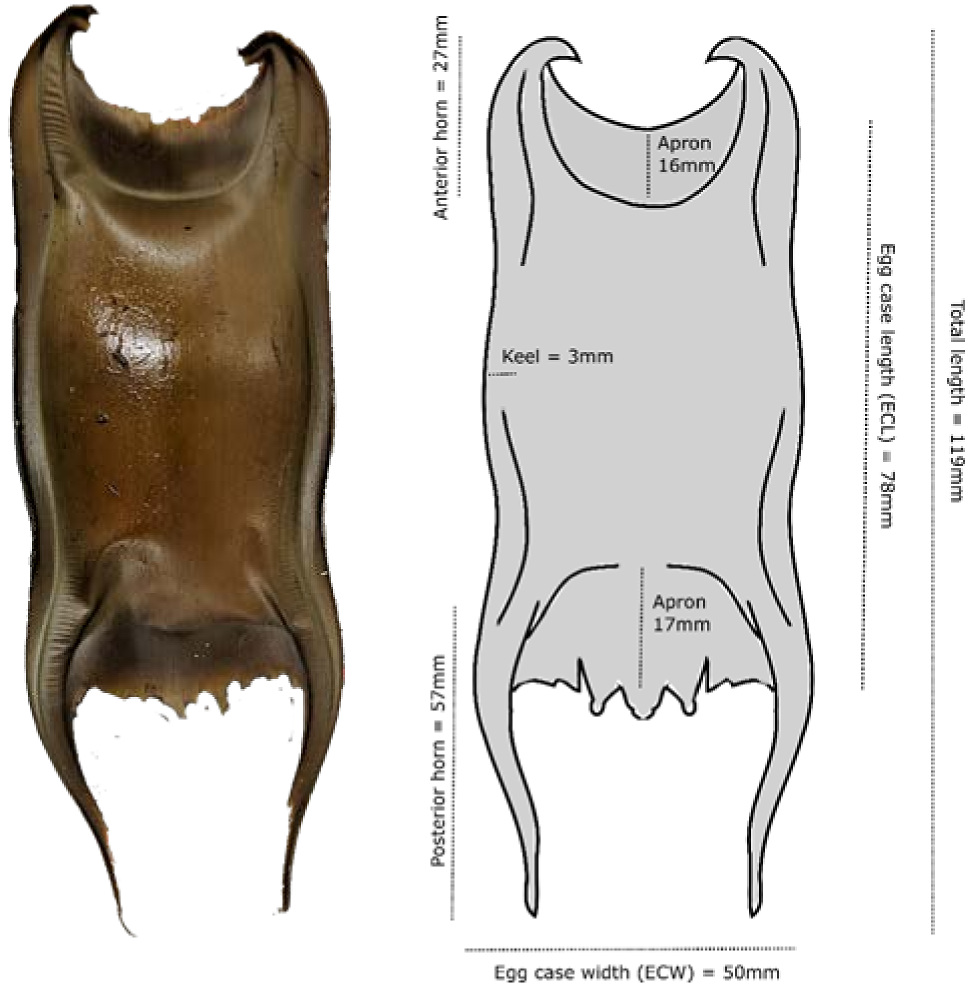

Fig. 7. Maugean Skate egg case. Photograph of a fresh egg (left) and diagram showing morphological features referred to in the text (right).

As noted above, there were significant logistical challenges associated with the collection of live Maugean Skate eggs. In total, only seven viable eggs were collected, of which three were found in the wild and four were laid by wild caught females held in captivity (Table 1).

The first viable egg was collected by beam trawl in November 2017 from the Swan Basin area at a depth of approximately 20 m. At the time of capture there was a visible embryo (~20 mm TL) between stages 3 and 4 of development (Musa et al., 2014) (Fig. 8). The embryo was slender with a long tail, the eyes were beginning to develop, and the respiratory channels of the egg capsule were still closed. This suggests that even though some time had elapsed since oviposition, the individual was still in the early stages of development. After approximately 10 weeks of rearing at ambient seawater temperature the embryo was deemed to have died after it stopped moving and the yolk had started to show obvious signs of decomposition (including bad smell). At that time, the embryo was between stages 5 and 6 of development (Fig. 8). The external gill filaments were almost gone, and the body more closely resembled that of an adult, but the yolk mass was still large. The egg was dissected and following examination, the embryo (80 mm TL) was preserved. Although the cause of death could not be determined it was decided to adapt the rearing protocol to simulate temperature conditions in Macquarie Harbour (at depths below the halocline) and to minimise handling and disturbance.

The second and third eggs were collected in Swan Basin at depths < 4 m in December 2018 (by beam trawl) and in March 2019 (by dive collection), respectively. At the time of capture, the yolk mass of both eggs was intact and looked healthy, but no visible embryo had developed. After 8 weeks, the yolk mass in both eggs had begun to decompose and the inside of the eggs had become cloudy. The contents of the eggs were examined and revealing no evidence of a developing embryo which suggests that both were either unfertilised or had become unviable very early in the development process.

Two wild-caught adult females being held for physiological trials in May 2019 each laid a pair of eggs in captivity. The females were in separate holding tanks with similar dissolved oxygen concentrations (~90% saturation) but different salinity levels (27% and 13.5 % respectively). Oviposition occurred overnight after the first day in captivity and was not directly observed. Neither of the females deposited any more eggs for the remainder of the captive observation period (5 days). At the conclusion of the physiology trials both individuals were euthanised, and their reproductive tract examined. No evidence was found of new developing eggs in-utero or within the oviducal glands. The egg capsules had near identical proportions to their sibling, with one pair much larger than the other. All four egg cases contained an intact yolk mass and no visible embryo. After 10 weeks the yolk inside the two larger capsules had begun to decompose and the eggs were deemed to be non-viable. The two remaining eggs were viable with developing embryos visible.

By 12 weeks following oviposition the developing embryos were ~20 mm in length (between development stages 3 and 4). At 15 weeks the gelatinous mass blocking the anterior horns had dissolved, allowing for water mixing between the inside and outside of the egg to occur through the respiratory fissures. After 20 weeks the embryos had taken on the appearance of a small version of the adult occupying most of the free space inside the egg capsule (between stages 5 and 6).

Approximately 24 weeks into incubation, both embryos were suspected to have died after several days without movement and no visible signs of continued development. One of the eggs was carefully opened and the embryo was carefully extracted. After about 10 minutes, the embryo was found to be alive after it responded to external stimuli. Once the yolk sack becomes fully vascularised in early development and the respiratory channels of the capsule open, it is possible to extract the embryo and continue development outside the egg. Following the methodology described in Tullis and Peterson (2000), the embryo was carefully transferred to a glass container and returned to its holding tank, where incubation continued with free water exchange (Fig. 9). The other embryo was assumed to also be alive and was left in the egg for the remainder of the incubation period. At week 28 the freed embryo was placed into an intermittent flow respirometry chamber to investigate oxygen consumption during gestation. Unfortunately, the embryo was injured during the course of the experiment and died the following day. The incident was reported to the animal ethics committee and the respirometry equipment was modified to avoid potential injuries in any future trials.

Under experimental conditions, hatching of the remaining embryo occurred 31 weeks (approximately 7 months) after oviposition (Fig. 10). Size at birth was 122 mm TL (total length), 85 mm DW (disk width) and a total wet mass of 11.1 gm. At birth, the yolk mass had been fully absorbed into the hatchlings body and a small internal yolk reserve was clearly visible. The internal yolk was fully consumed nine days after hatching and the skate commenced feeding two days later. Once feeding commenced the individual became more active and responsive to external stimuli.

Table 1. Capture development and captive rearing summary of live Maugean Skate eggs kept under controlled conditions

* At 24 weeks from oviposition the embryo was extricated from the egg case to continue gestation as per Tullis & Peterson (2000).

Date collected	Collection method	Location	Incubation time	Fertilised	Hatched	Comments
Nov-17	trawl	Swan Basin	10 weeks	yes	no	visible embryo ~20mm TL
Nov-18	trawl	Swan Basin	8 weeks	no	-	
Mar-19	dive	Swan Basin	8 weeks	no	-	
May-19	captive	NA	10 weeks	no	-	
May-19	captive	NA	10 weeks	no	-	
May-19	captive	NA	24 weeks *	yes	yes*	embryo died at 28 weeks
May-19	captive	NA	31 weeks	yes	yes	

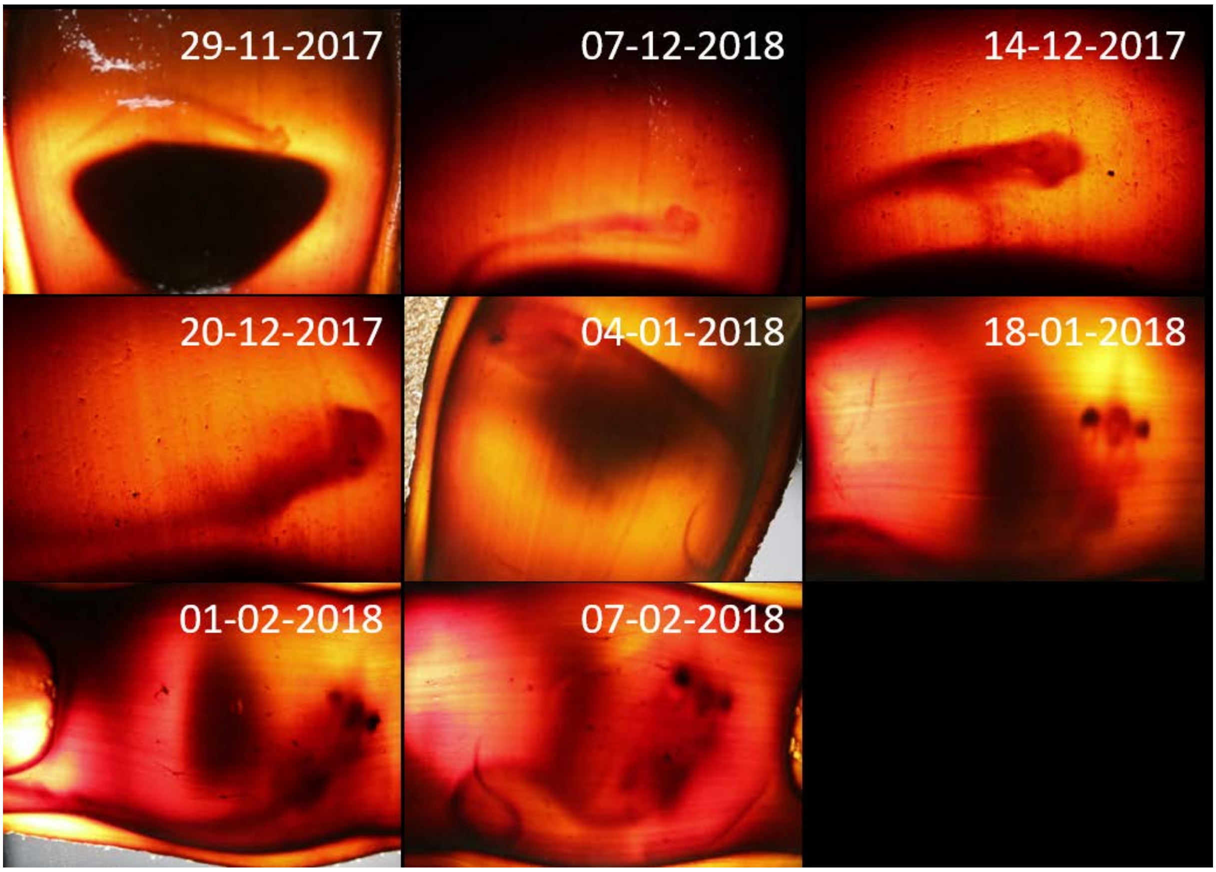

Fig 8. Bi-weekly pictures of embryo development for the first Maugean Skate egg held in captivity for 10 weeks.

Fig. 9. Photographs of the developing embryo after removal from the egg capsule. Dorsal view (left) and ventral view showing attached yolk sack (right).

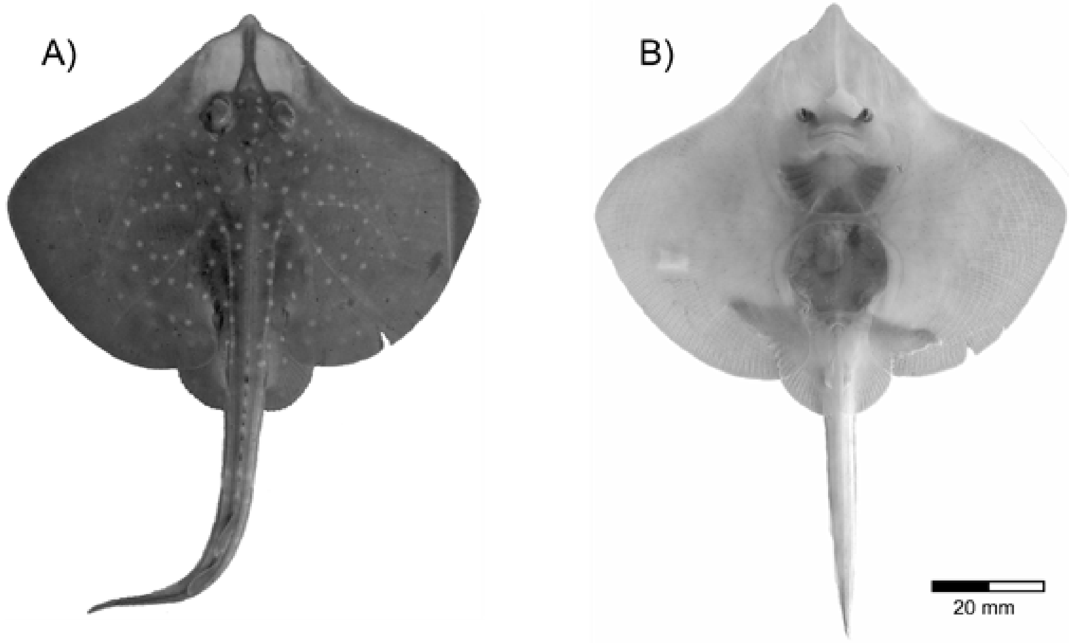

Fig. 10. A) Dorsal view of the developing embryo removed from the egg case (at 28 weeks), and B) Ventral view of a recently hatched (male) Maugean Skate.

Environmental data (water column)

Observations from the environmental string clearly showed a strong stratification of the water column that is characteristic of Macquarie Harbour (Fig. 11). The precise depth of the halocline oscillated throughout the year but appeared to follow a seasonal trend. The halocline was shallowest from the beginning of the austral summer in December until April (<2.5 – 6 m) compared to the rest of the year (5 -10 m). This pattern is likely correlated to rainfall levels and riverine flow from the Gordon and King rivers. Across the preferred depths for the Maugean Skate (7.5 – 12.5 m based UDs, see below), salinity was highly variable and ranged from different levels of brackishness to fully fresh-water.

Water temperatures beneath 10 m were relatively constant throughout the year, ranging between 13 and 15 °C. However, during summer, higher temperatures were occasionally recorded up to 20 m. Temperature in the upper 10 m was highly variable and ranged from 9 – 21 °C depending on the season.

Dissolved oxygen levels were mostly related to depth, with higher concentrations (80-100%) near the surface and declining quickly below the halocline. On the 20 March 2019 there was a sudden influx of oxygenated water caused by a strong westerly storm front. The large mass of oceanic water sank to the bottom of the harbour and caused an inversion event when the displaced low oxygen water was suddenly pushed up into the water column (Fig. 12). Following this event, bottom DO levels improved considerably up to July 2019, when levels began to drop once more.

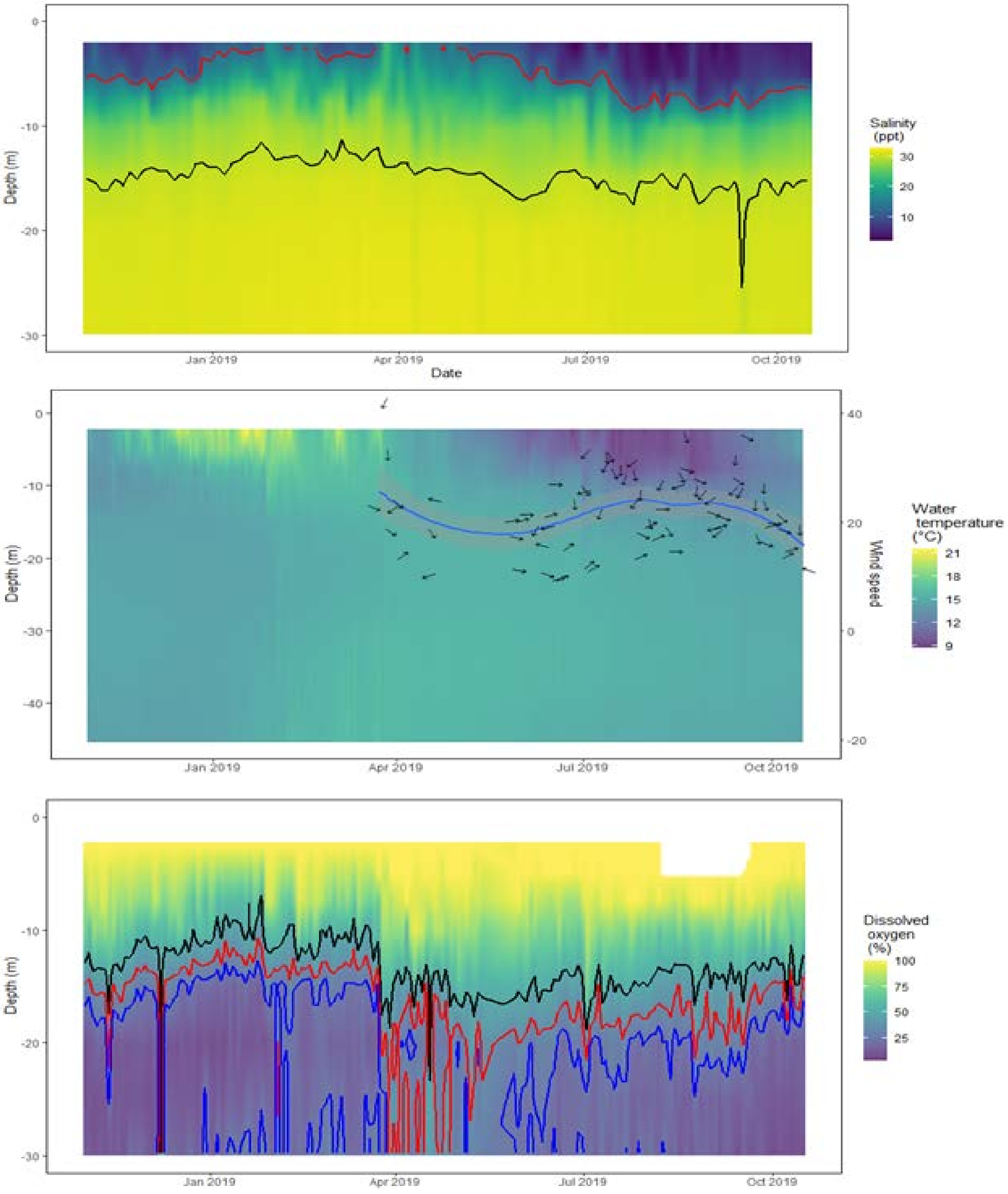

Fig. 11. Environmental profile showing water column conditions (by depth) near the centre of the acoustic array between November 2018 and November 2019. (Top) Water salinity (ppt) through time; the red line indicates the 13.5 ppt isocline and the black indicates 30 ppt. (Middle) Water temperature (°C) through time and loess regression of wind speed at the surface. Arrows indicate mean daily wind velocity (km/h) and direction (cardinal). (Bottom) Dissolved oxygen concentrations (%) through time; the black line indicates the 80% contour, red 50% and blue 25%.

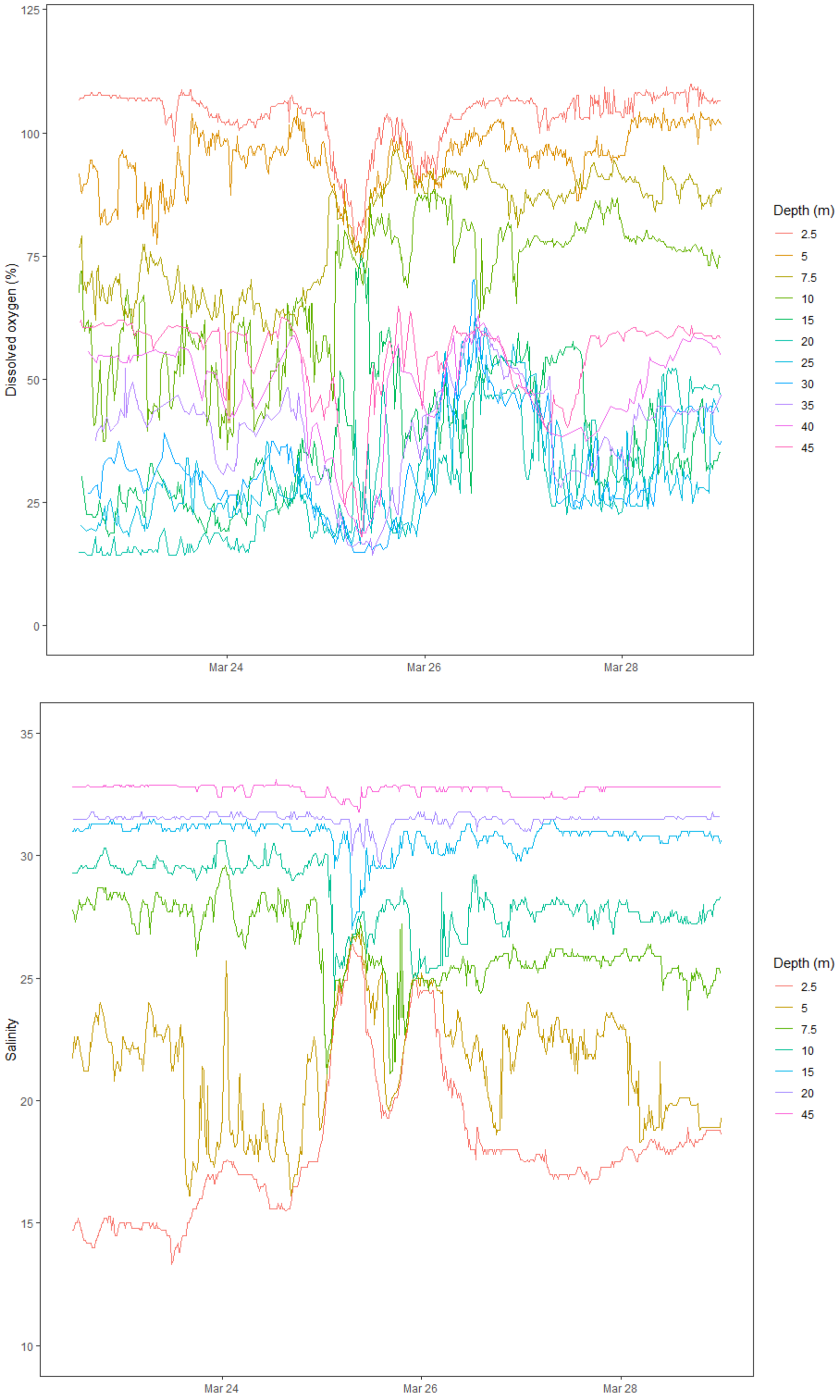

Fig. 12. Environmental conditions throughout the water column during a storm event in Macquarie Harbour in March 2019. Dissolved oxygen (top) and salinity (bottom).

Spatial ecology

Occurrence

The 25 tagged Maugean Skate ranged in size between 620 and 820 mm TL (females: N=11, range 690-820 mm; males: N=14, range=620-735 mm). Based on size, all individuals were judged to be mature (Bell et al., 2016) and were in excellent condition on release. There were detections on all receivers except for the one located at the harbour entrance, suggesting that none of the tagged skate left Macquarie Harbour during the study period.

Two of the tagged skate (MS21 and MS23) exited the array within a few days of release and were not detected again during the study period. An additional five individuals appeared to have exited the array area at different times during the study (exit times varied from 2 to 8 months after release) and were not detected again. Seven individuals stayed within the array area for the duration of the study, although most did have periods (ranging from hours to weeks) when they were undetected, suggesting the possibility of short-term excursions outside of the array. Eleven skate (eight males and three females, ranging in size from 670 to 795 mm TL) were suspected to have died during the study period. An assumed mortality event (AME) was defined to occur when detection data of an individual showed an abrupt change in movement patterns followed by a cessation of horizontal and vertical movement. Time of death was determined using multivariate change point analysis (see below) and all post-mortality periods in the data were removed from further analysis.

Prior to departure from the array or assumed mortality, most individuals had several months of high quality detection data and the overall daily detection rates were very high throughout the year. Summaries of detection data are provided in Table 2 and Fig. 13.

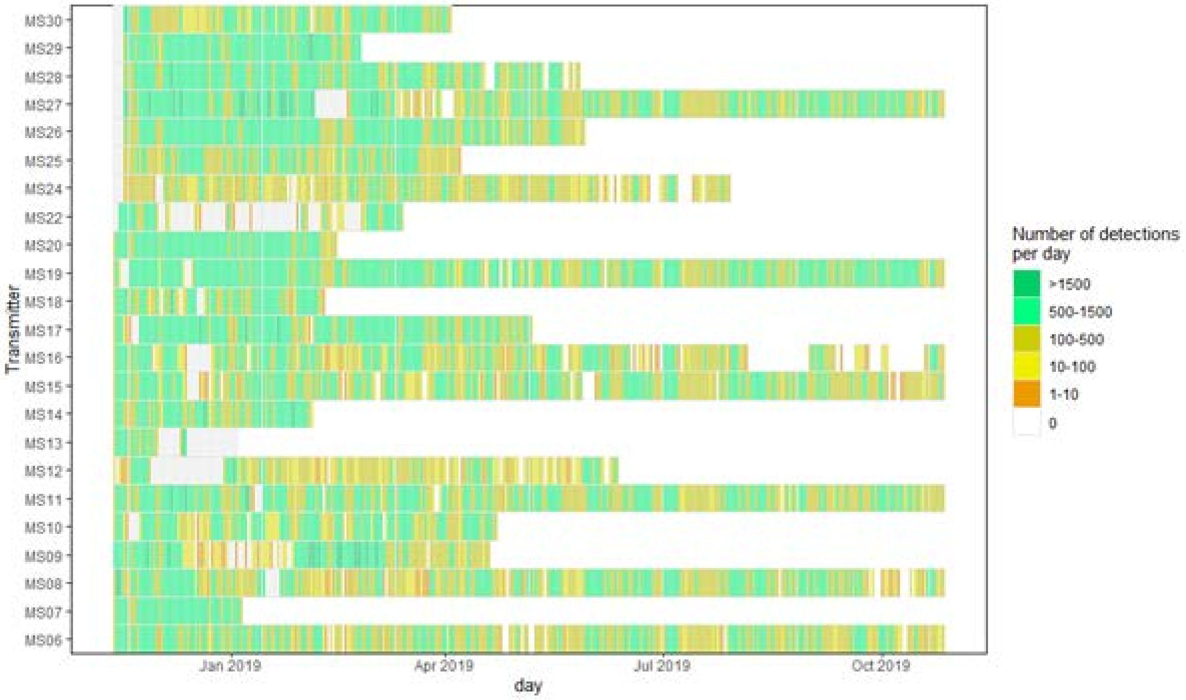

Fig. 13. Abacus plot showing daily detection information for all but two of the tagged Maugean Skate (the two excluded individuals were detected for < 2 days).

Table 2. Summary of detection and biological data of acoustically tagged Maugean Skate (N=25).

Detection index (DI) represents the proportion of days an animal was detected from the total days at liberty. DI values below 70 % are highlighted in red (non-resident). See methods section for a description of how mortalities and departures were determined.

Transmitter id	Tagging date	Last detection+	Sex	TL (mm)	Days at liberty	Days detected	Detections (Total)	Stations	DI* %	Observations
MS06	13/11/2018	27/10/2019	M	695	348	335	142941	26	96	
MS07	13/11/2018	05/01/2019	M	670	53	53	37486	23	100	mortality
MS08	13/11/2018	27/10/2019	M	620	348	328	150053	40	94	
MS09	13/11/2018	19/04/2019	M	725	157	142	84937	49	90	mortality
MS10	13/11/2018	22/04/2019	M	690	160	152	86921	32	95	mortality
MS11	13/11/2018	27/10/2019	F	710	348	342	187276	38	98	
MS12	13/11/2018	12/06/2019	M	705	211	169	50754	13	80	mortality
MS13	13/11/2018	13/12/2018	M	700	30	22	13180	31	73	departure
MS14	13/11/2018	04/02/2019	M	700	83	83	54831	19	100	mortality
MS15	13/11/2018	27/10/2019	M	735	348	326	158200	45	94	
MS16	13/11/2018	27/10/2019	M	680	348	276	115375	28	79	
MS17	13/11/2018	07/05/2019	M	665	175	171	116637	29	98	departure
MS18	13/11/2018	09/02/2019	M	715	88	84	44974	21	95	mortality
MS19	13/11/2018	27/10/2019	F	805	348	339	211098	49	97	
MS20	13/11/2018	14/02/2019	M	720	93	93	66007	27	100	mortality
MS21	14/11/2018	16/11/2019	F	780	2	2	<100	1	-	departure
MS22	14/11/2018	14/03/2019	F	765	119	57	27687	48	48	departure
MS23	15/11/2018	16/11/2019	F	820	1	1	<100	1	-	departure
MS24	17/11/2018	29/07/2019	F	780	254	231	67861	45	91	mortality
MS25	17/11/2018	07/04/2019	F	795	141	142	67812	46	100	mortality
MS26	17/11/2018	29/05/2019	F	775	193	194	123131	16	100	departure
MS27	17/11/2018	27/10/2019	F	725	344	323	202366	49	94	
MS28	17/11/2018	27/05/2019	F	690	191	183	119417	46	96	mortality
MS29	17/11/2018	24/02/2019	F	730	99	100	78891	26	100	departure
MS30	17/11/2018	03/04/2019	M	705	137	137	74791	32	100	mortality

+ For individuals that died, the last detection date represents the last day on which the individual was judged to be alive.

* For individuals that died or departed the array area permanently during the study, DI was calculated for the period spanning from release to the last detection.

Permanent departures

In the present study there were five individuals (two males and three females, ranging in size from 665 to 820 mm TL) that appeared to permanently depart from the study area at different times during the observation period. The last detection for all but one of these tags came from a receiver at the outer edge of the array, suggesting that the halt in detections resulted from the individuals leaving the array area and not from a failure of the tag (Fig. 14). Due to the spatially restricted array configuration used in this study, the fate of any skate that left the array cannot be established with any certainty.

Assumed mortality events

Assumed mortality events occurred 54 to 255 days after tagging and thus highly unlikely to have been directly linked to handling and tag application. Le Port et al. (2008) noted that in both captive and recaptured short-tailed stingrays tagged with similar dimension tags using the same methodology, the point of monofilament line insertion completely healed, and the animals were in healthy condition. This elevated mortality rate (44%) does, however, raise concerns so it was decided to expand the analysis of behavioural and environmental patterns to investigate the possible causes and implications of these deaths.

It is feasible that factors not associated with the cessation of movement of the tagged individual due to death could produce similar detection patterns to AMEs. Factors such as failure of the acoustic tags or detachment from the animal are possibilities that need to be considered prior to making inferences about the possible death of an individual. For each AME, the affected individual was within detection range of more than one receiver at the estimated time of death and all tags continued to record and broadcast data for the remainder of their battery life. Data from each of these skate confirmed that the environmental and depth sensors continued to function normally prior to and after the AME, discounting the possibility of tag malfunction or failure. Furthermore, almost all of the tags shifted depth on one or more occasions after the AME (horizontal movement was harder to detect due to the higher uncertainty of the location estimates when compared with the more precise pressure sensor used to estimate vertical movement). These ‘relocations’ were inconsistent with the typical movement of live skate and almost certainly reflect the neutrally buoyant tag being moved by currents, usually being slowly pushed down a slope into a deeper site. Notably, none of these relocations occurred until several weeks following the AME. Such a pattern is consistent with what would be expected if the tags were initially anchored (presumably to the corpse of a skate), only becoming ‘mobile’ once decomposition had advanced enough to release them. These observations provide a strong indication that instances where movement of individuals stopped abruptly, are very likely to reflect true mortality events and not failure of the tags or attachment method.

Sex and size did not appear to be factors in terms of which individuals died or relocated, and there were no apparent patterns linking the locations where the assumed mortalities occurred (Fig. 14). The earliest mortality occurred on the 6 January 2019 and the latest on the 31 July 2019, 54 and 255 days after tagging[2]. Most mortality and relocation events (14 out of 18) were clustered in one of two discreet time periods each lasting between three and eight weeks; the first from 1 January to 25 February (2 departures and 4 mortalities), and the second from 6 to 27 April 2019 (3 departures and 4 mortalities). The remaining three mortalities occurred on 30 May, 14 June and 31 July 2019, respectively (Table 2).

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[2] Note: The last detection date reported in Table 2 effectively represents the day prior to the mortality event.

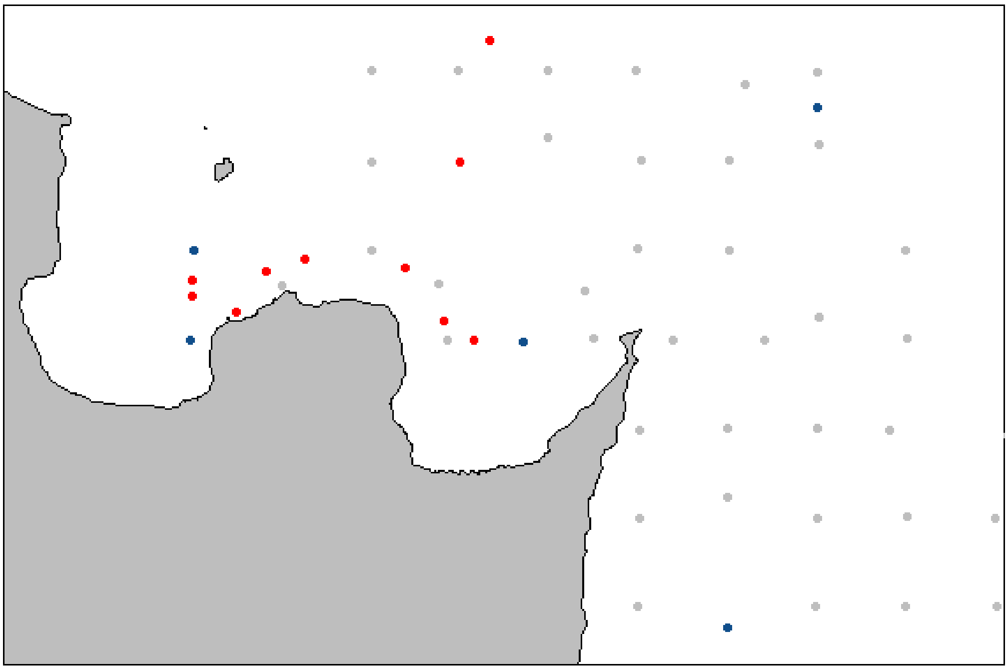

Fig. 14. Approximate locations of departure points (blue) and mortality events (red) for tagged Maugean Skate. Grey points show the location of listening stations in the acoustic array.

Behavioural change point analysis

Multivariate changepoint analysis detected significant inflection points in all individuals based on 3D-COAs (Lat, Long, and depth) calculated at 4 hr intervals. Time series with the estimated location of behaviour change points are shown for all individuals in Appendix 4. The timing of AMEs were detected as significant change points in each of the individuals that died. Time of death estimated empirically by the model was used as the cut-off point for which data to include in other analyses.

Overall, there was a high level of inter-individual variation in the timing of significant behaviour changes. However, there were four instances where behaviour changes (which include timing of AMEs) were temporally grouped and involved most of the available tagged individuals, some of which coincided with changes in environmental conditions, e.g. a period of high temperature and low DO during summer and a large storm in March. The first occurred during the first two weeks of December 2018, with 81% of the detected skate showing significant changes in behaviour (Fig. 15). The second coincided with one of the high mortality periods described above (January-February 2019), with 91% of the skate showing significant changes in behaviour (including AMEs). The third coincided with a large storm event on the 20 March 2019 and the subsequent three days, with 81% of the skate showing significant changes in behaviour. Lastly, the fourth coincided with the second high mortality period (April 2019), with 100% of the skate showing a significant change in behaviour.

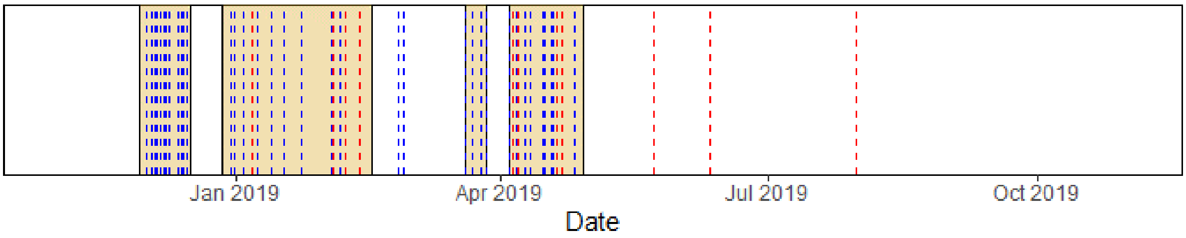

Fig. 15. Dates of mortalities (red) and behavioural changepoints (blue) based on multivariate change point analysis.

Horizontal space use

Home range modelling was performed on the seven individuals that were detected throughout the study period; an example of spatial analysis outputs is provided in Fig. 16. The variograms for the movement data showed a clear asymptote, suggesting that the Maugean Skate have true home ranging behaviour and individuals cover the full extent of their home range within one or two days (e.g. Fig. 16 B,C, and Appendix 5). All the home ranges overlapped considerably and consisted of the area adjacent to Table Head. The modelled core home range (50% UD) included a corridor which corresponds to a narrow channel that connects both sides of Table Head (e.g. Fig. 16A). The mean extent of the 95% UD was 9.1 km^2 (± 1.9 km^2 SE) and the 50% core UD was 0.6km^2 (± 0.07 km^2 SE).

Whilst detected, all individuals showed a high degree of site preference towards the Table Head area regardless of month (Appendix 5). Overall, the skate had small home ranges (< 10 km^2) through which they moved in a consistent manner. However, at different times of the year, most individuals displayed a change in behaviour where they briefly travelled to sites away from the home range in short excursions (< one week). This behaviour resulted in sudden increases in daily travelled distances. Generally, this alternative behaviour tended to be short lived and was followed by a return to the normal home ranging behaviour. For example, a male skate (MS15) remained largely within the same area from the time it was released until the end of the study, but in April 2019 it undertook several trips into deeper areas in the central basin of the harbour (Fig. 17). A summary of monthly site use and travel distance for all individuals is presented in Appendix 6.

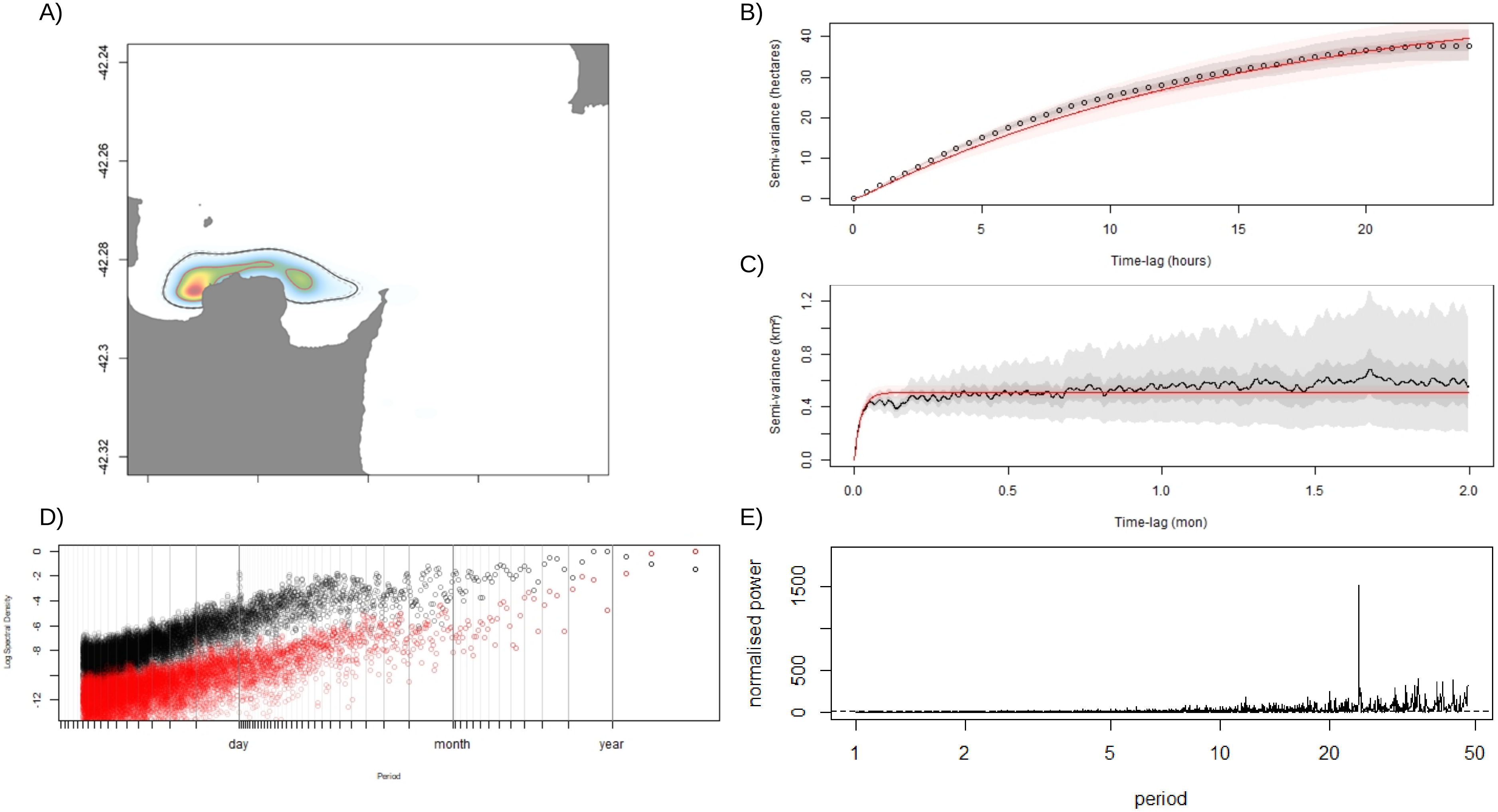

Fig. 16. Example of space use analysis for one of the electronically tagged Maugean Skate (MS06). (A) Utilization distribution showing core home range (50% UD) (red) and extended range (95% UD) (black) estimated using an OUF continuous time stochastic model. (B) Short term lag (hr) continuous time variogram for semi variance in ha with confidence and prediction intervals. Red line represents the fitted model. (C) Long term lag (Month) continuous time variogram for semi variance in ha^2 showing a clear asymptote at around 0.4 ha which is consistent with true home ranging behaviour. Red line represents the fitted model. D) Periodogram of movement variance (black) with diagnostic points (red) showing autocorrelated error due to the sampling interval. There is a clear peak at 1 day that suggests a diurnal pattern that is unlikely to be explained by stochasticity (noise) or sampling bias alone. E) Lomb-Scargle periodogram of depth showing a significant peak in normalised power at a period of ~24 hrs.

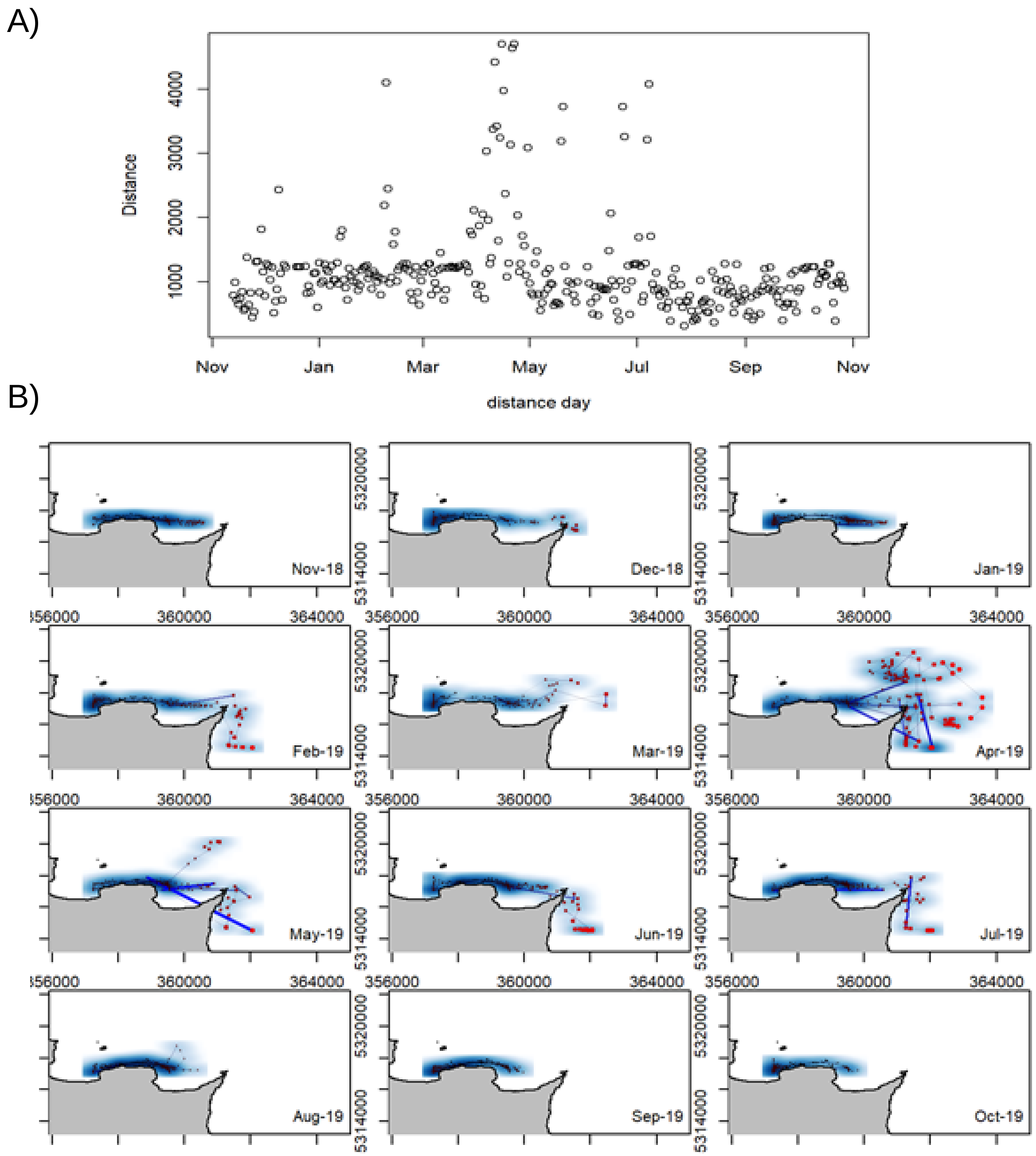

Fig. 17. A) Distance travelled per day (m) and B) monthly UDs (KUDs) showing horizontal activity areas for MS15. Movement paths represented inside the UDs to show the series of short trips taken by the individual towards the central basin in April and May 2019.

Short term patterns space utilization revealed similar trends. The extent of cumulative weekly 50 % UDs for the sample of tagged individuals (standard discreet time KUDs) did not change significantly through the year (One-way ANOVA: F=0.985, p=0.505) (Fig. 18A). In contrast, cumulative extended activity areas (95% UD) increased as the year went along (One-way ANOVA: F=3.01, p<0.001) (Fig. 18B). This analysis shows that when skate move into new sites the total extent of their activity area grows; however, because the time spent at these new sites is brief enough that the size of their core areas is not likewise expanded.

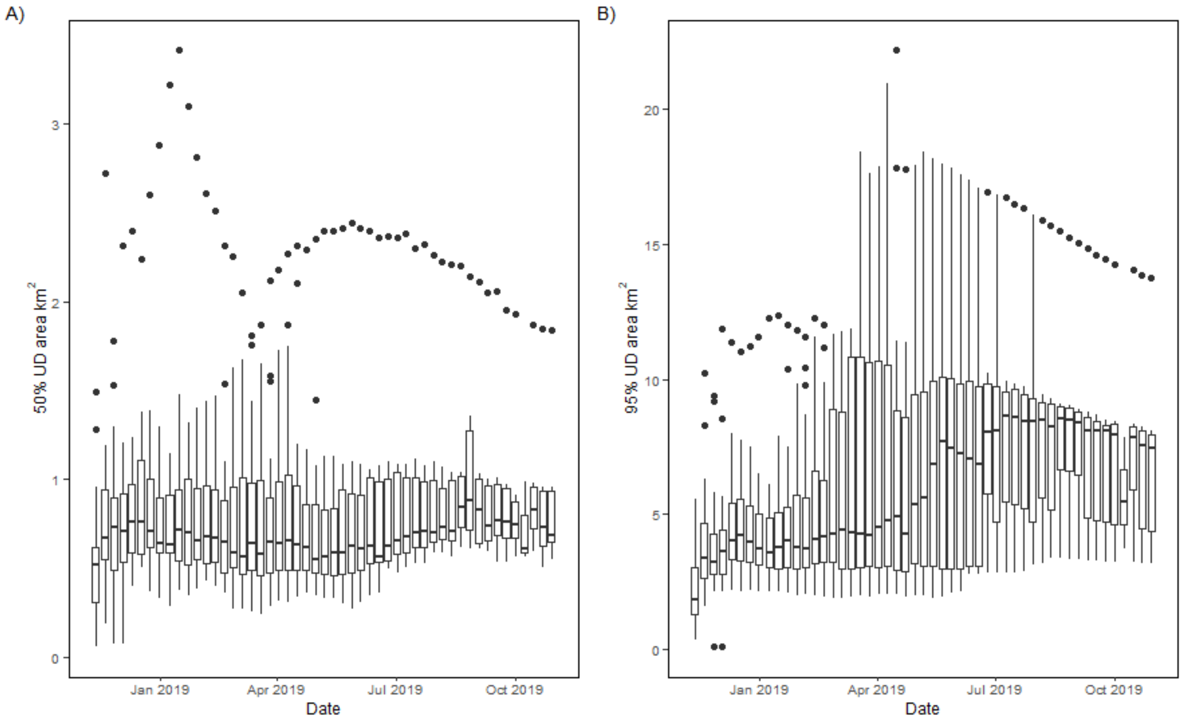

Fig. 18. A) Cumulative weekly core activity areas (50% UD) and B) extended activity areas (95% UD) for the seven Maugean Skate that were detected throughout the study period. Note the dots represent an outlier (MS27) that was unusually mobile throughout the study period.

Vertical space use

Skate were detected at depths ranging from <1 m to 55 m throughout the study period. Based on the 50% kernels, their preferred depth range is between 7.5 and 12.5 m. Average depth for the tagged individuals (~10 m) did not change throughout the year (Fig. 19A). The range and frequency distribution of depths did however vary depending on month. In January 2019, depth utilization patterns changed from a unimodal distribution around their preferred range, to a bimodal distribution indicating that individuals were spending more time at shallower sites (~5 m). In April and May, the amount of time that was spent at the shallow sites became even more significant, with none of the 50% kernel occurring within their preferred range (Fig. 19A). Between January and May, there was also an increase in short (< 1 day) back and forth trips into deeper sites, resulting in an overall increase in daily vertical activity areas (maximum depth – mean depth experienced by an individual every day) (Fig. 20).

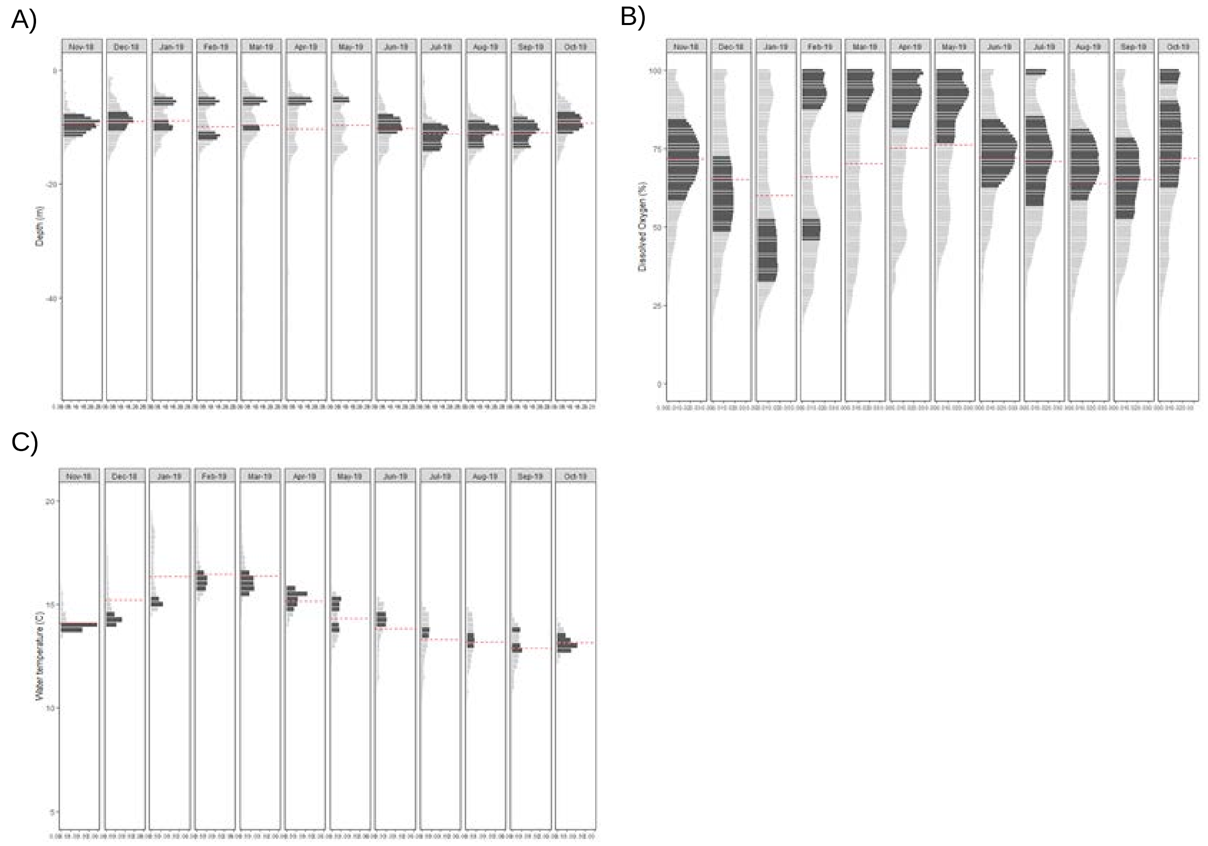

Fig. 19. A) Monthly depth (m), B) dissolved oxygen (%) and C) temperature (°C) activity spaces for the seven Maugean Skate that were detected throughout the study period. Distributions were calculated with a gaussian kernel estimator and weighted by individual based on number of detections. Dashed lines represent mean values per month and the darker area comprises the 50 % UD.

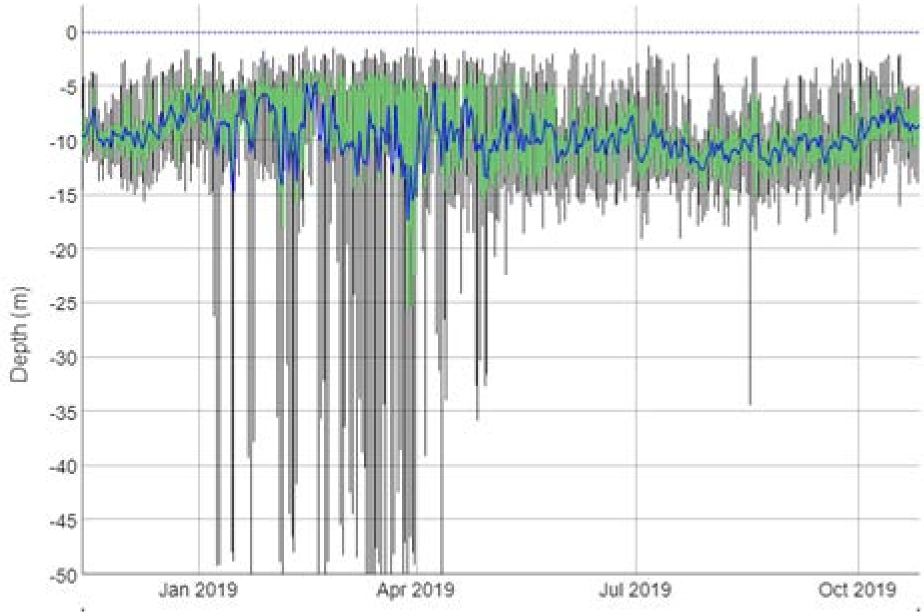

Fig. 20. Candle plot of mean depth range experienced every day by all individuals. The blue line represents mean depth and the green box the 75% quantiles.

Analysis of the discrete Markov chain model showed that there was a clear change in behaviour that influenced how skate moved between depths. The discreet Markov chain process is extemporaneous, so the changes in behaviour described by the model were independent of changes in time spent at different depths (see above). As such, this analysis does not describe what depths skate were most likely to occupy at any given time, but rather, how likely an individual is to move given its current state. Based on eigenvector centrality, the relative importance of shallow sites became increasingly greater from January to May, which resulted in a decrease in mean recurrence times (Table 3). This suggests that during this period there was an increase in vertical movement and that the skate were constantly travelling into the shallows.

Table 3. Network statistics for discrete time Markov chain models of vertical movement behaviour. Depths were classified into three states based on time spent at depth by all individuals during the study: shallow (0-7.5 m), preferred (7.5 – 12.5 m), and deep (> 12.5 m). Centrality shows the probability that an individual will end in each of the given states regardless of the starting state. Mean recurrence time was calculated in minutes and represents the average time that it takes an individual to return to a state once it departs.

State	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Eigenvector centrality												
shallow	0.17	0.38	0.54	0.53	0.48	0.48	0.40	0.20	0.10	0.06	0.10	0.30
preferred	0.77	0.53	0.37	0.33	0.38	0.27	0.34	0.60	0.61	0.64	0.67	0.62
deep	0.07	0.09	0.08	0.15	0.15	0.24	0.26	0.20	0.30	0.29	0.23	0.08
Mean recurrence time (mins)												
shallow	182	79	55	57	63	62	74	151	307	462	299	99
preferred	39	56	81	91	80	110	88	50	49	47	45	49
deep	446	335	354	207	203	123	118	151	101	103	129	364

Diurnal patterns of space use

Periodograms of movement variance for all individuals showed a significant peak at around 24 hrs (e.g. Fig. 16D, Appendix 7). Likewise, Lomb-Scargle periodograms for depth data showed a significant peak at a 24 hr period for all but one of the individuals detected for the entire study period (e.g. Fig. 16E, Appendix 7). However, the vertical and horizontal distributions of individuals did not appear to change much between day and night (Fig. 21). This indicates that even though the sites used by the Maugean Skate do not change throughout the day, there is a diurnal pattern of movement behaviour that reflects a difference in how individuals use the same spaces between night and day.

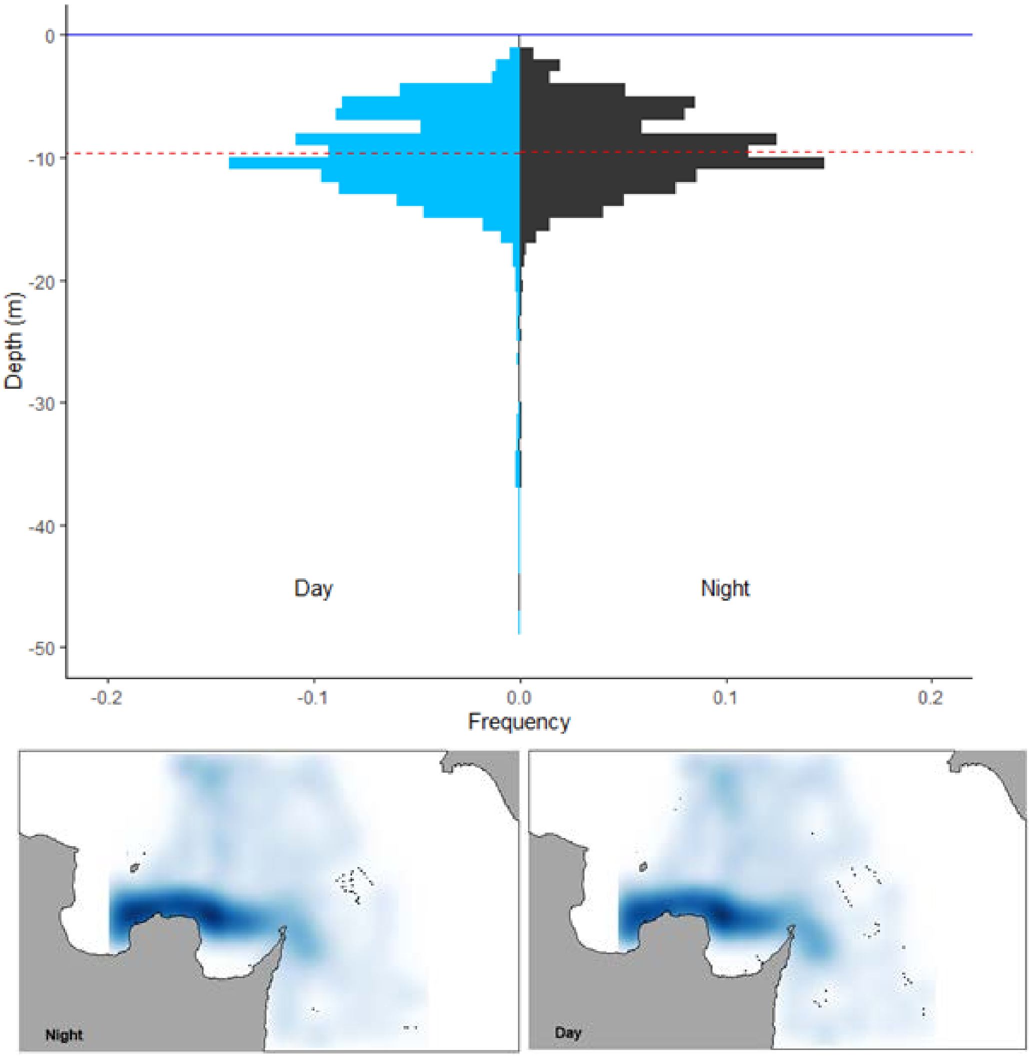

Fig. 21. Vertical (top) and horizontal (bottom) UDs for day and nighttime hours for all tagged individuals during residence periods within the array area. Red dotted line represents mean depth.

Environmental activity spaces

Through the monitoring period, Maugean Skate experienced DO concentrations ranging from 100% to < 1% (Mean: 71% ± 6.5, Median: 72% ± 6.9) and all individuals experienced conditions below 25% at some point. Mean DO levels experienced by the tagged skate varied by month and were lowest in January (~ 60%) and highest in May (~75%) (Fig. 19B). Monthly DO UDs for the tagged skate are shown in Fig. 22. Variability was much higher than anticipated (mean 40% daily range of DO) and all individuals experienced a difference of >75% in DO levels within the same day at least once. Daily DO variability was highest near the preferred depth range of the skate (7.5 -12.5 m) and became progressively more stable (and generally higher) at deeper and shallower sites (Fig. 22).

DO levels recorded by the tags (near the benthos) were comparable to the interpolated values (based on sensor string data) at equivalent depths in the water column for periods when the skate were alive (Fig. 23A). Tag data post-death for the eleven individuals that died within the array was also compared to the water column data. These tags remained in a static position and acted as sentinels at or very close to the substrate-water interface. Only data from the first month after death was used, since the tags are likely to have been slowly covered by sediments which would affect DO readings. In the main, DO values recorded by these static tags were typically lower than concentrations at equivalent depths in the water column (Fig. 23B), suggesting that DO levels at or near the water-benthos interface may not be homogeneous by depth and/or that some readings may have been impacted by sedimentation effects. If the former, the data imply that at given depths skate may actively select areas where DO is higher or, conversely, avoid areas of low DO.

Available temperatures as well as the mean temperature experienced by the skate varied depending on time of year and largely followed a seasonal pattern. Individuals experienced water temperatures ranging from 8 to 21 °C. The daily temperature variation experienced averaged 1.9 °C, although some individuals experienced temperature differences as high as 7.8 °C on certain days. Location of the 50% kernels for the monthly temperature UDs show that the skate had a strong preference for temperatures between 13-15 °C (Fig. 19C). During the summer months, when ambient temperatures were above that range, individuals spent most of their time at the cooler available sites. The opposite was true during winter, when individuals favoured sites within the preferred range over other much cooler areas that became available.

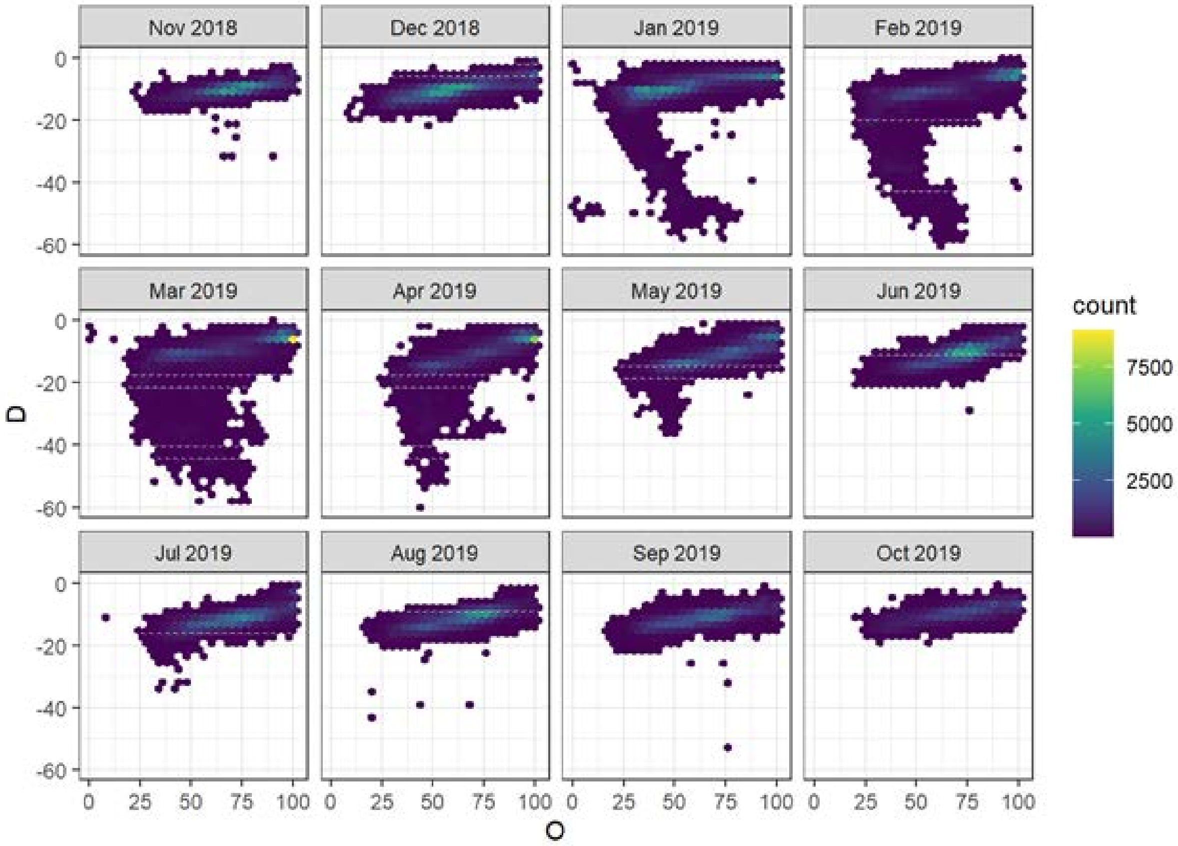

Fig. 22. Two-dimensional distribution of depth and DO activity spaces experienced by the seven Maugean Skate that were detected throughout the study period.

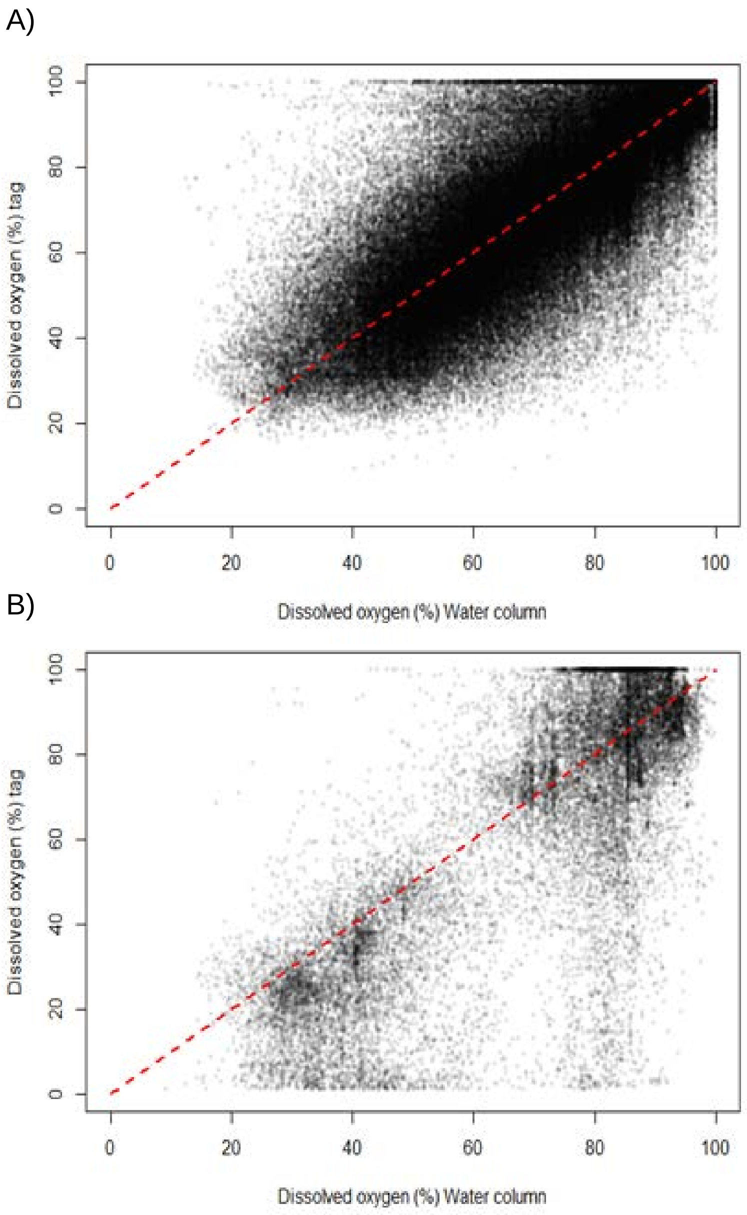

Fig. 23. Comparison of dissolved oxygen concentrations recorded by tags and interpolated based on sensor string data at equivalent depths. A) Data for skate whilst alive. B) Tag data transmitted one-month post-mortality event. Red dotted line represents 1:1 correlation.

Environmental drivers of space use

Analysis of the daily drivers of activity area showed that for both the 50% and 95% UDs, the best fitting model included sex, total length, dissolved oxygen, temperature, salinity, and their interactions (Table 4). For weekly activity spaces the best fitting model had the same parameters except for salinity (Table 4). Notably, most models that included DO across all response variables differed significantly from the null model, suggesting that DO at the preferred depth of the skate has an effect on the extent of activity areas. This consistency suggests that results were not driven by the sampling period chosen and reflect a true link between environmental variables (particularly DO and temperature) and space use patterns of the tagged Maugean Skate. However, models with only environmental variables performed worse than those with environmental variables, sex, and size, suggesting that space use is likely determined by a combination of biological and environmental factors.

Table 4. Models showing the effects of biological and environmental factors on the core and extended UD areas of the Maugean Skate calculated daily and weekly. Akaike's information criterion (AIC) of all candidate models is provided and the best fitting (most parsimonious) model is highlighted in red. The Null model included only individual as a random factor. Asterisks indicate level of significance of chi squared test comparing the candidate models to the Null model**.**

Model formula				**Daily**		**Weekly**	
				50 UDs	**95 UDs**	**50 UDs**	**95 UDs**
				AIC	**AIC**	**AIC**	**AIC**
fit 0	√area	~	1	11270	12019	1760.8	1782.1
fit 1	√area	~	Date	11257 ***	12011 **	1765.5 .	1783.8
fit 2	√area	~	Sex	11272	12021	1762.7 ***	1783.9
fit 3	√area	~	TL (mm)	11271 ***	12120 ***	1762.5 ***	1783.3 ***
fit 4	√area	~	DO (air%)	11189 ***	11952 ***	1739.2	1756.1 ***
fit 5	√area	~	Sal (ppt)	11266	12016	1758.6	1780.5
fit 6	√area	~	Temp (°C)	11267	12016	1761.8	1784
fit 7	√area	~	Sex * TL	11273	12022	1762.6	1784
fit 8	√area	~	DO * Temp	11189 ***	11950 ***	1741.1 ***	1754.6 ***
fit 9	√area	~	DO * Sal	11173 ***	11934 ***	1740.5 ***	1754.9
fit 10	√area	~	DO * Temp * Sal	11154 ***	11916 ***	1746.3	1759.3
fit 11	√area	~	Sex * TL * DO * Temp	11150 **	11917 *	**1736** ***	**1748** ***
fit 12	√area	~	Sex * TL * DO * Temp * Salinity	**11128** ***	**11891** ***	1755.5	1764.1

Eco-physiology

Adults

Oxygen consumption was measured before and after 96-hour exposure for the three treatments. The rate of oxygen consumption (MO_2) for each skate was calculated at either 20% DO, 27 ppt (hypoxia treatment) or 90% DO, 13.5 ppt (hyposaline treatment) and compared to the corresponding pre-exposure control as well as the normoxic treatment control (Fig. 24). Hypoxia acclimated skate exhibited a significantly lower metabolic rate compared to the acutely exposed control ($p = 0.006$) when oxygen consumption was measured at 20% DO. The hyposaline group exhibited lower values approaching significance ($p = 0.062$) post exposure compared to the control group.

Plasma lactate concentrations were calculated as a proxy for anaerobic metabolism. The hypoxia exposed group demonstrated significantly higher ($p < 0.001$) lactate concentrations than their control (at 90% DO), but these values remain extremely low overall (Fig. 25). The hyposaline group did not differ significantly from its control (at 27 ppt). Given that animals can store lactate in tissues, lactate concentrations in heart and white muscle were also measured. No significant difference from the controls were found for either treatment ($p = 0.99$ and $p = 0.061$ for hypoxic and hyposaline treatments, respectively) (Fig. 25).

To understand if there were any changes in ketone body oxidation as a cellular fuel, both acetoacetic acid (AcAc) and β-hydroxybutyrate (BOH) were measured and added to calculate total ketone body concentrations (Fig. 25) in plasma samples from before (control) and after (treatment) exposure. The hypoxia exposed group had a significantly lower plasma ketone concentration than their control ($p < 0.001$) while the hyposaline group had a significantly higher plasma ketone concentration than their control ($p = 0.008$).

Plasma osmolality declined significantly from the control following exposure to the hyposaline treatment ($p < 0.005$) (Fig. 25).

Glycogen concentrations in the liver and white muscle were investigated to elucidate glucose use as a metabolic fuel. Concentrations in both tissues (liver and muscle) decreased compared to the control following exposure to the hypoxic and hyposaline treatments (Fig. 25).

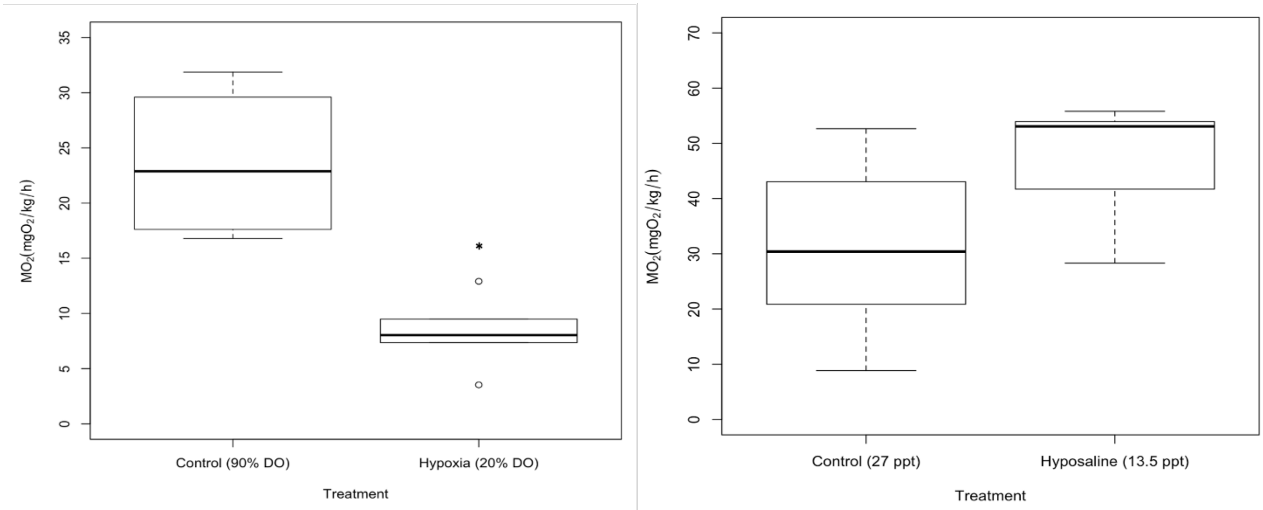

Fig. 24. Boxplot of Maugean Skate metabolic rate (MO2 mgO2/kg/h) before (control) and after 96-hour exposure to the hypoxic (left) and hyposaline (right) treatments. Boxes represent the median and standard quantiles**.**

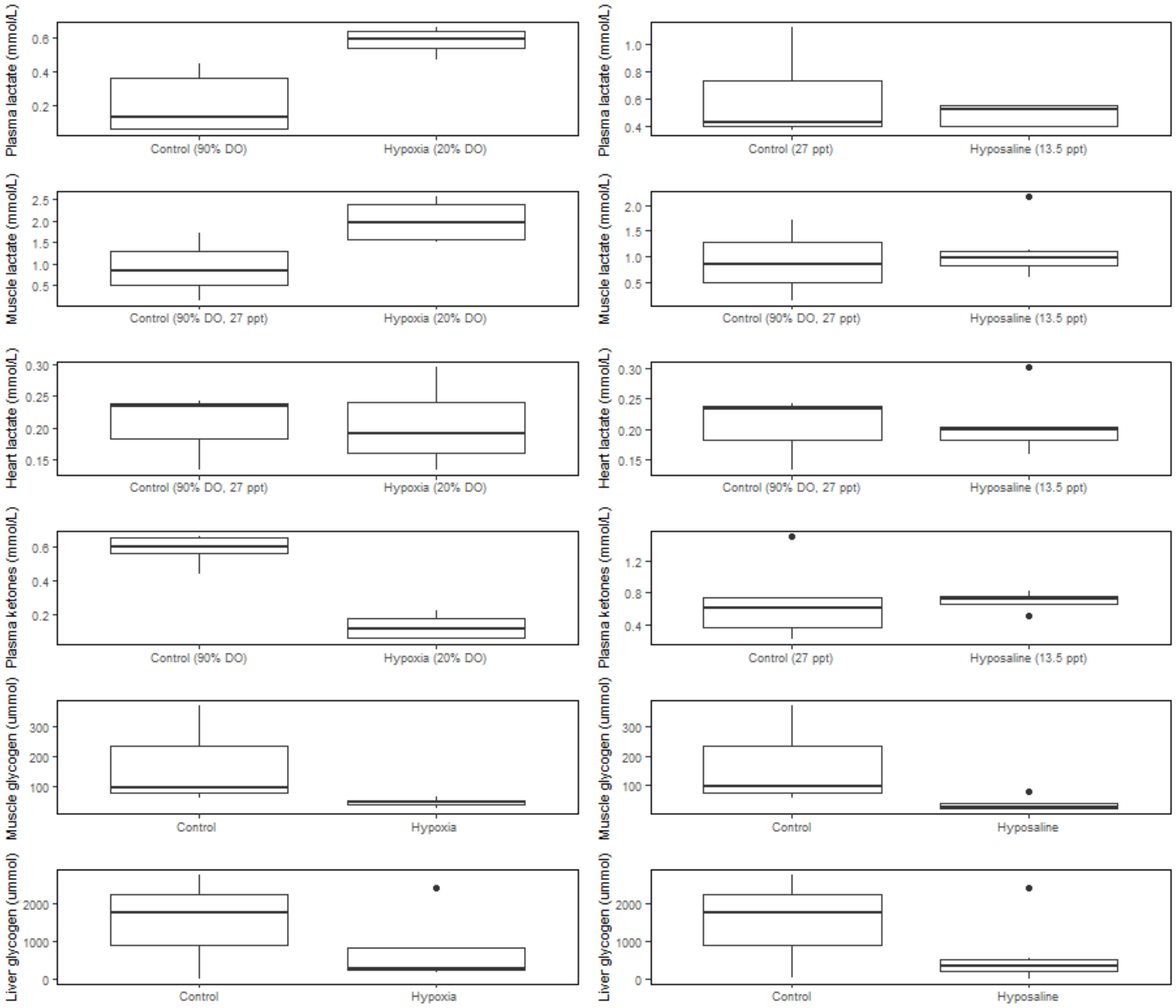

Fig. 25. Results of haematological tissue assay analysis measuring multiple metabolites before (control) and after 96-hour exposure to the hypoxic (left) and hyposaline (right) treatments. Boxes represent the median and standard quantiles.

Neonate

Oxygen consumption of the newborn skate was measured using intermittent respirometry one month after hatching and three weeks after the individual began feeding (TL=12.9 mm, TW=11.45 g). Linear regression fitted separately to each closed period showed R^2 values > 0.9. Base aerobic metabolic rate (MO_2) corrected for volume, mass, and background was estimated from the average slopes as 30.25 $mgO_2\,kg^{-1}\,h^{-1}$ (± 2.01 SE) at 15 °C.

Baseline blood metabolite levels were measured before the pre-exposure static respirometry trial. Lactate was 0.6 mg L, glucose levels were below the detection threshold of the reader and remained so for the remainder of the experiment (< 1.1 mol L) and haemoglobin was 28 g L. O_2 levels during the trial were allowed to drop until consumption became negligible, reaching a final concentration of 66% DO (acute hypoxic exposure). Following acute hypoxic exposure, lactate levels were 2.3 mg L and haemoglobin declined to 23 g L. The skate was found dead three days into the 96 hr exposure treatment and haematological analysis were conducted a final time, showing lactate levels of 6.35 mg L and haemoglobin levels of 16 g L.

MO_2 levels at 5% DO intervals were calculated from the two static respirometry trials and averaged. Metabolic rate of the individual declined linearly with oxygen availability (Fig. 26). Critical oxygen partial pressure (P_{crit}), the DO level below which the rate of oxygen consumption can no longer be maintained, was estimated to occur at 66.33% DO (± 0.44 SE).

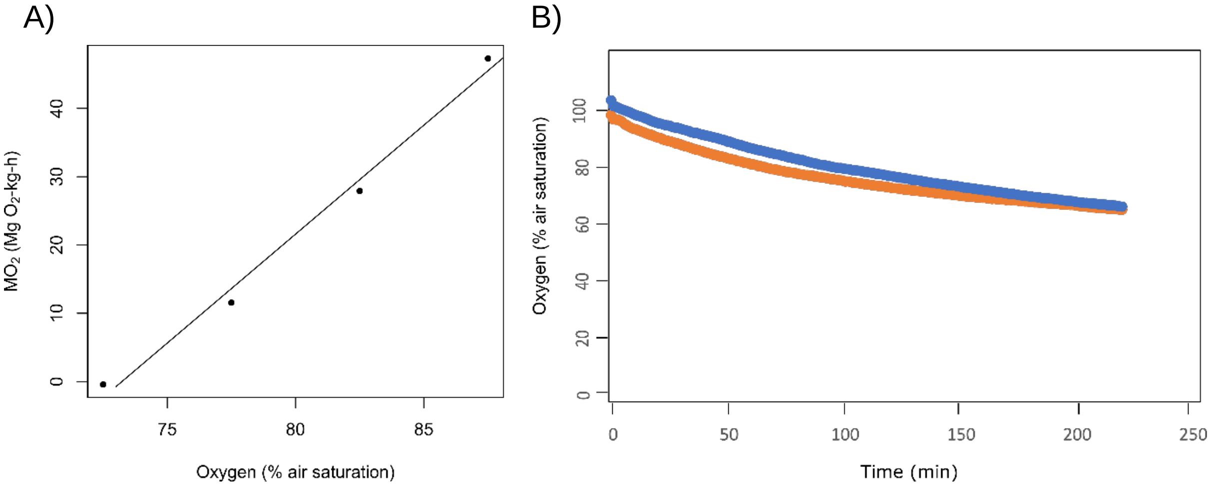

Fig. 26. A) Rate of oxygen consumption (MO_2) across a range of environmental dissolved oxygen concentrations, and B) oxygen consumption curves for two static respirometry experiments in a neonate Maugean Skate.

Size composition

There was a total of 284 individual gillnet deployments in the Table Head/Liberty Point area between February 2017 and November 2018, leading to the capture of 103 individual skate (females=46, males=57). The smallest individual captured was 450 mm TL and the largest was 830 mm TL (Fig. 27). Data were also available for 84 individuals sampled in 2012 (FRDC2010-016) and 125 individuals sampled in 2013 and 2014 (FRDC2013-008) (Fig. 27.)

Median size and size distributions did not differ between the 2012 and 2013-14 samples for either sex (Wilcoxon rank sum test [Males, W=1230, p=0.29; Females, W=1180.5, p=0.98], Kolmogorov-Smirnov test [Males, D=0.21, p=0.12; Females, D=0.14 , p=0.63]), but there was a significant difference between 2013-14 and this study for both median size and size distributions (Wilcoxon rank sum test [Males, W=1387, p=0.02; Females, W=957.5, p=0.01], Kolmogorov-Smirnov test [D=0.29, p<0.01; Females, D=0.25, p=0.05]). Furthermore, there was a significant difference in the proportion of juvenile and sub-adult size classes (≤ 600 mm) (Bell et al., 2016) between samples (χ^2=7.03, p=0.03), with the lowest representation (7.8%) in the current study, compared with 18-20% in the earlier studies (Fig. 27).

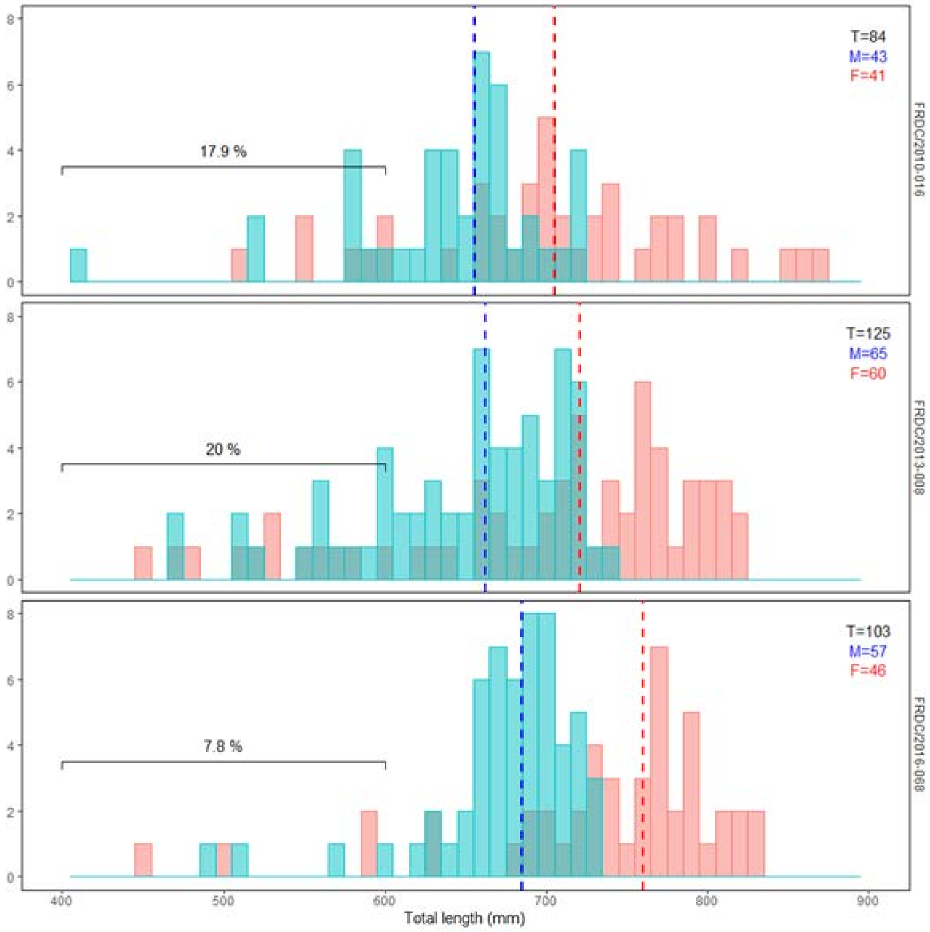

Fig. 27. Size distribution of male (blue) and female (red) Maugean Skate sampled from Table Head/Liberty Point during 2012 (top panel; FRDC2010-016), 2013-14 (centre panel; FRDC2013-008) and 2017-18 (bottom panel; current project FRDC2016-068). Vertical dashed lines indicate median lengths of males (blue) and females (red). Sample sizes are indicated (T= sexes combined, M = males, F = females), value above the horizontal bracket indicates the proportion of individuals < 600 mm present in each sample.

Discussion

This study has contributed to a greater understanding of the ecology, physiology and life history of the Maugean Skate and describes for the first time some of the behavioural and physiological adaptations that have allowed the species to survive in such a unique and challenging environment. Our results suggest that the species can mitigate some changes in the environment by altering their movement behaviour; as well as being capable of tolerating moderate to severe long-term exposure to hypoxia through metabolic depression. However, a recent shift in the size structure of research gillnet catches, the vulnerability of early life stages to the prevailing environmental conditions and an unexpectedly high rate of mortality amongst tagged individuals that appears to be linked to environmental events, are suggestive that the skate population may be under stress and may have reached the limits of their tolerance to environmental change. Collectively, these findings highlight the vulnerability of the Maugean Skate to the degraded and varying environmental conditions in Macquarie Harbour.

Early life history

This study provides the first observations on the distribution and abundance of eggs, hatching success rates, egg predation rates, incubation period and captive hatching of the Maugean Skate.

Maugean Skate egg cases were found across a wide range of depths (2.5 - 33 m), being most abundant at depths < 10 m, and tended to be clustered into a small number of localised areas rather than widely distributed throughout the study area. However, making inferences about spatial patterns of oviposition using egg capsules has inherent limitations. The lack of tendrils or attachment structures on the capsules means that eggs may be passively displaced by water currents (Cox et al., 1999) and thus the location where eggs are collected does not necessarily reflect the site of oviposition. For example, the locations of egg ‘hotspots’ in Swan Basin and Table Head/Liberty Point consisted of narrow channel systems which probably focus water flow in these areas. It is thus unclear if these sites are being selected by female skate for oviposition, or if the structural complexity of the channels function as collection-points where eggs tend to accumulate. Furthermore, virtually all capsules were empty and thus had been in the system for protracted periods during which they are likely to have been displaced from the site of their deposition. Regardless, the higher density of eggs seen at these sites suggest that these channels might be useful as a sampling baseline to monitor seasonal patterns in the future.

Prior to this study, all knowledge about Maugean Skate eggs and possible preferred locations or depths for egg laying, came from a pair of partially formed eggs found in the shell gland of a pregnant female (Treloar et al., 2006) and a pair of eggs (one empty case and one live-egg) incidentally captured during a gillnet survey (Treloar et al., 2017, Kirkpatrick et al., 2019). Given that the later were collected from a deep region of the harbour in the vicinity of the World Heritage Area (> 30 m) and that egg cases had not been previously observed during dive surveys at shallow sites, it was hypothesized that Maugean Skate may exclusively deposit eggs in deeper water as a strategy to reduce predation and provide more stable environmental conditions (especially temperature and salinity) (Treloar et al., 2017). Results of the present study clearly demonstrate the Maugean Skate lay eggs their eggs across a wider range of depths, including some unexpectedly shallow habitats (~2.5 m).

Observations of egg development and successful hatching of one individual represent the first account of the duration of incubation (~7 months) and size at birth (122 mm TL) in the Maugean Skate. The incubation period of skates can vary from a few months to multiple years depending on the species and environmental conditions. For example, a meta-analysis of published incubation rates for the Alaska Skate (*Bathyraja parmifera*) found that a mean temperature increase of 0.5 °C could reduce incubation by almost 6 months (16% of the estimated mean gestation of 3.5 years) (Hoff, 2008). The Maugean Skate egg successfully reared under laboratory conditions was kept under constant temperature conditions and thus incubation duration in the wild is likely to differ depending on environmental temperature and variability, which in Macquarie Harbour is closely associated with depth. However, the captive rearing trial provides a first empirical approximation of how long this crucial period of the species life history can last. Along with the size at birth data, these observations fill a crucial knowledge gap in early life-history traits for the species that will contribute to understanding age and growth (Bell et al., 2016) and population dynamics; ultimately serving as a valuable reference point for future assessments of the stock and conservation status of the species. In addition, this result demonstrates that it is possible to successfully rear Maugean Skate under captive conditions, providing a protocol that could be used as an active conservation tool for the species in the future, i.e. captive breeding.

There is some evidence that elasmobranch egg membranes (Evans, 1981) and yolk mass (Takagi et al., 2017) may act as an osmotic and ionic barrier between the embryo and the external environment at the earliest stages of development. However, after the respiratory slits of the egg become unblocked, the individual is fully exposed to external water conditions for the remainder of the gestation period. Unlike adults, eggs are not able to relocate as a mechanism to mediate environmental conditions, noting, however, that the observed mortalities of adults demonstrate that the ability to relocate may not always provide relief from adverse environmental conditions. Furthermore, although results from the newborn experiment should be considered as preliminary, they suggest the possibility that at the earliest life stages, the species' tolerance limit to hypoxia may be much lower than that of adults, a phenomenon that has been observed in other taxa. For example, lower tolerance thresholds to hypoxia, temperature and salinity during larval and early juvenile phases were reported in the Red Sea Bream (*Pagrus major*) as a consequence of the higher metabolic demands associated with the elevated growth rates seen at these early life stages (Ishibashi et al., 2005). Therefore, the environment conditions that the developing embryo is exposed to could present significant physiological challenge, conditions that are linked in part to the depth at which the egg is deposited. At deeper sites, eggs are likely to experience stable temperature and salinity conditions but be regularly exposed to hypoxic or even anoxic conditions for prolonged periods. At shallower sites, eggs will be exposed to highly variable temperatures depending on the season and hyposaline conditions.

Based on the condition of the eggs collected in the wild, a hatching success rate of just over 40% was estimated for Maugean Skate, which is comparable with other temperate skate species (Smith and Griffiths, 1997, Frisk et al., 2002, Cox et al., 1999). There is a growing body of research showing that tolerance thresholds to abiotic factors in oviparous elasmobranchs are likely to be affected by interactions with other stressors, therefore affecting survivorship during incubation. For example, elevated temperature was shown to increase embryonic development rates and improve fitness at the time of hatching (although at a smaller size at birth), but when reared under hypoxic conditions, egg mortality increased and the relationship between increased fitness and temperature was reversed (Musa et al., 2020). Likewise, event related changes in environmental conditions could have other indirect effects during gestation. For example, in some fish species, hypoxia may result in premature hatching, lowering the fitness of the individual and forcing it to begin feeding earlier (Di Santo et al., 2016). Therefore, these multi stressor interactions and their effect on the early life physiology of the Maugean Skate needs to be further examined in order to better understand the vulnerability of this critical life stage, as any factor affecting recruitment through reduced egg survivorship will have important consequences for the population.

Egg predation rates reported for multiple skate species ranged from 14 to 40% with an average of 24% (Lucifora and Garcia, 2004). This suggests that the minimum predation rate of 20% calculated in the current study from egg cases is close to the average of what is commonly experienced by other species. The least common of the two types of predation damage seen in egg capsules of the Maugean Skate was similar to boreholes often reported for other skate species that have been linked with boring gastropods (Lucifora and Garcia, 2004, Cox et al., 1999, Smith and Griffiths, 1997). The more prevalent type of predation damage consisted of straight cuts or tears that resulted in a large slit. Macquarie Harbour is naturally depauperate and there are only a few species that could commonly predate on Maugean Skate eggs (Edgar et al., 1999). The most common large predator in the system is the White-spotted Dogfish (*S. acanthias*), but dietary analysis showed no evidence of feeding on eggs (Moreno 2018). Based on the characteristics of the tears and cuts seen on the egg cases, it is likely that they were caused by one or more of the three crab species common in the estuary (Richardson et al., 1997).

Use of estuarine nurseries is a well-documented trait of the biology and behaviour of elasmobranch species, although only a few studies so far have focused on batoid species. A nursery area must offer early life-history individuals advantages such as protection from predation or access to additional resources, preferential habitat or environmental conditions. A nursery can be broadly defined as an area where: (i) newborn or young individuals are more likely to be encountered than in other areas (ii) newborn or young individuals are likely to remain or return for prolonged periods, and (iii) newborn or young individuals repeatedly occur across multiple years (Heupel et al., 2007). If the area overlaps with the spatial range of adults then the advantages are conferred to the whole population and the site cannot be considered a nursery (Knip et al., 2010). Furthermore, for oviparous species such as the skate, development requirements for eggs and juveniles will not necessarily overlap and therefore juvenile nurseries and egg case nurseries can differ (Martins et al., 2018). Both egg case nurseries and juvenile nurseries (usually simplified to nurseries) can be of critical importance for a population, highlighting the need to properly define and identify them. Martins et al. (2018) proposed, using the criteria set by Hoff (2016), that an egg case nursery must show: (i) high densities of eggs and egg cases in contact with the benthos or substrate, (ii) adults must use the area to lay eggs repeatedly throughout multiple years (however, the area must be segregated from the normal range of the adults), and (iii) newborn and young of the year individuals must leave the area shortly after hatching.

Egg case nurseries have been identified for some skate populations, in particular deep-water species that use sites along the continental slope, including the sister species to the Maugean Skate, *Z. chileniensis* (Amsler et al., 2015, Hoff, 2016, Vazquez et al., 2016). These egg case tend to be restricted to relatively small, well delimited areas and can in some cases reach egg densities as high as 500,000 eggs/km^2 (Vazquez et al., 2016). Using the criteria stated above, there was no evidence in this study to support the existence of a Maugean Skate egg-case nursery. Distribution of adults and egg cases largely overlapped, and in fact, egg densities tended to correspond with sites and depths favoured by the adults. Given the naturally low predation rates in Macquarie Harbour, the high environmental variability and defined stratification of the water column, the lack of a defined nursery area likely affords the Maugean Skate an evolutionary advantage by limiting the proportion of eggs that are exposed to depth specific stressors (e.g. low bottom DO in mid waters or elevated temperatures at shallow sites during the summer months). However, anthropogenic habitat degradation could negate the potential advantages of such a strategy. Similar differential strategies have been hypothesised in other skate species that show seasonal peaks in egg deposition in winter and summer (Palm et al., 2011), representing a trade-off between longer gestation times but low predation rates due to low temperatures, and faster gestation and larger size at birth in warmer water, but at the cost of higher mortality rates.

Movement

Distribution and habitat utilisation

Acoustically tagged Maugean Skate exhibited a high level of site selectivity and site fidelity across a relatively small spatial extent. Despite some individual variation in long-term residency, noting that seven individuals appeared to permanently exit the study area sometime after tagging, the skate showed a clear predilection towards the Table Head and Liberty Point areas. Furthermore, home ranges calculated using continuous time movement models (only seven individuals had sufficient data for this analysis) showed a very high degree of spatial overlap in the core home ranges of each of the individuals. This analysis suggests that the species behave in a manner consistent with true home ranging behaviour (Fleming et al., 2014) and that this pattern remains constant throughout the year. In other words, despite changes in extended activity areas and short-term movement patterns, the core home range of these individuals revealed no evidence of seasonal shifts, expansion, or migratory movement. Similar patterns of long-term residency and limited dispersal are common (but not exclusive) in other small bodied skate species (Dulvy and Reynolds, 2002) and range-restricted coastal elasmobranchs (Thorson, 1982, Martin, 2005).

Individual utilisation distributions (UDs) indicate that Maugean Skate resident at Table Head and Liberty Point most frequently utilise a narrow band of habitat that runs parallel to the coast around the Table Head headland. Even though the models did not include explicit information about the topography of the area, this predicted area of increased use corresponds with a channel approximately 100 m wide and up to 13 m deep. Additionally, while increased densities of batoid species have been documented near aquaculture cages in other places (Martins et al., 2018, Wearmouth and Sims, 2009), skate in this study did not frequent sites near any of the aquaculture leases that overlap with the study area. These findings are consistent with previous knowledge about the movement patterns of Maugean Skate in Macquarie Harbour (Bell et al., 2016), providing further evidence that the Table Head and Liberty Point areas constitute an important habitat for the species (particularly the channel system) and that direct spatial interaction with aquaculture infrastructure continues to be limited.

While results from the generalised mixed effects models suggest that size and sex had an effect on the size of activity areas, the small number of individuals and size classes represented were insufficient to confer much weight to the predictive relationship. However, size and sex-based difference in space use are common in elasmobranchs (Spaet et al., 2020) and it seems reasonable that such a pattern may also exist for the Maugean Skate. Such differences could result from size-specific foraging success (i.e. competition) or sex-specific interactions (e.g. female avoidance of males to limit mating harassment). Importantly, although the extent of activity areas may differ between individuals, the core UDs overlap significantly, suggesting the possibility of a behavioural mechanism for partitioning instead of a spatial one. A similar result was reported in the other common elasmobranch species found in the estuary, the White-spotted Dogfish (Moreno, 2017).

All tagged Maugean Skate left their home range at some stage during the observation period, inferred from movement patterns and periods of non-detection within the acoustic array. These latter ‘departures’ could be classified into one of two distinct behaviours, short-term excursions lasting from one day up to a week followed by a return to the Table Head/Liberty Point region, and permanent relocations where the individual did not return to their initial home range for the remainder of the study. Previous research into the distribution and movement of the skate throughout the harbour demonstrated that adult Maugean Skate sometimes relocate to different areas and adopt a new home range (Bell et al., 2016). For instance, out of 30 Maugean Skate[3] tagged in the Table Head/Liberty Point region, Bell et al. (2016) observed that 19 (63%) were also detected by receivers outside of the area (confirmed excursions), with nine individuals (30%) exhibiting ‘permanent’ relocations. The present findings are consistent with these observations, namely that while there is a degree of mobility within the skate population, most individuals exhibit strong site fidelity at least within the timeframe of these studies. Notably, there was no evidence of movement outside Macquarie Harbour based on the absence of detections at the listening station near the estuary entrance. These observations provide further evidence to the hypothesis that Maugean Skate in Macquarie Harbour likely represent an isolated population (Weltz et al., 2018).

For the short-term excursions there was a high degree of individual variation regarding timing and duration of the events (see below for further discussion about the link between excursions and environmental conditions). A few of these events occurred outside the detection range of the receivers at the western edge of the array in Table Head, near the boundary of the predicted UDs. This area comprises a shallow (<1.5 m) expanse, which hampered deployment of listening stations. Therefore, it is likely that the UDs presented in this study may slightly underestimate the overall importance of this area.

Maugean Skate were detected at depths ranging from <1 to 55 m, but showed a clear preference for depths between 7.5 and 12.5 m. For coastal benthic swimmers such as the skate, vertical movement tends to correspond with horizontal displacement perpendicular to the shore (Siskey et al., 2019).

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[3] Mortalities that occurred within three weeks of tagging have been excluded as they are likely to have been tag induced, noting that tags were surgically implanted, a more invasive procedure than that used in the present study.

However, in this study this only occurred during periods of large-scale vertical movement during excursions away from the home range area in the direction of the central basin of the estuary. This type of short-term, large-scale vertical movement has been linked with searching behaviour (for prey availability or improved environmental conditions) in other species (Wearmouth and Sims, 2009). Conversely, when skate stay within their home range, the area around Table Head and Liberty Point is not topologically complex and therefore provides a limited range of vertical habitats.

Bell et al. (2016) reported a diurnal pattern in vertical area use as well as in activity rates of the Maugean Skate based on their overall dynamic body acceleration (calculated from a subset of skate tagged with tri-axial accelerometers). In the present study, harmonic analysis identified a diurnal pattern in both vertical and horizontal movement, which corresponds with observations from the past accelerometry data. However, vertical activity areas of the skate tagged in this study did not differ between night and day as reported in Bell et al. (2016). Detection efficiency of acoustic receivers can be heavily biased by burial behaviour and during low activity times, particularly in arrays with lower receiver densities (Grothues et al., 2012). Bell et al. (2016) reported higher detection frequencies during the night-time that may suggest a bias in depth UDs recorded during the low detection periods. This may explain the discrepancy between the studies and was almost certainly the result of differences in design between the two acoustic arrays (i.e. the array used in this study had a much higher receiver density spread over a smaller total area, making it less susceptible to temporal biases). Even so, these results indicate that even though the day-night cycle does not affect the vertical range of individuals, how they use and move across that space does differ between night and day. For example, at night the Maugean Skate appear to be more active and utilise a slightly larger horizontal activity area, a pattern commonly seen across multiple elasmobranch species (Humphries et al., 2017, Sousa et al., 2019) that has been linked with nocturnal foraging.

Environmental drivers of behaviour

Although comparatively rare, elasmobranchs that utilise euryhaline areas - either facultatively or obligatorily - are subject to similar conservation concerns pertinent to marine species (i.e. conservative life histories, vulnerability to overfishing, long recovery times and ongoing gaps in fundamental knowledge for several species). Nonetheless, euryhaline species are subject to additional constraints that may further their vulnerability, such as more variable water chemistry and localised effects of anthropogenic habitat alteration (Martin, 2005, Knip et al., 2010). Advances in electronic tagging technology have enabled researchers to study the influence of environmental factors in terms of site selectivity and movement of elasmobranchs, particularly salinity (Heithaus et al., 2009, Simpfendorfer et al., 2011), temperature (Matern et al., 2000, Grubbs et al., 2007) and more recently dissolved oxygen (Heithaus et al., 2009, Coffey and Holland, 2015).

Deoxygenation is likely to be of particular importance for elasmobranchs as they are relatively large bodied and occupy important trophic roles across a broad range of habitats as mid and top level predators, resulting in comparatively high oxygen demands (Laffoley and Baxter, 2019). However, given that dissolved oxygen concentrations are highly dependent on local conditions (Limburg et al., 2020), assessing the oxygen levels experienced by an individual as it moves through the environment has traditionally required extensive environmental datasets or complex oceanographic modelling. The integration of miniaturised oxygen sensors into bio-logging packages (Bailleul et al., 2015) and now passive telemetry tags (as used in this study) will significantly reduce these restrictions, providing an improved understanding of how elasmobranch species respond to changes in DO concentrations. Furthermore, tagged animals can potentially act as mobile oceanographic data collection platforms that may help better map oxygen dynamics in data deficient ecosystems (Laffoley and Baxter, 2019). Notably, to our knowledge, this study represents the first use of animal-borne acoustic sensors to monitor long-term dissolved oxygen conditions experienced by a coastal elasmobranch and their potential link with vertical and horizontal movement.

This study showed that despite occupying a restricted spatial range, skate are subject to wide ranging fluctuations in water chemistry. Oxygen levels experienced by tagged individuals ranged from normoxic (50-100%), hypoxic (20-50%) and to near anoxic (~0-20%). This constitutes the first confirmation that skate regularly use and move through areas that are severely oxygen deficient. Dissolved oxygen levels in Macquarie Harbour are variable across the year due to changes in temperature, river flow, rainfall and wind levels that affect vertical stratification, oxygen mixing and replenishment. This seasonality heavily influenced the conditions experienced by the skate. However, the daily range of DO concentrations experienced by all individuals regardless of time of year was much higher than anticipated. On average individuals experienced fluctuations of nearly 50% DO levels and on some occasions as high as >90% within the same day. Analysis of DO levels experienced by skate showed that conditions were the most variable at their preferred depths (7.5 to 12.5 m). Furthermore, tags attached to skate that died throughout the study showed considerably different DO levels than the water column sensors at the same depth; suggesting that DO conditions within the skate's preferred habitat appear to fluctuate dynamically by depth and horizontally across relatively small spaces. Importantly, these results indicate that even when DO concentrations are highest in the harbour, skate are intermittently exposed to hypoxic conditions on a regular basis.

Behavioural change point analysis in the current study showed that despite some individual variation, there were four instances when a concurrent change in the behaviour of most individuals occurred within a very short amount of time. Differences in vertical and horizontal movement patterns have been reported for several coastal skate species and have been linked to life history processes (e.g. reproduction), seasonal environmental changes (e.g. deep water wintering) or ontogeny (e.g. nursery areas) (Hoff, 2008, Siskey et al., 2019). However, these changes tend to involve coordinated movement (can be sex or life stage specific) of individuals over a wide area, usually in, and offshore into, deeper waters (Hunter et al., 2006, Frisk, 2010). In contrast, changes in the movement behaviour of the Maugean Skate occurred over small spatial and temporal scales and could be classified into three distinct types; (i) an increase in short term excursions away from the core home range area, (ii) a shift in depth distribution and vertical movement, and (iii) permanent exits from the study area. Moreover, these changes in behaviour corresponded with periods of high variability in environmental conditions within the harbour, such as a sharp increase in surface water temperature in December, declines in mid- and deep water dissolved oxygen levels in January and a large storm surge in late March and subsequent oxygen recharge in April.

Bottom oxygen levels in Macquarie Harbour dropped significantly during the summer months at depths including and below the preferred range of the skate (7.5 to 12.5 m). Not surprisingly, tag data showed that mean oxygen concentrations experienced by the individuals declined considerably during these times. At this time, the skate altered their vertical distribution by increasing the amount of time spent at shallow sites around 5 m in depth. These sites are oxygen rich and likely act as a refuge from increased hypoxic stress during the summer months. Several studies have shown the importance of dissolved oxygen as a driver for movement and abundance in elasmobranch species (Heithaus et al., 2009, Espinoza et al., 2011, Drymon et al., 2014). It has been hypothesised that one of the likely detrimental effects that deoxygenation will have on elasmobranch species will be a contraction of their vertical range (Laffoley and Baxter, 2019). However, despite their increased used of shallow sites, the core or preferential depth range of the Maugean Skate remained unchanged during these periods. By contrast, the overall depth range broadened as a result of the increased shallow area use as well as the skate undergoing occasional, short term excursions into much deeper waters before returning to their core home range. The occasional use of areas outside the core UD by elasmobranchs has been linked with searching behaviour for additional resources, more suitable habitat or more favourable conditions (Knip et al., 2010). Use of these sites outside the preferential range of the skate (both shallow and deep) increased following an oxygen recharge (caused by the large influx of marine water during a storm in March) and continued until May.

Surface waters during the summer months were 5 to 10 °C warmer and had > 10 ppt lower salinity than the deeper sites usually favoured by the skate. Skate experimentally exposed to hyposaline conditions during the physiological experiments showed an increased metabolic demand associated with osmoregulation (see below). Not surprisingly, increasing temperatures have also been shown to result in higher oxygen consumption rates in elasmobranchs (Di Santo and Bennett, 2011). Therefore, while shallow sites may provide Maugean Skate access to improved oxygen conditions, they also expose them to thermal and osmotic stress, potentially causing them to continuously move back into deeper waters where temperature and salinity conditions are more stable. Skate that underwent excursions outside their core home range usually moved into sites with depths >30 m, where they experienced DO levels slightly higher than in mid water (15–20 m) due to the stratification profile of Macquarie Harbour.

There are many challenges to understanding the mechanistic links between behaviour and abiotic factors. Particularly because movement and environmental data are naturally correlative and the connection between animal behaviour and abiotic factors tend to be fundamentally complex (Schlaff et al., 2014). The results from the present study show there is an intricate link between movement of the Maugean Skate and environmental conditions in Macquarie Harbour. The species appear to be able to behaviourally modulate environmental stressors through movement, likely balancing optimal habitat requirements against the energetic cost of chronic hypoxia in deeper waters and increased thermal and osmoregulatory stress at shallow sites. Such behavioural plasticity may constitute a key adaptation that has enabled the species to tolerate such a challenging environment. Similar mechanisms have been described in the Epaulette Shark (*Hemiscyllium ocellatum*), another species commonly subject to large environmental fluctuations and which can use movement to alleviate the stress of increased water temperatures around coral reef systems (Gervais et al., 2018).

The aforementioned behavioural plasticity of the skate appears to be limited to their extended use areas (95% UDs) and throughout the study individuals continued to utilise the same core UD and preferential depth range, indicating that site selection and fidelity patterns of the species are only partially driven by environmental factors. Conditions within those preferred sites were subject to the largest daily fluctuations within the harbour (particularly DO). Therefore, the site attached behaviour of the skate suggest that these core sites likely provide an advantage that outweighs the cost of exposure to unfavourable environmental conditions, such as access to prey that may not be as readily available elsewhere within the estuary. Batoid species with high physiological tolerance thresholds to hypoxia have been reported to exploit hypoxic zones for foraging (Craig et al., 2010) when reduction-oxidation conditions (chemical reactions in which oxidation states of atoms are changed) in the substrate force burrowing organisms to emerge (Genz et al., 2013). It is possible that Maugean Skate, which are specialised benthic hunters that feed primarily on crustaceans and other in-faunal organisms could utilise a similar strategy. On-going anthropogenic activities, coupled with the effects of climate change, are expected to continue to influence the environmental conditions in the harbour and challenge the effectiveness of behavioural mediation mechanisms.

Mortalities

Mortality is a crucial life-history process with implications on the dynamics and structure of a population (Mollet and Cailliet, 2002). Therefore, understanding the rate and causes of mortality is of great importance for rare and vulnerable species, as the loss of individuals will affect the reproductive output of the population, but may also help identify disturbances and stressors affecting it. However, documenting non-fisheries mortality events in the wild can be extremely challenging and there is little available information about how, when, and why these events happen in elasmobranch fishes (Heupel and Simpfendorfer, 2002).

The rapid expansion of underwater acoustic telemetry along with improvements in battery-life and multi-sensor technology, provide unprecedented long-term records of detailed animal behaviour. This information can also be useful for identifying and analysing possible mortality events (Khan et al., 2015). There are several factors that may result in a tagged animal dying, such as tagging related injuries (Rechisky and Welch, 2010), predation (Klinard et al., 2019), natural causes (Khan et al., 2015) or fishing (Wiig et al., 2013). Tagging induced mortalities usually occur within the first month or so after initial release (Cooke et al., 2011). Previous research on Maugean Skate using surgically implanted tags experienced a mortality rate of 22% within 18 days post-tagging (presumed tagging induced), subsequent assumed mortality events (AMEs; a further 10%) occurred between 36 and 178 days post-release (Bell et al, 2016). By contrast, in present study all AMEs occurred between 54 and 255 days post-tagging and thus are unlikely to have been directly linked to tagging effects. Furthermore, while no tagged individuals were recaptured and thus wound healing could not be directly assessed, there is a large amount of evidence that elasmobranchs show rapid tissue healing around external tag attachment points (Heupel et al., 1998) as well as to much larger wounds such as boat strikes (McGregor et al., 2019). The attachment method used in this study was developed specifically for use in dorso-ventrally flattened batoids (Le Port et al., 2008) and tested on captive individuals. The authors were also able to recapture a wild tagged individual following detachment of the tag, reporting complete wound healing around the insertion points with no added complications for both the recaptured and captive individuals. Therefore, while complications from the tagging procedure cannot be definitively ruled out as a contributor to death, this seems highly unlikely and thus alternative factors are likely to have played a role in these mortalities.

All spatial analyses conducted in this study showed that up to the point of death, behaviour of these individuals was comparable to that of animals that survived the entire study. Post tagging behaviour showed no erratic patterns, suggesting that individuals resumed normal behaviour shortly after release. Behavioural patterns seen in these animals were also comparable to those reported in Bell et al. (2016), suggesting that the different and less invasive tag attachment method had no noticeable effects on the behaviours of tagged skate.

Sudden changes in behaviour followed by an abrupt stop in detections can indicate a predation event (Khan et al., 2015). Only few species capable of preying on Maugean Skate are known to enter the estuary, these include seven gill sharks (Lyle et al., 2014), fur seals and dolphins. However, given that the movement ecology of these species varies considerably to that of the skate (assuming consumption of the tag),the absence of erratic behaviour immediately preceding death tends to discount predation as a likely cause for the observed mortalities.

Results clearly show that all but three of the mortalities were clustered in two discrete periods, January-February and April 2019. Each of these periods corresponded with severe and rapid changes in environmental conditions within the core habitat used by the skate. During the first, bottom oxygen levels dropped considerably and remained consistently below 50% (and as low as 0-20% DO at times) within the species preferred depth range, while surface water temperatures reached upwards of 20 °C. The second period included a large westerly storm event (20 March 2019) that pushed a large mass of dense oceanic water into the harbour, displacing low DO water up into the water column. In the weeks following this inversion, conditions stabilised and oxygen levels throughout the harbour saw a recharge. The two periods when the mortality events occurred were preceded with changes in behaviour (based change point analysis) in the skate that died as well as the surviving individuals, implying that virtually all of the tagged individuals simultaneously had exhibited a response to an external (environmental) factor. Therefore, although inherent limitations in acoustic telemetry technology mean that causes of death cannot be established with certainty, given the timing of the events and associated behaviour changes, it is probable that the deaths of at least eight of the eleven tagged Maugean Skate in this study were related in some way to stresses caused (directly or indirectly) by significant changes in the environmental conditions of the harbour.

The tagging experiment in this study was designed to gain an improved understanding of the environmental conditions experienced by the skate. These results suggest that stress on the population may be higher than anticipated and that action is required to provide critical data needed to assess the true conservation status of the species. Particularly since the small population size and conservative life history traits of the Maugean Skate suggest that mortality events like those seen could have long-term implications for the future viability of the population. However, currently there does remain some uncertainty as to the ultimate driver/s of these mortalities.

Inversion events of the magnitude seen in the current study (March/April) are uncommon in Macquarie Harbour but have been reported in the past. For example, a similar event occurred in July 2014 when several acoustically tagged Maugean Skate were being tracked by Bell et al. (2016). Results from that study showed a dramatic change in movement behaviour associated with the storm event but, unlike the current study, no mortalities occurred in the following months. Even before human interaction, Macquarie Harbour was a challenging environment with naturally low DO, large influx of fresh water and depauperate conditions. A growing body of work (including the present study) has shown that the Maugean Skate has some unique adaptations that have allowed it to survive under such conditions. However, most of this work has focused on single stressors and general conditions in Macquarie Harbour have only shown minor improvement since 2016, with DO levels remaining well below 2009 levels. Multiple environmental stressors can have additive or synergistic effects on individuals (Gunderson et al., 2016). For example, increased temperatures have been shown to result in lower tolerance thresholds to hypoxia in coastal skates (Schwieterman et al., 2019). Likewise, the coping ability of individuals after exposure to stressful conditions will be determined by their ability to return to homeostasis (stability of a biological system), so the timing, duration and magnitude of subsequent stressors can heavily impact survivorship of a species (Feidantsis et al., 2015). Ultimately, the high feeding specialization of the species (Weltz et al., 2019), restricted spatial range and limited relocation ability, and narrow site selectivity patterns limit the life history plasticity of the Maugean Skate, which may restrict their capacity to cope with disturbances beyond their natural tolerance thresholds.

Eco-Physiology

Hypoxia

The Maugean Skate is one of few species that can survive chronic hypoxia of this severity (<20% DO) and duration (>48 hrs) by using metabolic depression as a survival strategy. Decreases in metabolic demands limit the need for anaerobic adenosine triphosphate (ATP) production and thus lengthen the amount of time an organism can survive on its limited stores of fermentable substrate (Richards, 2009). Consequently, the degree to which animals can suppress their metabolic rate, and the quantity of fermentable fuel stores they possess can have a large impact on duration of survival to long-term hypoxia (Richards, 2009). During metabolic depression, aerobic metabolic rate is typically lowered to between 5 and 40% of an animal’s resting metabolic rate, with little to no change to the internal environmental of the animal’s cells (Storey and Storey, 2004). The Maugean Skate is no exception, lowering its aerobic metabolic rate to 34% of control values on average. One common mechanism of metabolic suppression is reversible protein phosphorylation, which regulates fuel metabolism, ion channel arrest, and suppresses protein synthesis (Storey and Storey, 2004). For the epaulette shark, elevated adenosine levels trigger the cessation of expensive cellular processes, slowing energy consumption while increasing oxygen-independent ATP production (Renshaw et al., 2002). Hypoxia tolerant animals use a variety of combinations of aerobic and anaerobic metabolism and metabolic depression to cope with exposure to low oxygen conditions (Mandic and Regan, 2018). The crucian carp depresses metabolic rate but maintains enough brain electrical activity to allow for marginal movement and thus individuals are able to search for higher DO conditions (Nilsson and Renshaw, 2004). In contrast to the Maugean Skate, however, the crucian carp is also able to maintain high levels of glycolysis during periods of anoxia by producing and excreting ethanol as an end-product, avoiding lactate toxicity. The Acara-açu (*Astronotus ocellatus*) also depresses its metabolic rate to cope but does so in response to acute hypoxia, postponing anaerobic glycolysis (Muusze et al., 1998) instead of using anaerobic metabolism as a first defence like the Maugean Skate.

When faced with shorter periods of hypoxic exposure, the Maugean Skate has been shown to rely on anaerobic metabolism, resulting in increased lactate production (Morash et al., 2020). However, results presented here show that when this exposure becomes chronic, the skate likely depresses its overall metabolism to limit energy expenditure. While plasma lactate levels were significantly higher in the hypoxia exposed group, concentrations remained very low (0.58 mmol/L on average) compared with those in skate acutely exposed to similar DO concentrations (48 h), which ranged from 7-10 mmol/L (Morash et al., 2020). This suggests that production of lactate for use in anaerobic respiration may be limited. It is possible that the skate store some amount of lactate in white muscle, given that the noted increase in concentration in this study is approaching significance, but this concentration was still minimal (~2.0 mmol/L). In Rainbow Trout exposed to 25% dissolved oxygen for 90 minutes, hypoxia stimulated an increase in lactate disposal, accumulating up to 19.2 µmol/g in white muscle (Omlin and Weber, 2010). The authors calculated that without this increase in deposition blood lactate concentration would have doubled, and thus is essential to reducing lactate load in circulation. Given that plasma lactate levels remained low and there was no change in tissue lactate concentrations, these results suggest that under chronic hypoxia exposure lactate production in the Maugean Skate is extremely limited.

Metabolic depression in the Maugean Skate as a response mechanism to chronic hypoxia will occur at the cost of other energy intensive life history processes, such as growth, foraging and reproduction (Speers-Roesch and Treberg, 2010). Given the species' late maturation and asynchronous reproductive strategy (Bell et al., 2016), lengthy periods of depressed metabolic rates could greatly reduce its growth/maturation rate and capacity to reproduce, limiting any increases in the population under these conditions. As such, metabolic depression cannot be sustained forever, and the skates may seek oxygen in the shallower waters of the harbour where they will encounter hyposaline conditions and increased temperatures in the summer.

Considering the cost of metabolic suppression as a survival mechanism to cope with chronic hypoxia, it should be expected that individuals become less active (and therefore less mobile) during the severe hypoxic stress experienced in the summer months. However, analysis of the telemetry data showed an increase in vertical movement and an expansion of extended horizontal activity areas during these times. This might suggest a decoupling between behavioural and physiological responses to hypoxia in the species. A similar effect has been shown to occur in oviparous (egg laying) elasmobranchs during development when the eggs are exposed to lower DO levels (Di Santo et al., 2016). Exposure to low DO prompts a decline in metabolic rate of the embryo, but also an increase in tail beating activity, which increases water circulation and improves oxygenation. For the adult skate, the increase in mobility outside their core home range could constitute searching behaviour for improved conditions or refuge areas. However, this constitutes a high risk strategy, as the increased movement will result in a higher load for the already depressed metabolic rate of individuals.

Hyposalinity

Maugean Skate are unusual in that they are the only known skate species to live exclusively in a euryhaline environment. Observations in this and previous studies clearly show that throughout their vertical distribution, the species is constantly exposed to a wide ranging haline scope, which distinguishes them even from their closest known relatives (*Zearaja chileniensis* and *Zearaja nasuta*) which are only known to inhabit fully marine waters (Bustamante et al., 2012, Concha et al., 2012, Bennett and Randhawa, 2019). Therefore, while very little is known about the physiology of the species, it was generally assumed that Maugean Skate must be capable osmoregulators. Results from the present study show that levels of plasma osmolality decreased significantly in individuals following 96 hr acclimation to hyposaline conditions. Differences in plasma osmolality levels between freshwater and saltwater acclimated individuals has been reported for several euryhaline and partially-euryhaline elasmobranchs such as the Little Skate (*Leucoraja ocellata*), freshwater sawfish (*Pristis pristis*, as *P. microdon*) and Bull Shark (*Carcharhinus leucas*) (Sulikowski et al., 2003, Pillans et al., 2005). Lower osmotic concentration experienced by euryhaline elasmobranchs result from increased urinary excretion and/or overall reductions to production and retention of urea, trimethylamine oxide and other organic nitrogenous compounds (Hammerschlag, 2006). It is likely (but still untested) that like these other species, the Maugean Skate maintain internal conditions that are hyper-osmotic relative to the external medium when moving into increasingly dilute seawater despite reductions in plasma osmolarity (Hammerschlag, 2006). Although not included in the current study, tissue samples from the gills, rectal gland, brain, heart, and muscle tissue of these individuals were collected and are currently being analysed. These will provide further insight into the mechanisms used by the Maugean Skate for osmotic and ionic regulation as well as their plasticity and potential tolerance thresholds.

Routine metabolic rate of individuals appeared to be higher following acclimation to hyposaline conditions, although the corresponding statistical test only showed near-significance ($p=0.06$). However, total ketone body concentrations were significantly higher and tissue glycogen levels significantly lower than for their controls. Glycogen can be liberated into the blood as glucose for use as metabolic fuel (Richards, 2009). Likewise, elasmobranchs rely heavily on liver produced ketone bodies as a high energy metabolic fuel (Speers-Roesch and Treberg, 2010). Accordingly, depletion of glycogen stores and increases in ketone body concentrations provide additional evidence of an increased metabolic load following exposure to hyposaline conditions. Studies on other euryhaline batoids have found similar increases in oxygen consumption associated with brackish water (Meloni et al., 2002), suggesting that there is a significant energetic cost related to osmoregulation in lower salinity environments. The higher metabolic cost of living in freshwater could have important ecological implications for the Maugean Skate, such as higher food consumption requirements, or failing to accomplish this, a decrease in vital processes such as growth and reproduction. Moreover, individuals in this experiment were exposed to brackish conditions (~13.5 ppm); however, the vertical distribution of the tagged animals suggests that skate in the wild experience much lower salinities, presumably with a higher metabolic cost for osmoregulation. This may be particularly important if as suggested by results from the tracking experiments, individuals utilise shallow areas as refuges during low DO periods and in particular during summer, when surface waters reach higher temperatures that will result in additional metabolic stress.

Early life history

Static respirometry trials showed that like the adults (Morash et al., 2020), early life stage Maugean Skate are oxyconformers. That is, routine oxygen consumption levels are proportional to environmental oxygen concentrations. Levels of lactate in plasma rose considerably following exposure to decreased oxygen concentrations. Lactate is an important marker of anaerobic metabolism and similar build-up was detected in adult skate after acute exposure to hypoxia (Morash et al., 2020). This suggests that even at an early age, Maugean Skate may be able to tolerate short-term hypoxic events through an increased reliance on anaerobic metabolism. However, lactate build-up can have severe detrimental consequences in elasmobranchs such as blood and tissue acidosis (Ballantyne, 1997), and can be up to 30-fold less efficient than aerobic metabolism (Rogers et al., 2016). Therefore, anaerobic responses are not likely to provide a viable long-term survival strategy for coping with chronic hypoxia.

Experimental respirometry and data from electronic tagging clearly shows that adult skate are capable of tolerating a wide range of oxygen conditions. Morash et al. (2020) reported that for adults, the point below which oxygen uptake became negligible occurred between 10 and 25% DO. By contrast and based on two trials conducted on the neonate skate in this study, that threshold occurred at ~ 66% DO. Accordingly, a conservative DO level was chosen for the chronic exposure treatment of 75%. However, even at this relatively high concentration, the neonate unexpectedly died three days into the four-day treatment. Interestingly, blood lactate levels taken at the time of death were elevated, suggesting that unlike the adults in the chronic exposure experiments, the neonate continued to rely on aerobic metabolism instead of metabolic depression. The effects of hypoxia exposure in early life stage elasmobranchs remain poorly understood, and it is unknown if there is an ontogenetic component to the mechanisms that results in the species' high environmental tolerance thresholds or if environmental *conditions* during development may play a role in the expression of these traits. Given that all respirometry measurements and haematological analysis of newborn skate came from a single individual, these results should be considered with great care, in particular because the skate was reared in a controlled environment under consistent temperature, salinity and oxygen conditions. Nonetheless, these findings highlight the need for an improved understanding of the oxygen physiology of the Maugean Skate during its early life-stages, as lower tolerance thresholds as seen in this individual could have important implications for the survivorship of early life stages, especially in relation to the variable environmental conditions experienced in Macquarie Harbour.

Population structure

Deriving an estimate of the effective population size of the Maugean Skate in Macquarie Harbour has proven challenging due to their sparsity (Weltz et al., 2017), low genetic diversity (Weltz et al., 2018), and restricted sampling options linked to the species' protected status. Therefore, there is a high degree of uncertainty associated with current estimates that limit their applicability in monitoring the continuing status of the population. Bell et al. (2016) used multiple models to calculate population size from recapture data and the best fitting model resulted in an estimated population size of 3177 individuals. However, this prediction had considerable uncertainty (95% prediction interval 1827–6247), and none of the models used accounted for the strong stratification in the sampling protocols. Accordingly, the authors urged that these results be treated with caution and considered indicative in terms of population size order of magnitude. While useful as a baseline, a better understanding of the population status and trajectory will be crucial for the effective management of the Maugean Skate, particularly given the potential implications of the changing environmental conditions in what is possibly the species sole remaining stronghold.

Catch data from the present study, Lyle et al. (2014) and Bell et al. (2016) represent a near continuous sampling record of the species in the Table Head/Liberty Point area since 2012. Size composition data indicated that the median size of both males and females has increased since 2012, particularly between the 2013-14 and 2017-18 surveys. Likewise, the overall length frequency distribution has shifted to the right, reflecting a reduction in the relative abundance of juvenile and sub-adult individuals (≤ 600 mm) and as well as an increase in the size of the larger individuals (adults) in the catches. The gillnets used in these surveys are size selective and most Maugean Skate tend to be lightly entangled in the meshes by the rostrum (and rostral spines), such that some individuals have been observed to drop out of the meshes while the net is under tension during hauling. Small individuals (< 500 mm TL) with a less pronounced snout (and very small rostral spines) are thus less likely to be caught or retained in the meshes and thus expected to be underrepresented in the gillnet samples even when present. However, given that the same nets have been used in all three studies, any size selectivity bias is expected to have been constant through time, justifying an examination of temporal trends in size composition for inferences about changing population status.

Without a better understanding of size-specific movement and distribution patterns of the skate population throughout Macquarie Harbour, it is not possible to definitively determine whether the observed shifts in the size structure of skate in Table Head/Liberty Point area are reflective of localised factors or indicative of population-wide changes. At least in terms of adult behaviour, the general consistency between in the present study and that conducted in 2013-14 (Bell et al. 2016), in terms of high site fidelity, restricted home ranges and tendency to return to preferred sites following temporary relocations, do not support an obvious behavioural change.

Despite uncertainty as to the significance of the changes in catch size structure, a recent decline in recruitment, possibly due lower hatching success or juvenile survival, coupled with the growth of the adult could result in changes like those observed. Unvalidated estimates of age and growth for the Maugean Skate (Bell et al. 2016) are not inconsistent with the observed data in that if a period of reduced recruitment is assumed, then the continued growth of juveniles and adults over a 3-4 year period (e.g. between 2013-14 and 2017-18) would give rise to a comparable change in size structure.

While the analysis presented herein is not definitive, the possibility that changes in population structure could have occurred in the Maugean Skate population within the past 10 years should be considered seriously, particularly within the context of the other findings in this study, namely; (i) the vulnerability of developing embryos to the abiotic conditions experienced during the protracted incubation period, and (ii) the vulnerability of early life stages to environmental stressors, and (iii) the evidence of adult mortalities potentially linked with environmental factors. Accordingly, an immediate priority needs to be an evaluation of options to estimate and monitor population status and health along with consideration of potential conservation actions, as greater certainty about the status of the population is gained. Any such monitoring approach should aim to be minimally disruptive, highly replicable and able to work within the challenging conditions of Macquarie Harbour.

Conclusion

Maugean Skate eggs were found across a wide range of depths but appear to be most abundant in relatively shallow sites (< 10 m). An egg reared under laboratory conditions hatched 31 weeks after oviposition, with respiratory channels open after 15 weeks, implying that developing embryos may be exposed to external environmental conditions for over half of the gestation period. The actual dissolved oxygen, salinity and temperature conditions experienced by the embryos will largely depend on the depths at which eggs are deposited. The implications of such exposure on development and survival are unknown.

This study represents the first use of animal-borne acoustic sensors to monitor long-term dissolved oxygen conditions experienced by a coastal elasmobranch and the links with behaviour. The results showed that skate are subject to wide ranging fluctuations in water chemistry. For instance oxygen levels experienced ranged from normoxic (50-100%), hypoxic (20-50%) and to near anoxic (~0-20%), with individuals often experiencing substantial variation (as high as > 90%) in the range of DO levels experienced within the same day.

The present study shows that there is an intricate link between movement of the Maugean Skate and environmental conditions in Macquarie Harbour. The species appear to behaviourally modulate environmental stressors through movement, likely balancing optimal habitat requirements against the energetic cost of chronic hypoxia in deeper waters and increased thermal and osmoregulatory stress at shallow sites. Such behavioural plasticity may constitute a key adaptation that has enabled the species to tolerate to such a challenging environment. However, since individuals continued to utilise the same core home range and preferential depth range, despite these areas being subject to the largest daily fluctuations in water chemistry conditions experienced by the skate, this behavioural plasticity appears to be limited to their extended use areas. Therefore, the site attached behaviour of the skate suggest that these core sites likely provide an advantage that outweighs the cost of exposure to unfavourable environmental conditions, such as access to prey that may not be as readily available elsewhere within the estuary.

Physiological experiments demonstrated that adult Maugean Skate are quite capable of surviving chronic exposure to hypoxic conditions (< 20% DO) by using metabolic depression as a survival strategy. Metabolic depression does, however, occur at the cost of other energy intensive life history processes, such as growth, foraging and reproduction. Maugean Skate are unusual in that they are the only known species of skate to live exclusively in a euryhaline environment and, based on their vertical distribution behaviour, are constantly exposed to a wide range of salinities. Routine metabolic rate of individuals appeared to be higher following acclimation to hyposaline conditions, although the corresponding statistical test only showed near-significance.

Physiological data based on a single individual suggests that neonates are oxyconformers, implying that even at an early age, Maugean Skate may be able to tolerate short-term hypoxic events through an increased reliance on anaerobic metabolism.

During this study an unexpectedly high proportion of tagged individuals died. Although causality cannot be established, the mortalities were unlikely to have been a direct consequence of tagging. Most mortalities were clustered in two discrete periods that corresponded with marked changes in environmental conditions within the core habitat used by the skate. In addition, behavioural changes (based on change point analysis) were observed at the same time in virtually all of the surviving individuals. It is feasible, therefore, that the deaths may have been related to stress caused (directly or indirectly) by the significant changes in the environmental conditions of the harbour. If this is the case, then recent changes in the environmental health of the harbour (especially dissolved oxygen levels), coupled with the consequences of climate change (including occurrence of extreme weather events), may already be challenging the skate’s capacity to cope with the environmental conditions in Macquarie Harbour.

Analysis of research gillnet data collected since 2012 suggests a reduction in the relative abundance of juvenile and sub-adult individuals and as well as an increase in the size of the larger individuals (adults) in the catches through time. Despite uncertainty as to the significance of the size composition data in terms of representing trends in population status, a recent decline in recruitment, possibly due lower hatching success or juvenile survival, coupled with adult growth represent plausible scenarios that could produce the changes observed.

In conclusion, this study provides a greater understanding of the ecology and life history of the Maugean Skate and describes for the first time some of the behavioural and physiological adaptations that have allowed the species to survive in such a unique and challenging environment. Our results suggest that the species can mitigate some environment variability by behavioural (movement) responses and physiological capability. However, the vulnerability of early life stages to the changing environmental conditions, potential changes in the size structure of the population, and the mortality of some tagged individuals following significant environmental events, are indicative of a stressed population. Collectively, these findings highlight the vulnerability of the Maugean Skate population in Macquarie Harbour and the need to consider further conservation action to support the persistence of this unique micro-endemic skate.

Implications

This study has highlighted the vulnerability of the Maugean Skate to the changing environmental conditions in Macquarie Harbour and, therefore, the environmental health of the system, in particular levels of DO in the bottom waters, is likely to represent a crucial factor in the future well-being of the skate population. The various anthropogenic activities impacting Macquarie Harbour - aquaculture, mining, hydro-electricity generation (river flows) and coastal development - along with the effects of climate change all play a role in shaping the environmental conditions of this unique system. Thus, the maintenance of best environmental practices by industry, supported by effective monitoring and environmental management policies, will be crucial if humans and the Maugean Skate are to coexist in perpetuity.

Furthermore, uncertainty surrounding the current status of the Maugean Skate population emphasises the need to consider further conservation action, including the development of a management plan to support the persistence of this unique micro-endemic species. As a start, there is an urgent need to better understand the biological significance of the observed changes in the size structure of the gillnet samples through further targeted sampling. In addition, there is need to investigate alternative, cost-effective and non-invasive approaches to estimate population size and mortality rates, which can be applied to track population status and health over time. If as suggested here, recruitment success may have already been impacted (potentially due to reduced egg survival), future consideration may also need to be given to captive rearing and restocking to enhance population recovery. Importantly, managing and reducing anthropogenic impacts on the environmental health of Macquarie Harbour, both in terms water column and benthic conditions, will ultimately prove crucial to the success of any conservation strategy for the Maugean Skate.

Recommendations

Results from this study contribute to the growing body of knowledge regarding the Maugean Skate and highlight the continued uncertainties regarding the species management and conservation status, as well as several important remaining research gaps.

The site-attached behaviour displayed by the Maugean Skate means that spatial management strategies are likely to be highly effective in mitigating certain impacts (e.g. fishing) if implemented at an adequate scale and adequately enforced. In 2015 gillnetting was prohibited in the Table Head area and in waters deeper than 5 m throughout Macquarie Harbour as a measure to minimise the incidental capture of Maugean Skate. While it is unclear as to how effective this measure has been, there were two separate incidents during this study where the research team was contacted about Maugean Skate carcases found discarded at the Strahan boat ramp. It is likely that these individuals were landed by fishers unaware of their protected status and discarded once at the ramp where there is signage with relevant information about the species. At the very least these incidents highlight a need for improved methods of education and signage to ensure that members of the general public that fish this area are aware of the presence and protected status of the skate.

However, environmental conditions are not localised, and managing these impacts will require a strategy involving the entire estuary. Accordingly, future conservation efforts for the species can be classified into four categories, namely retrospective population analysis, population monitoring, prospective population analysis, and environmental health.

- *Retrospective population analysis*:
  In trying to understand the impacts of natural and anthropogenic stressors on the population the absence of pre-impact information represents a major uncertainty. Retrospective analysis refers to research attempting to understand how the species reached current conditions.

  This includes using information gained in this and a previous study (Bell et al., 2016) to refine estimates of crucial life-history features for the species, including age, growth and reproductive dynamics. These data can be used to construct demographic models and conduct elasticity and sensitivity analysis to identify research priorities as well as help direct conservation efforts in a way that maximises the effects on the population.

  Microchemical fingerprinting using laser ablation inductively coupled plasma mass spectrometry on existing hard tissue samples might be helpful in reconstructing long term (life-long) ecology and environmental patterns (Smith et al., 2013). This could be particularly useful to gain a better understanding of movement and habitat use patterns around the poorly understood early stages of development.

- *Population monitoring*:
  Conditions described in this study suggest that the Maugean Skate population in Macquarie Harbour is likely subject to higher environmental and demographic stress than previously thought. Therefore, extension and expansion of the time series of size composition information (via research netting) along with an investigation of alternative and minimally invasive approaches to monitor population trends and health should be considered a high priority.

  The unusual physical characteristics of Macquarie Harbour restrict the usefulness of many video or diver-based approaches while the use of sampling gear that necessitates the capture of the skate poses a risk of physiological disruption or incidental mortality. Acoustic video or other acoustic imaging techniques may offer a viable option to undertaking regular population surveys (e.g. Becker et al., 2011).

Any monitoring program should be integrated with ongoing conservation efforts and also provide a way of tracking the health of the population. For example, monitoring of long-term environmental interactions and mortality rates (e.g. Villegas-Rios et al, 2020) with long-life animal-borne acoustic sensors, body condition analysis (e.g. bioimpedance analysis), and monitoring of reproductive status (e.g. ultrasonography) among others.

- *Prospective population analysis*:
  In lieu of the potential demographic impacts suggested in this work (e.g. reduced recruitment), the tools developed by the retrospective component can be used as simulation tools to explore possible effects that those impacts may have on the population going forward, as well as evaluating potential management scenarios and actions, which could include consideration of captive rearing and restocking to enhance population recovery.

- *Environmental health*:
  Effective management of anthropogenic impacts on the environmental health of Macquarie Harbour, both in terms water column and benthic conditions, should also represent a crucial component of any conservation strategy for the Maugean Skate. Nutrient load and river flows have been identified as key drivers of dissolved oxygen dynamics in the harbour (Andrewartha and Wild-Allen, 2017; Ross and MacLeod, 2017). A comprehensive water quality and benthic monitoring program (Ross et al., 2020) together with tighter regulation of farming practices, including reduction in salmonid biomass determinations and trials of waste capture methods (Marine Solutions, 2018), have been implemented as a strategy to manage the impacts of excess nutrient load arising from salmonid farming. Hydrodynamic modelling (Andrewartha and Wild-Allen, 2017) could also potentially provide a useful support tool when making decisions around the timing and volume of releases associated with hydro-electricity production, thereby ameliorating rapid fluctuations in dissolved oxygen levels within the system.

Extension and Adoption

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

- The project steering committee met on four occasions - 27 July 2017, 30 May 2018, 11 July 2019 and 22 September 2020. Progress updates were presented to the committee and advice and direction in dealing with some of the challenges encountered during the course of the study was sought by the project team. Milestone reports were routinely provided to the committee.

- Jeremy Lyle and Jeff Ross (IMAS) provided science briefings to the West Coast Community Aquaculture Forum in Strahan on 16 August 2017. The focus of the briefing was the environment in Macquarie Harbour and the status of the Maugean Skate, including the objectives of the current project.

- In February 2019 Jeremy Lyle presented an overview of the conservation status and state of knowledge, including current project objectives, to a Shark Conservation Summit held at The University of Sydney.
- Jeremy Lyle and David Moreno (IMAS) presented a project update to Tassal staff on 20 February 2019.
- In April 2019 Jayson Semmens presented on the project to the Japanese National Institute of Polar Research (Tokyo), presentation title was: *Influence of dissolved oxygen levels on*

Maugean Skate (Zearaja maugeana) habitat use established using animal-borne environmental sensors.

- In June 2019 Jayson Semmens and David Moreno (IMAS) presented talks related to this project at the 5th International Conference for Fish Telemetry held in Arendal, Norway:
    - *Influence of dissolved oxygen levels on Maugean Skate (Zearaja maugeana) habitat use established using animal-borne environmental sensors* (Semmens)
    - *Understanding the movement of two elasmobranch species with contrasting spatial dynamics using a fixed-array design* (Moreno).
- In August 2019 Jeremy Lyle presented on the project to the Norwegian Institute of Marine Research (Bergen), presentation title was: *Endangered skate, salmon farming and degraded estuarine environment: balancing development and conservation*.

- In November 2019 Jeremy Lyle and David Moreno presented details of the study at a west coast community forum held in Strahan to an audience of more than 100 people that included community leaders, representatives from the local council, representatives from the aquaculture companies as well as members of the general public.

- Preliminary results were presented by the research team to the FRDC and Tasmanian government representatives (EPA Tasmania, Marine Resources, Natural and Cultural Heritage Division) on 20 Feb 2020.

- Jeremy Lyle provided an informal project briefing to the Tasmanian Salmon Growers Association Technical Reference Committee on 27 April 2020.

Project coverage

- An article was published about the project in *The Saturday Mercury* on 15 July 2017: https://www.themercury.com.au/news/tasmania/probe-into-fish-farms-risk-to-the-endangered-maugean-skate/news-story/3c643c3e325b2dd82c9faa89f876ead9

- IMAS issued a media release on 8 September 2017 outlining the proposed research and objectives for this project: http://www.imas.utas.edu.au/news/news-items/new-macquarie-harbour-projects-to-help-protect-maugean-skate-and-monitor-oxygen-levels

- Jeremy Lyle and Jeff Ross were interviewed about the current project and environmental research in Macquarie Harbour for Southern Cross TV News (8 September 2017): https://www.youtube.com/watch?v=xzP_7R-rpBY

- *The Saturday Mercury* reported on the project (9 September 2017): http://www.themercury.com.au/news/tasmania/underwater-mapping-project-to-assess-fish-farms-effect-on-macquarie-harbour-oxygen-levels/news-story/0a293408d0a4cd3c1cdf3ce90162305d

- ABC TV Tasmania interviewed members of the project team to discuss the tagging research and findings of a recently published genetics study on the Maugean Skate. The interview was aired on ABC News (Tasmania) on 2 December 2018. https://www.abc.net.au/news/2018-12-02/skate-study-endangered-fish-waters-tasmania/10572918

Appendix 1: Project staff

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Assoc Prof Jeremy Lyle
Prof Jayson Semmens
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Graeme Ewing
Dr Jaime McAllister (to late 2018)
Dr Kilian Stehfest (to early 2019)
Assoc Prof Neville Barrett

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Dr Andrea Morash

Appendix 2: Intellectual Property

The research relating to this project is for the public domain and the report and any resulting publications are intended for broad dissemination and promotion.

Appendix 3: References

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Appendix 4: Individual change point analysis

Individual summaries

Change point analysis performed on 6 hr COAs on x,y,z data (divisive non-parametric multivariate change point algorithm). Candlestick plots show daily aggregates of depth as well as daily number of detections

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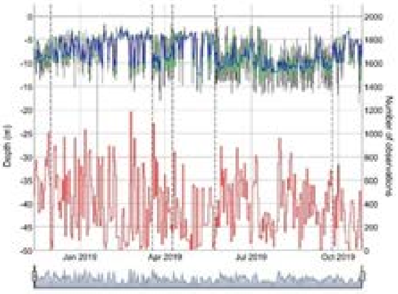

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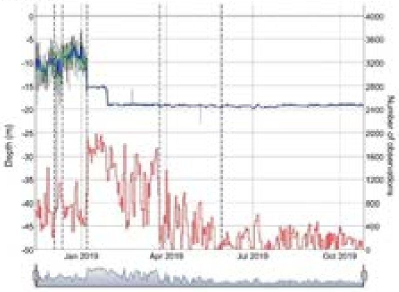

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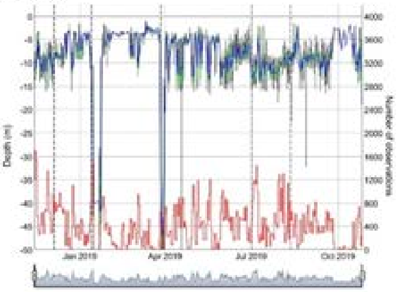

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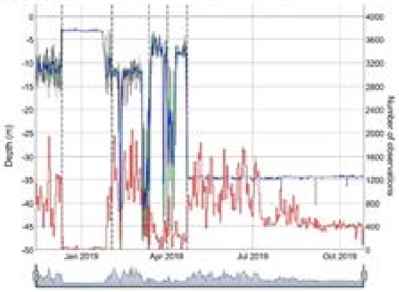

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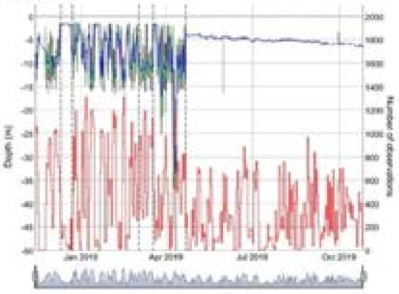

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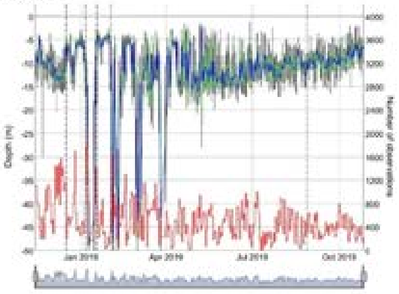

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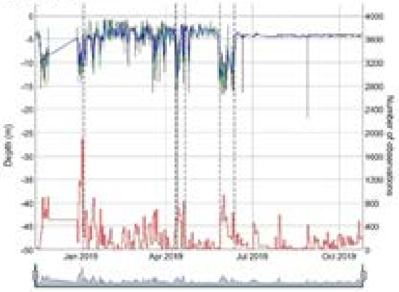

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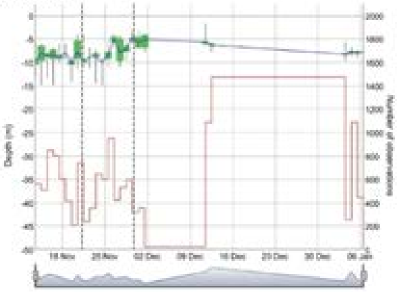

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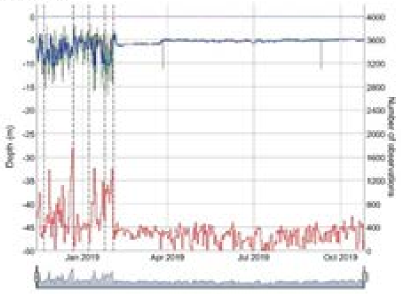

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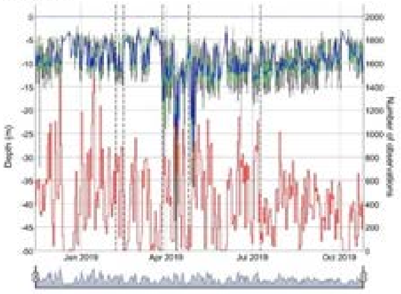

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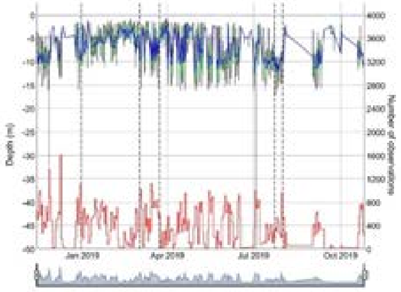

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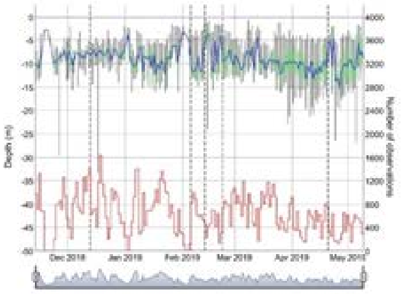

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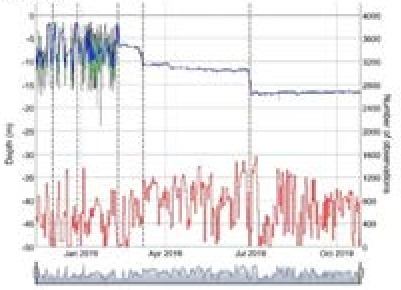

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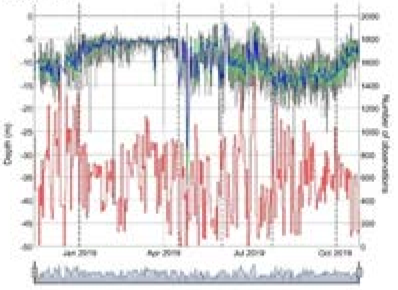

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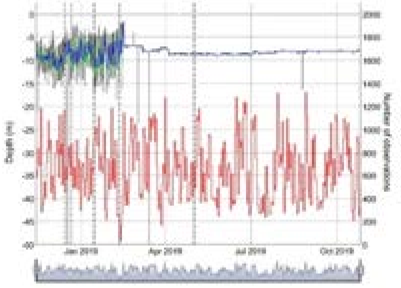

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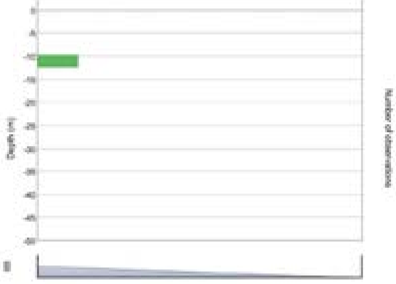

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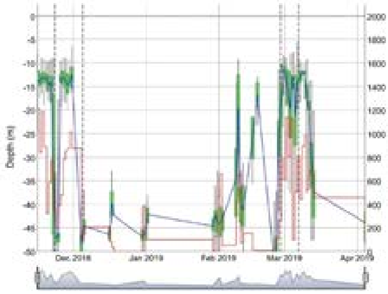

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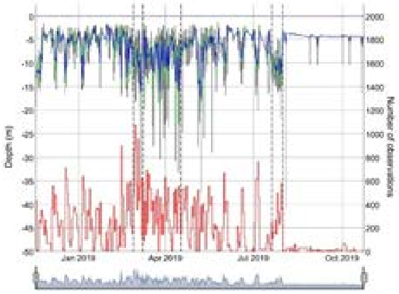

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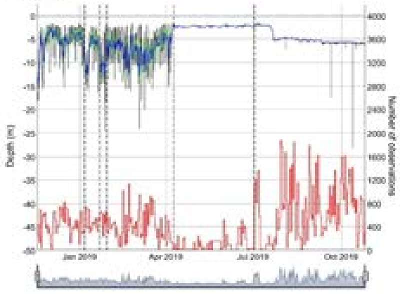

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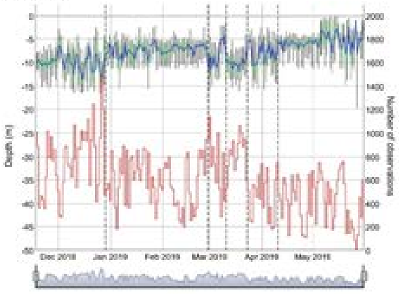


Appendix 5: Individual spatial analysis for tagged Maugean Skate showing long term residency within the study area

MSK06

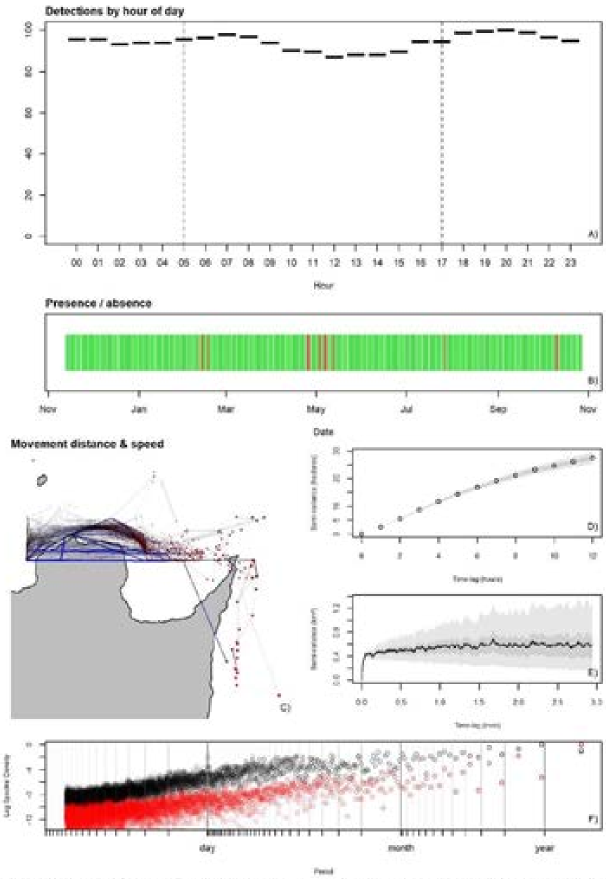
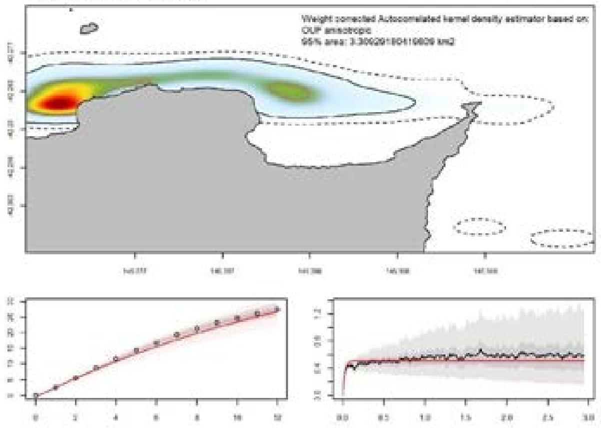


Figure 1: "**A)** Detectability (%) by hour of the day. Dotted lines represent average dusk and dawn times. **B)** Daily presence (green) or absence (red) of the individual within the array.__C)__ COA pseudo-locations for the whole study period (red dots). Line colour represents travel speed, where those lines with a deeper blue huw have the highest speeds (used for outlier detection). **D)** Short term lag (hr) continuous time variogram for semivariance in ha^2 with confidence and prediction intervals. **E)** Long term lag (Month) continuous time variogram for semivariance in ha^2 showing a clear asymptote at around 8 ha^2, consistent with residential behaviour. **F)** Periodogram of movement variance (black) with diagnostic points (red) showing autocorrelated error due to the sampling interval. There is a clear peak at 1 day that suggests a diurnal pattern that is unlikely to be explained by stochasticity (noise) or sampling bias alone. This pattern is consistent with the ocilatory pattern in the variogram (E). "

Continuous time stocastic model:



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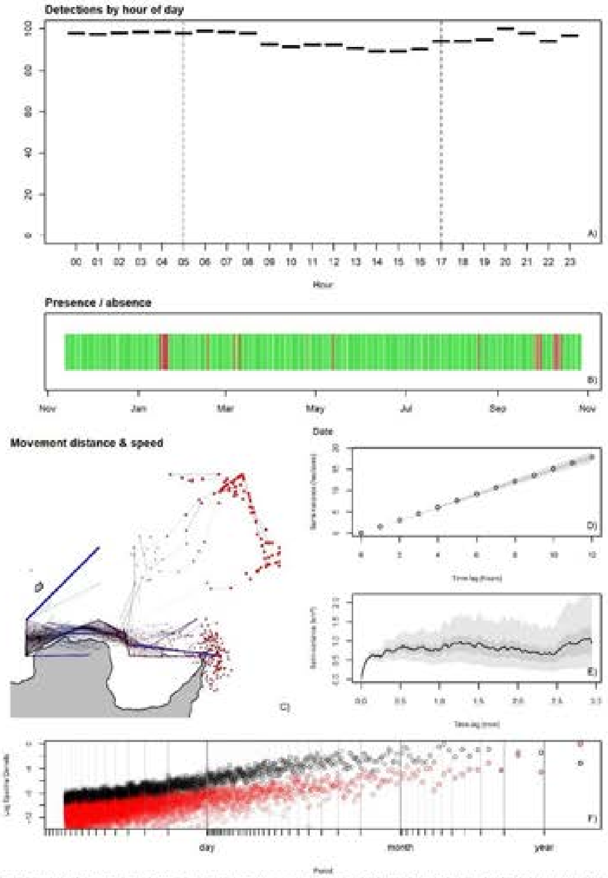

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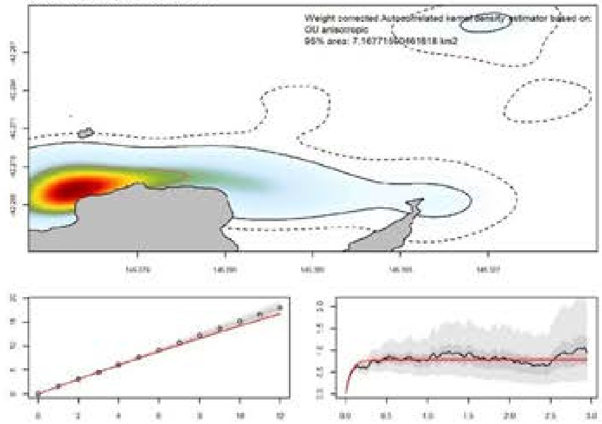
Continuous time stocastic model:



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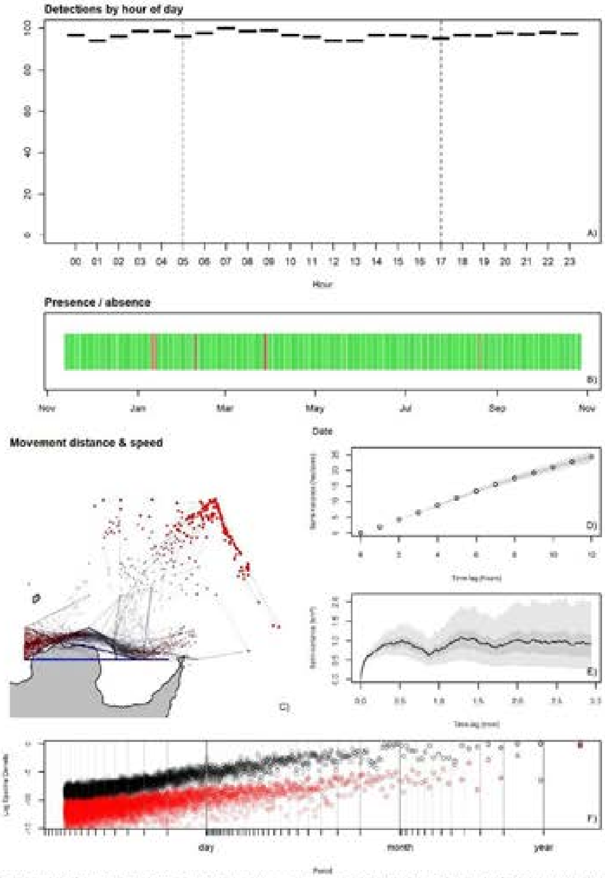

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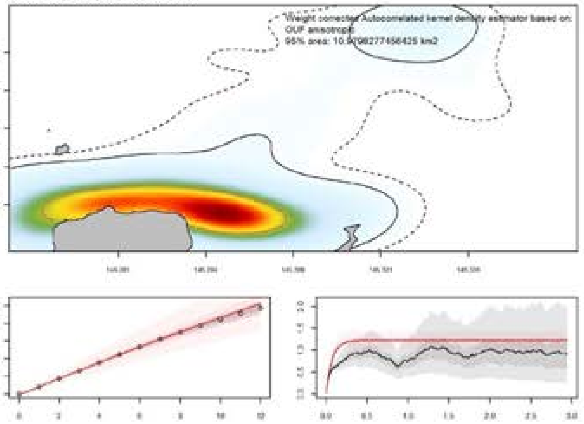
Continuous time stocastic model:



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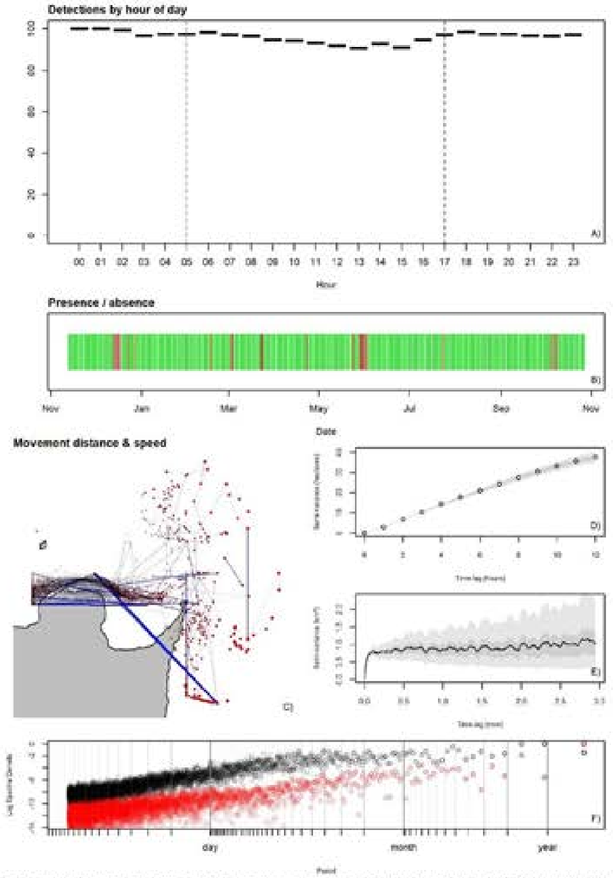
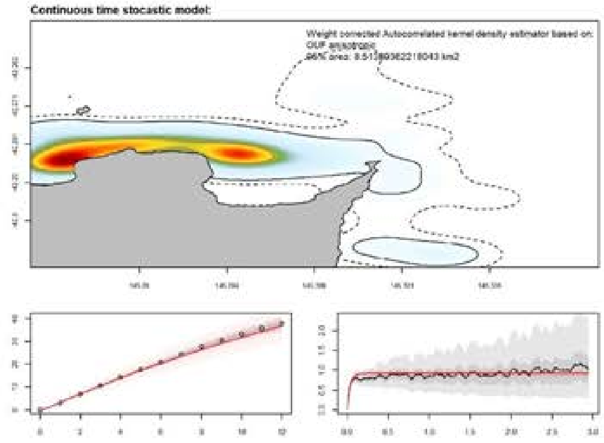


Figure 1: "**A)** Detectability (%) by hour of the day. Dotted lines represent average dusk and dawn times. **B)** Daily presence (green) or absence (red) of the individual within the array.__C)__ COA pseudo-locations for the whole study period (red dots). Line colour represents travel speed, where those lines with a deeper blue huw have the highest speeds (used for outlier detection). **D)** Short term lag (hr) continuous time variogram for semivariance in ha^2 with confidence and prediction intervals. **E)** Long term lag (Month) continuous time variogram for semivariance in ha^2 showing a clear asymptote at around 8 ha^2, consistent with residential behaviour. **F)** Periodogram of movement variance (black) with diagnostic points (red) showing autocorrelated error due to the sampling interval. There is a clear peak at 1 day that suggests a diurnal pattern that is unlikely to be explained by stochasticity (noise) or sampling bias alone. This pattern is consistent with the ocilatory pattern in the variogram (E). "



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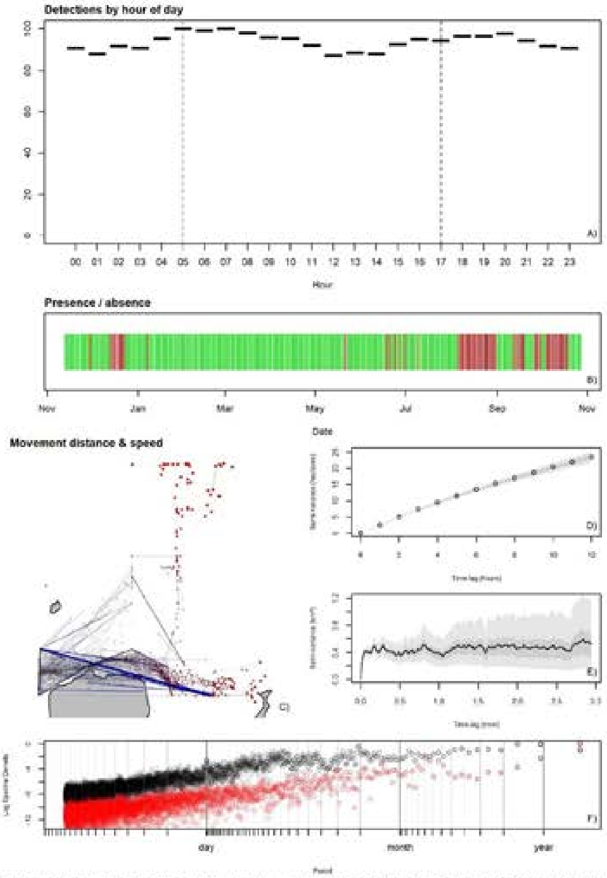
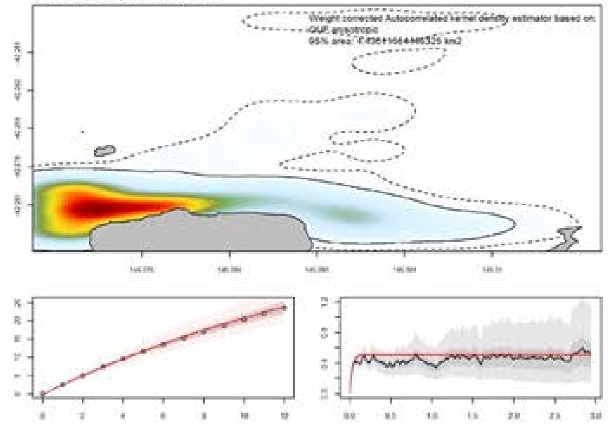


Figure 1: "**A)** Detectability (%) by hour of the day. Dotted lines represent average dusk and dawn times. **B)** Daily presence (green) or absence (red) of the individual within the array.__C)__ COA pseudo-locations for the whole study period (red dots). Line colour represents travel speed, where those lines with a deeper blue huw have the highest speeds (used for outlier detection). **D)** Short term lag (hr) continuous time variogram for semivariance in ha^2 with confidence and prediction intervals. **E)** Long term lag (Month) continuous time variogram for semivariance in ha^2 showing a clear asymptote at around 8 ha^2, consistent with residential behaviour. **F)** Periodogram of movement variance (black) with diagnostic points (red) showing autocorrelated error due to the sampling interval. There is a clear peak at 1 day that suggests a diurnal pattern that is unlikely to be explained by stochasticity (noise) or sampling bias alone. This pattern is consistent with the ocilatory pattern in the variogram (E). "

Continuous time stocastic model:



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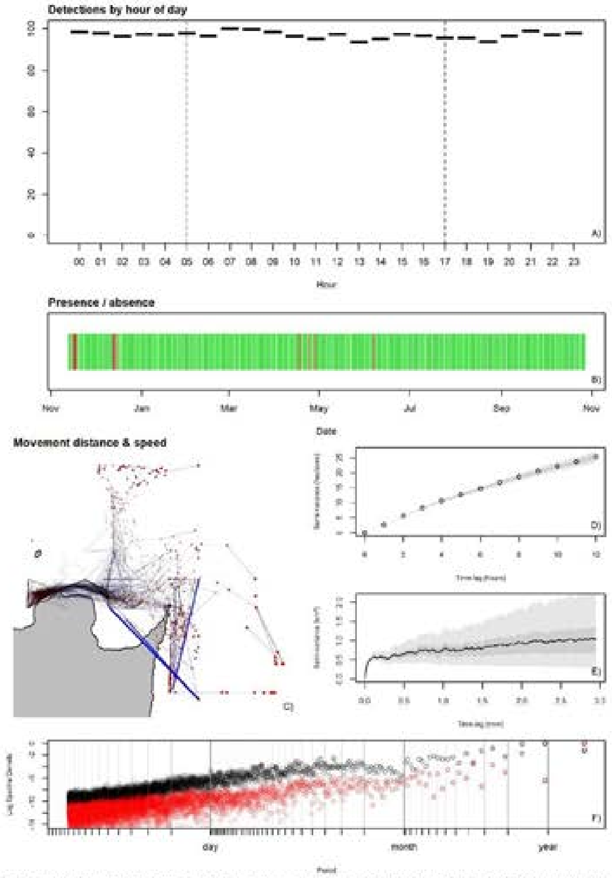
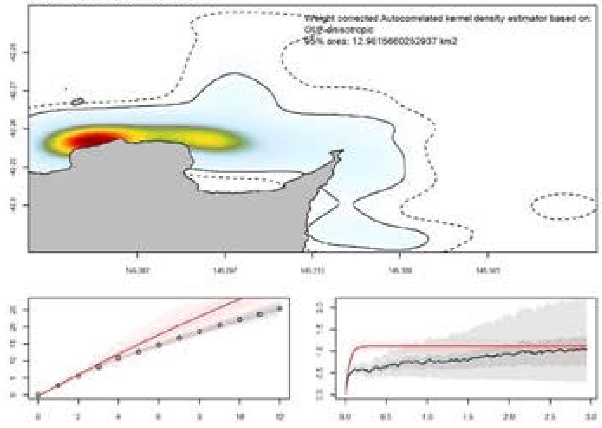


Figure 1: "**A)** Detectability (%) by hour of the day. Dotted lines represent average dusk and dawn times. **B)** Daily presence (green) or absence (red) of the individual within the array.__C)__ COA pseudo-locations for the whole study period (red dots). Line colour represents travel speed, where those lines with a deeper blue huw have the highest speeds (used for outlier detection). **D)** Short term lag (hr) continuous time variogram for semivariance in ha^2 with confidence and prediction intervals. **E)** Long term lag (Month) continuous time variogram for semivariance in ha^2 showing a clear asymptote at around 8 ha^2, consistent with residential behaviour. **F)** Periodogram of movement variance (black) with diagnostic points (red) showing autocorrelated error due to the sampling interval. There is a clear peak at 1 day that suggests a diurnal pattern that is unlikely to be explained by stochasticity (noise) or sampling bias alone. This pattern is consistent with the ocilatory pattern in the variogram (E). "

Continuous time stocastic model:



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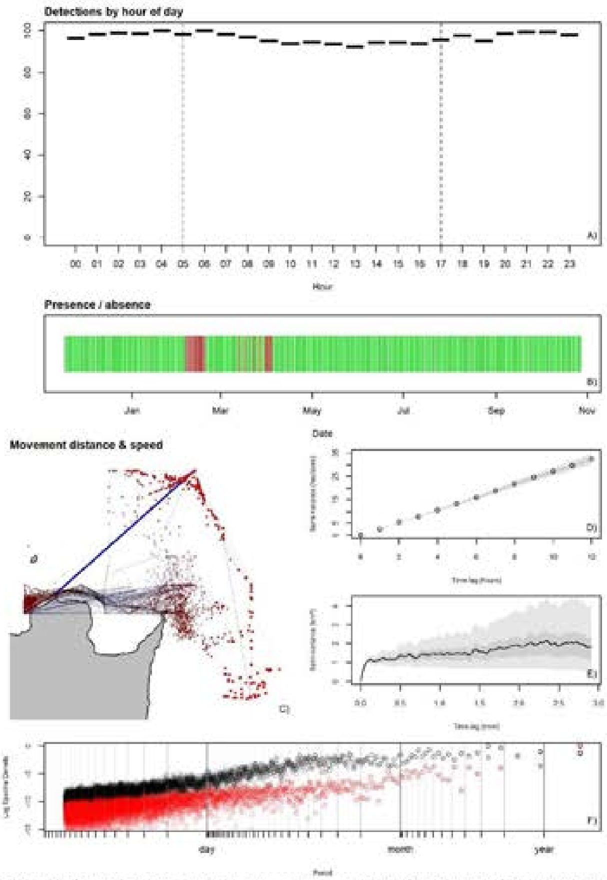
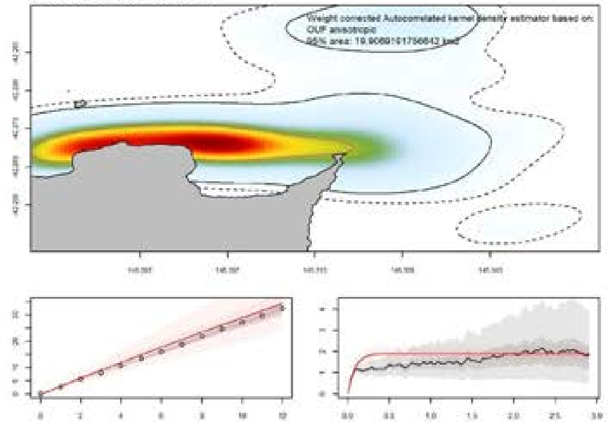


Figure 1: "**A)** Detectability (%) by hour of the day. Dotted lines represent average dusk and dawn times. **B)** Daily presence (green) or absence (red) of the individual within the array.__C)__ COA pseudo-locations for the whole study period (red dots). Line colour represents travel speed, where those lines with a deeper blue huw have the highest speeds (used for outlier detection). **D)** Short term lag (hr) continuous time variogram for semivariance in ha^2 with confidence and prediction intervals. **E)** Long term lag (Month) continuous time variogram for semivariance in ha^2 showing a clear asymptote at around 8 ha^2, consistent with residential behaviour. **F)** Periodogram of movement variance (black) with diagnostic points (red) showing autocorrelated error due to the sampling interval. There is a clear peak at 1 day that suggests a diurnal pattern that is unlikely to be explained by stochasticity (noise) or sampling bias alone. This pattern is consistent with the ocilatory pattern in the variogram (E). "

Continuous time stocastic model:



Appendix 6: Monthly space use and travel speed for all acoustically tagged individuals

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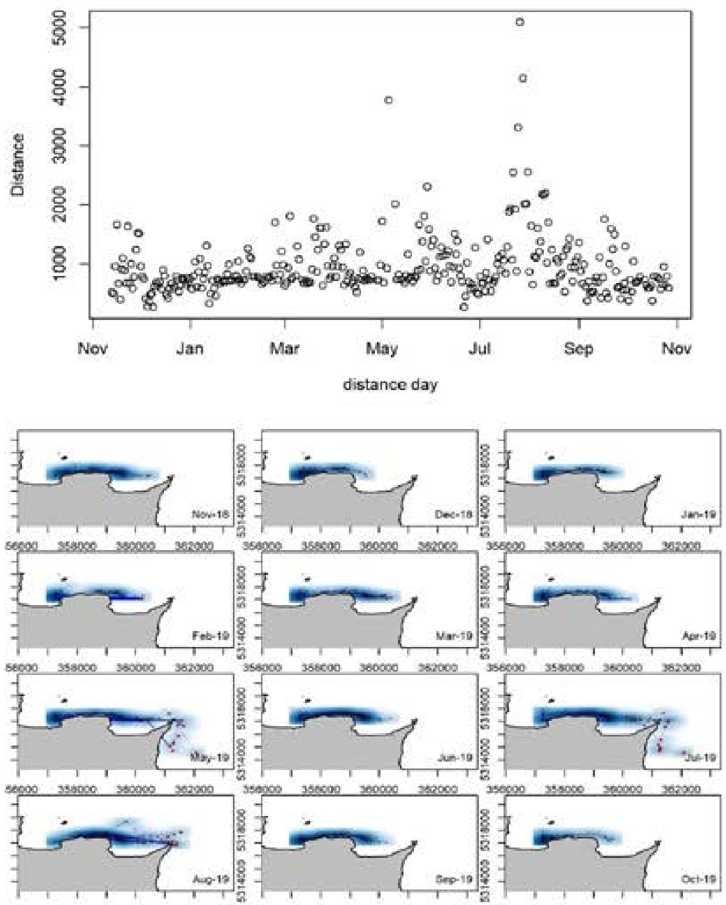

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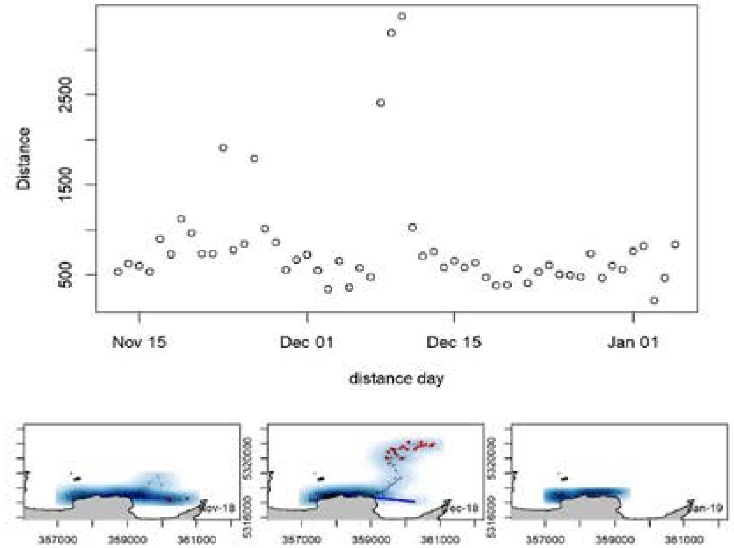

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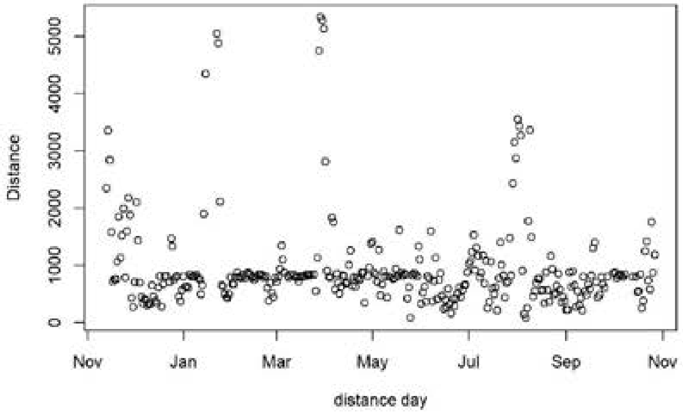
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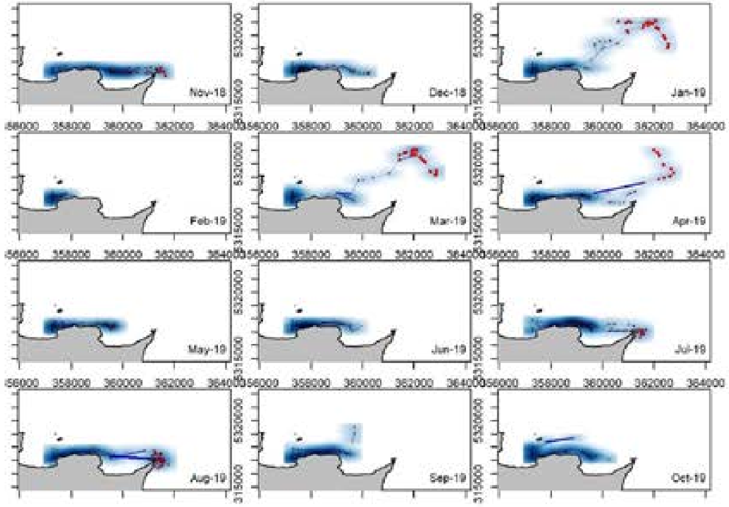
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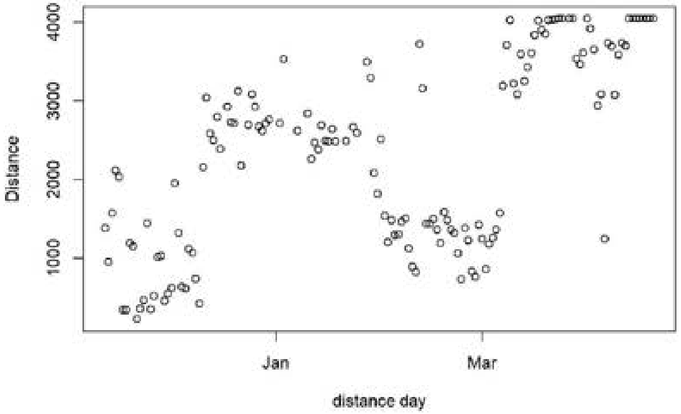
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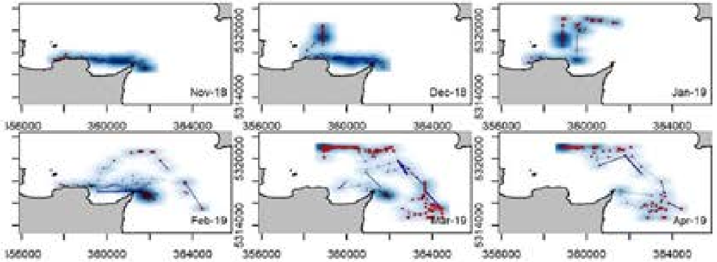
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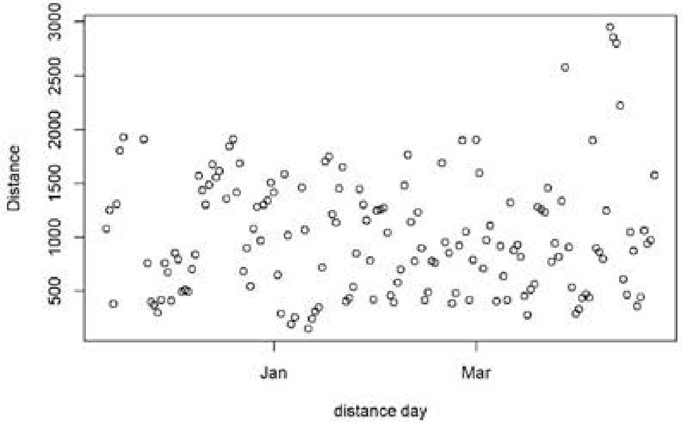
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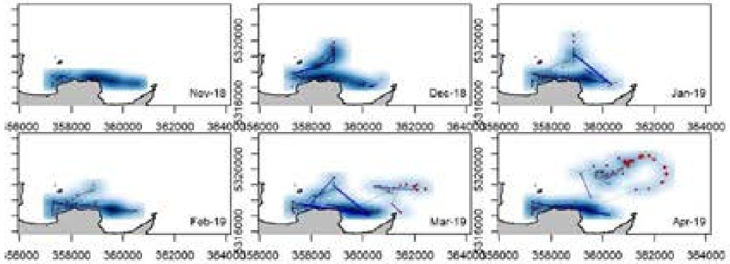
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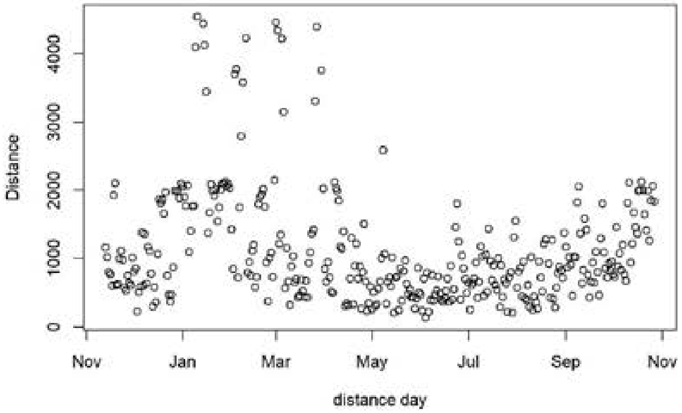
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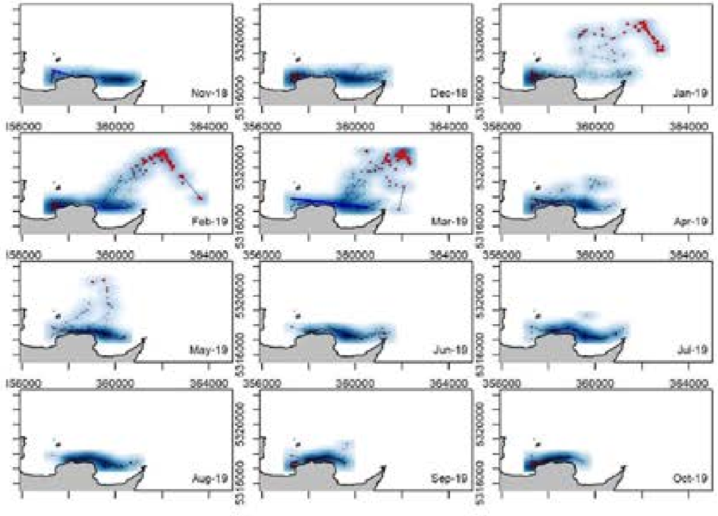
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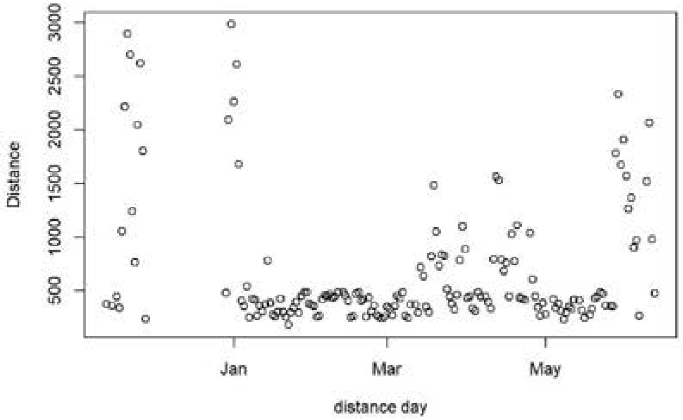
MS12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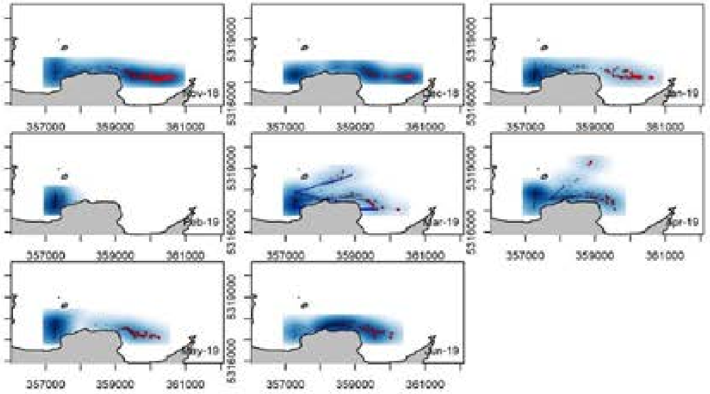
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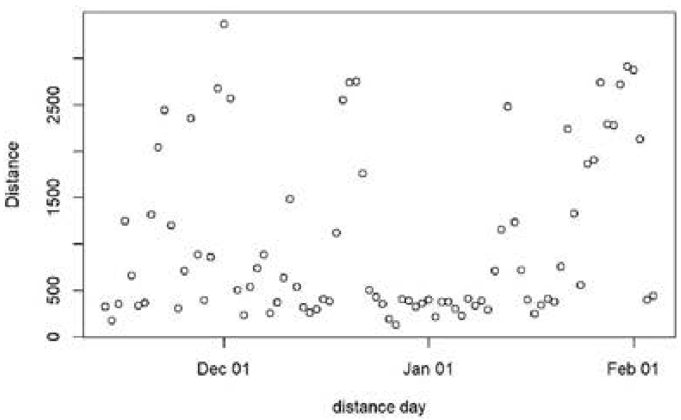
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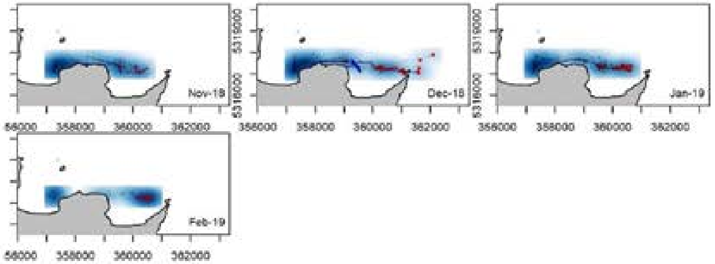
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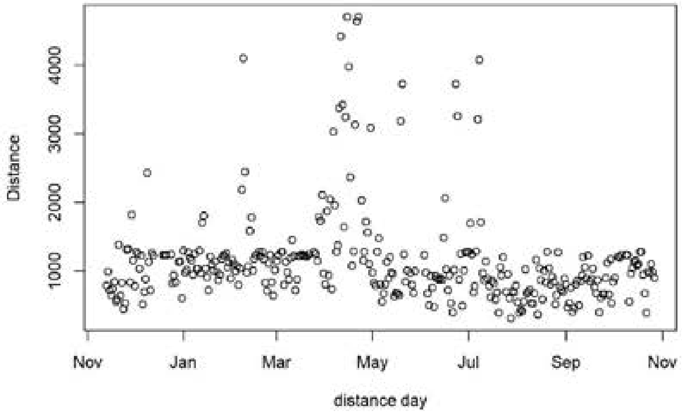
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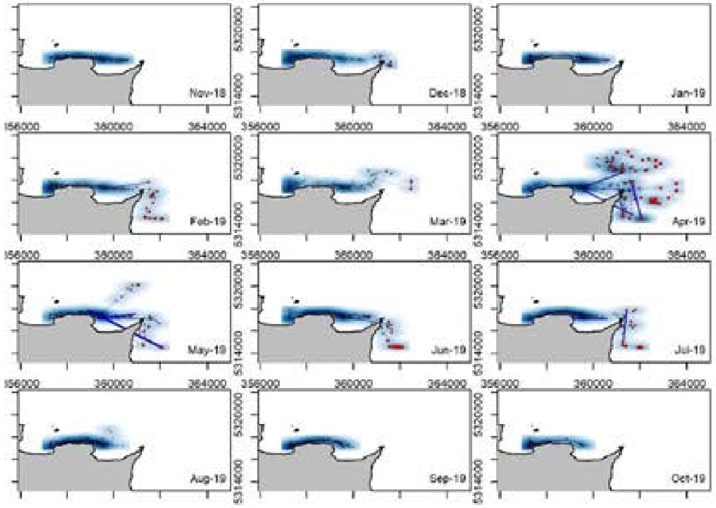
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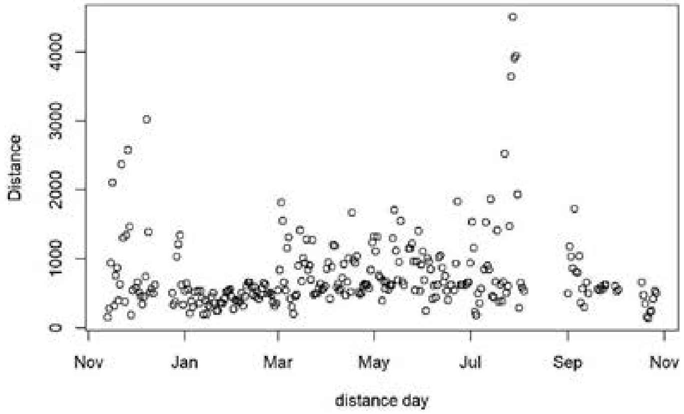
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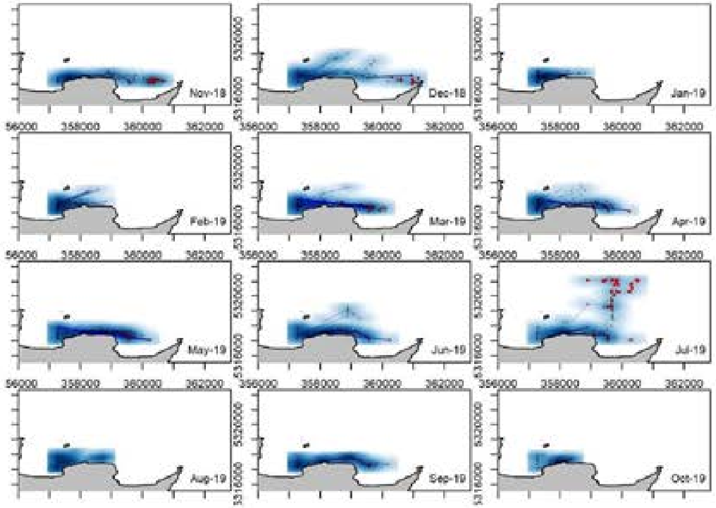
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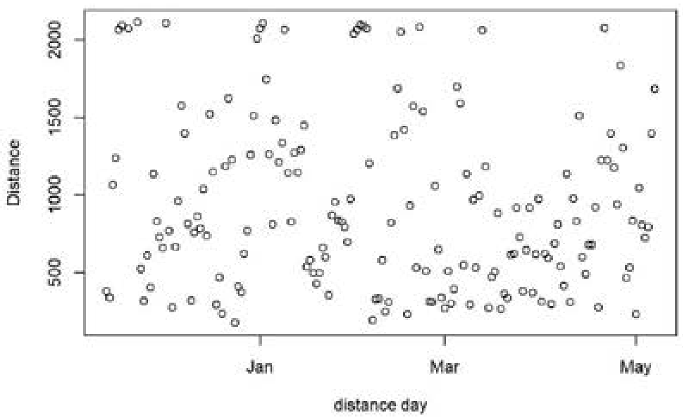
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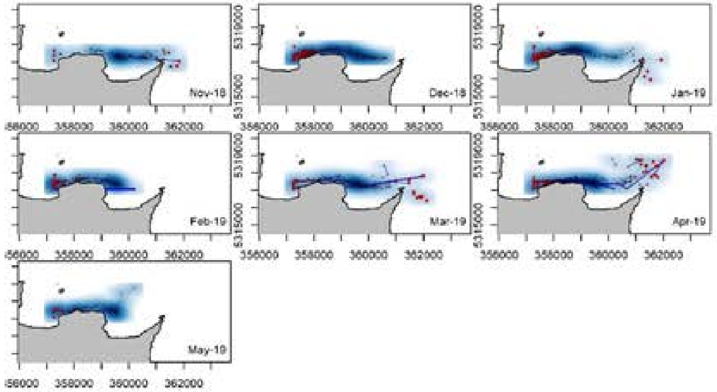
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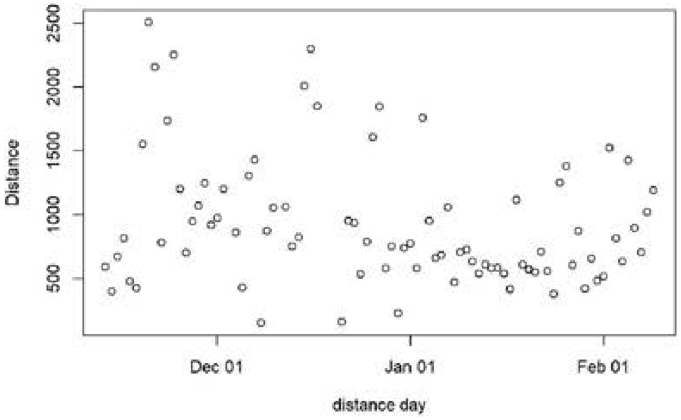
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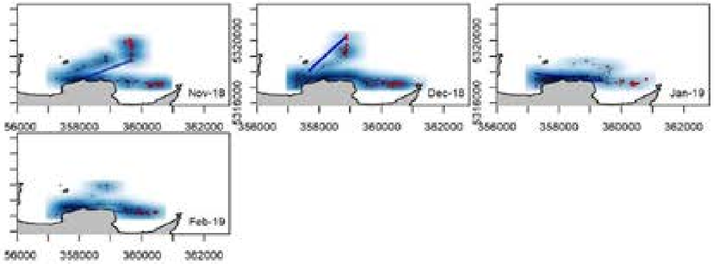
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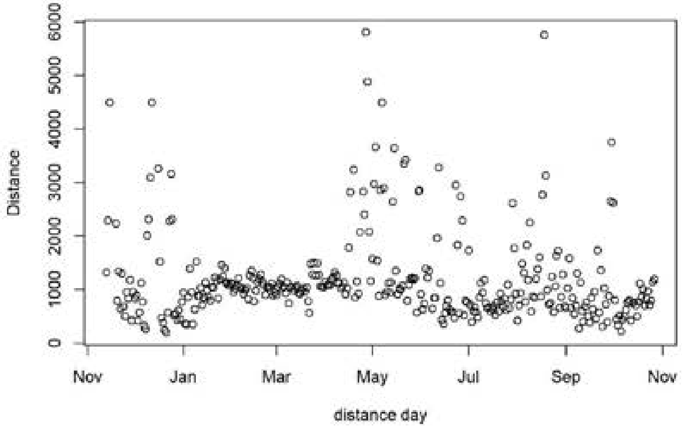
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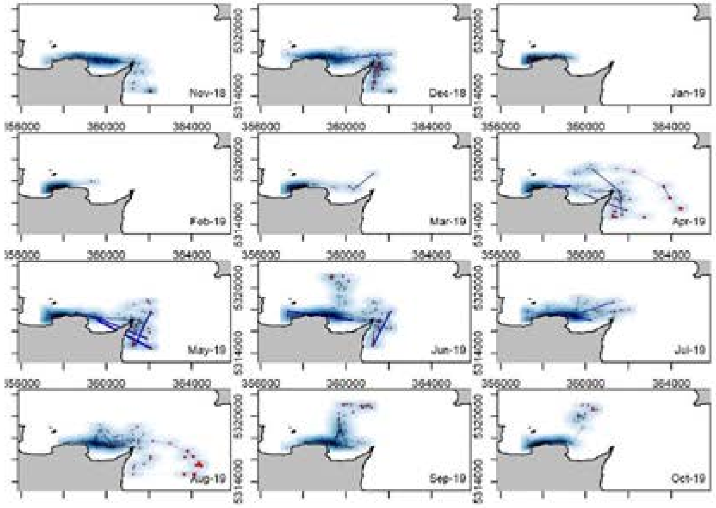
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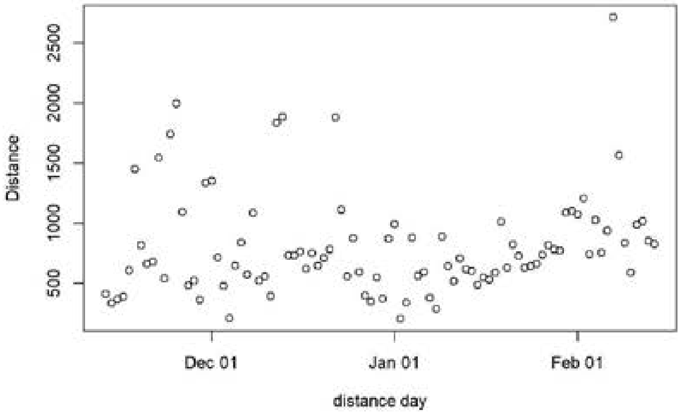
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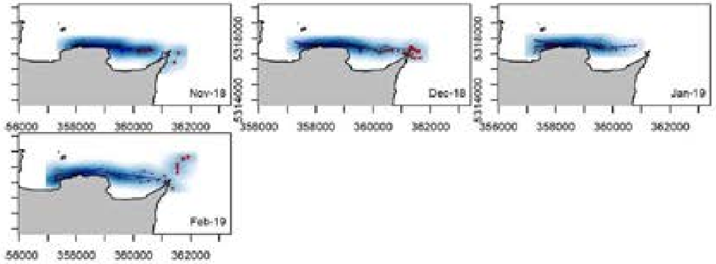
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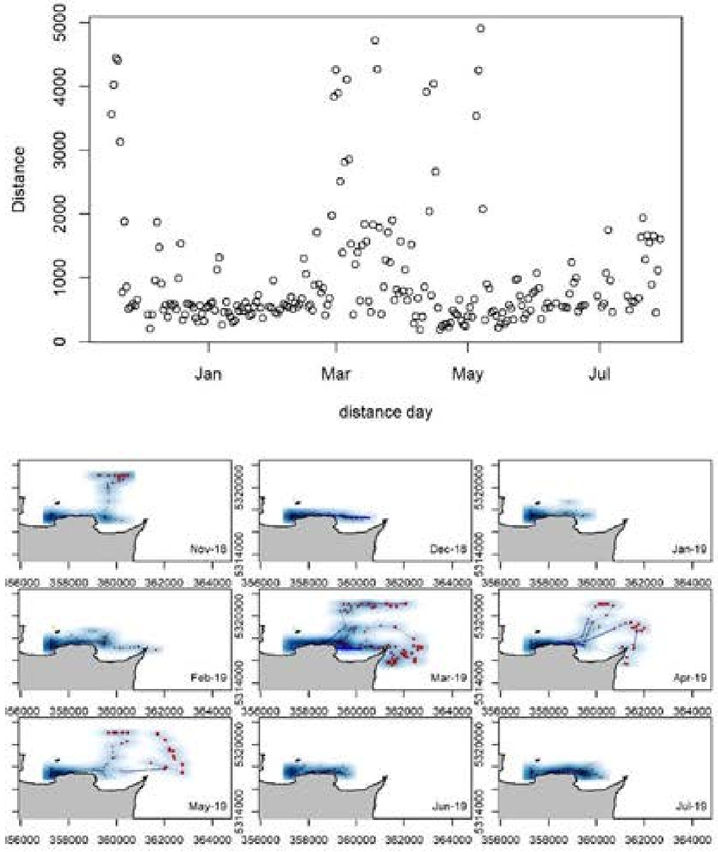
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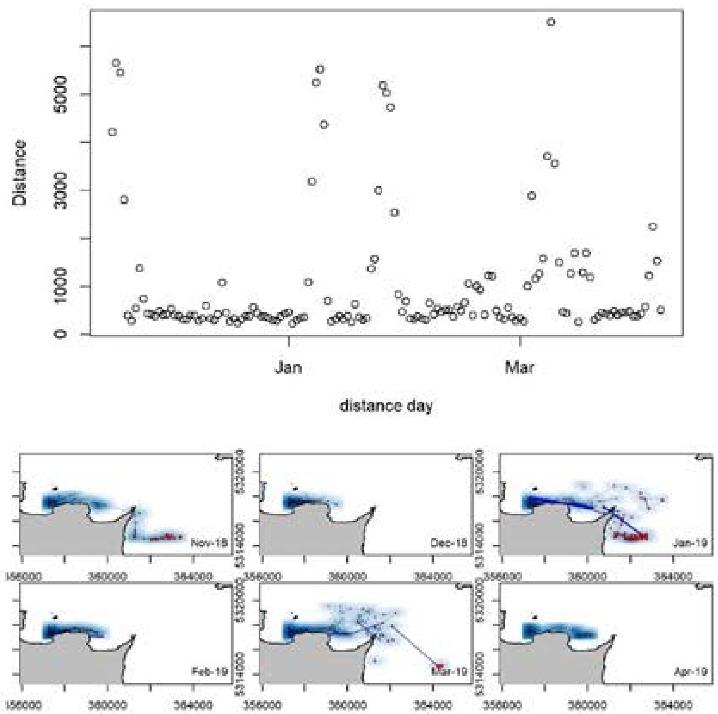
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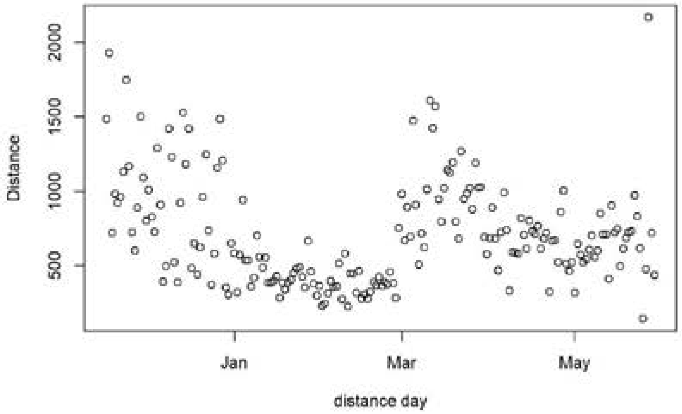
MS26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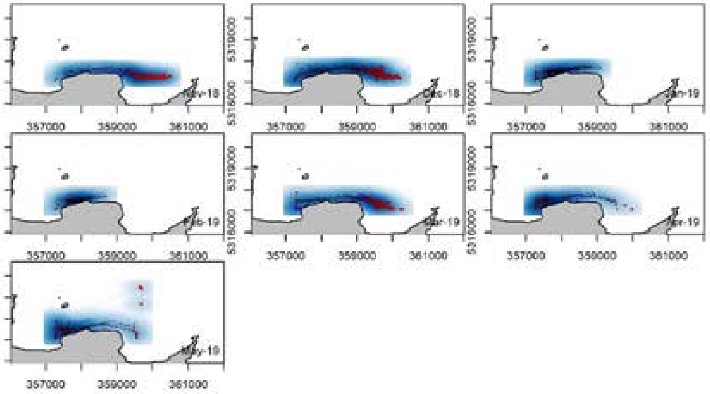
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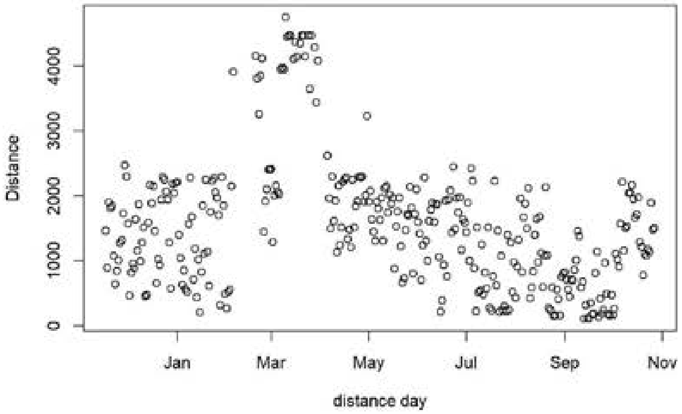
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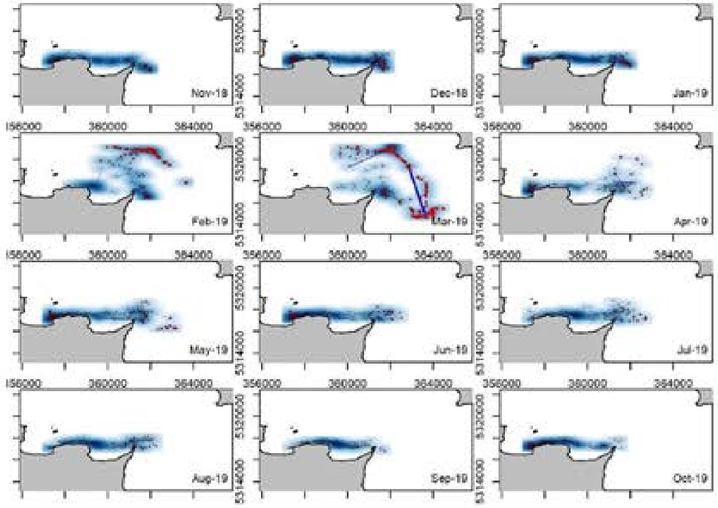
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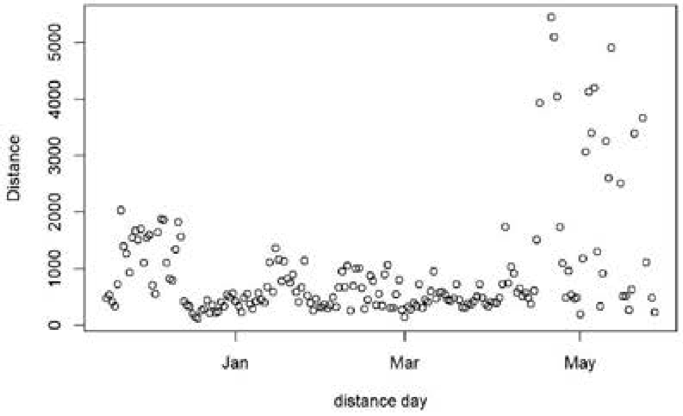
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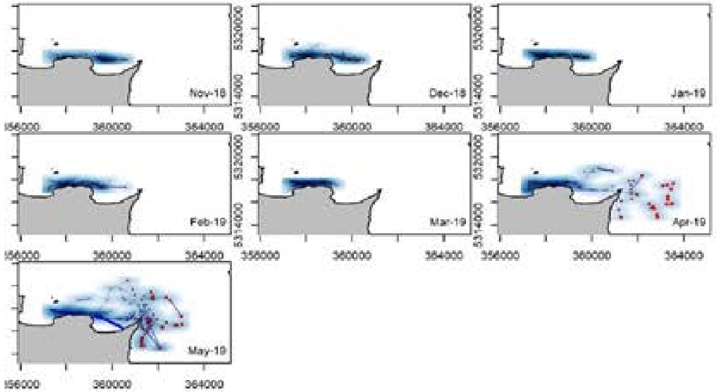
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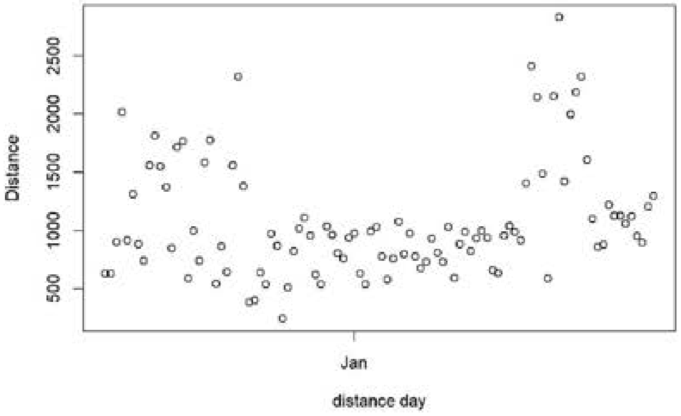
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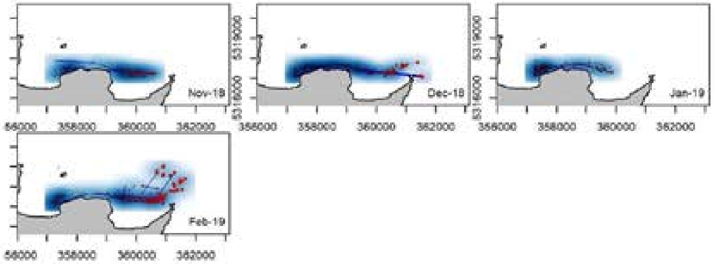
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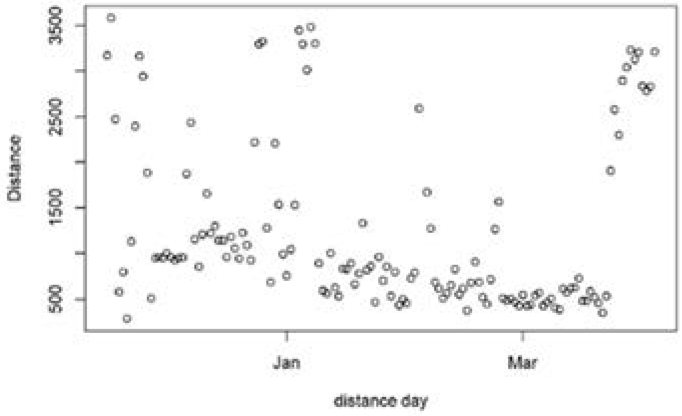
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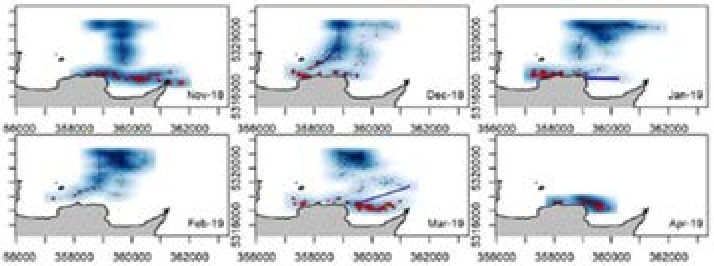
distance day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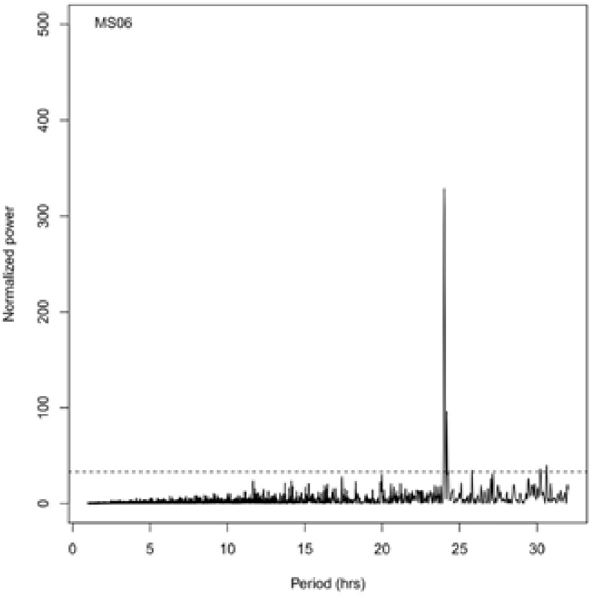
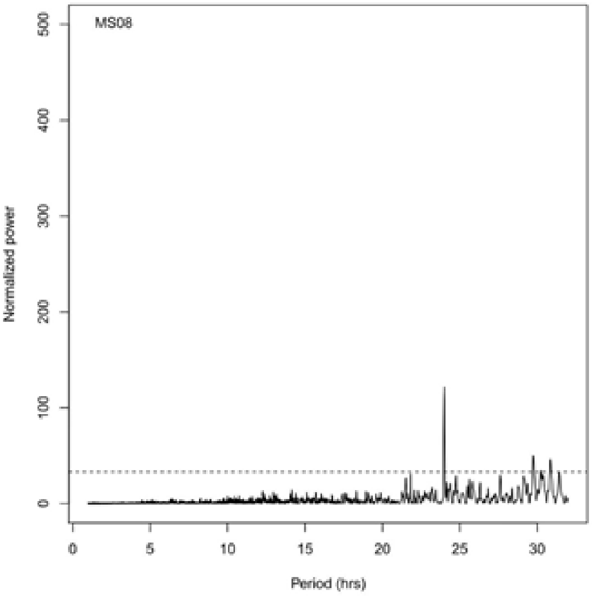
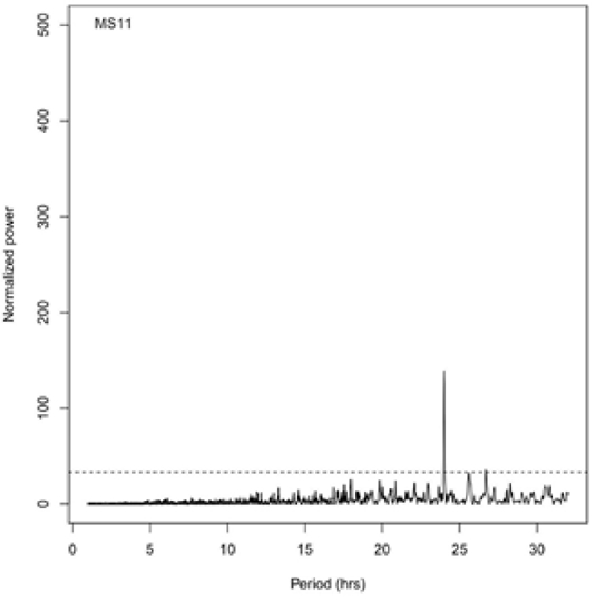
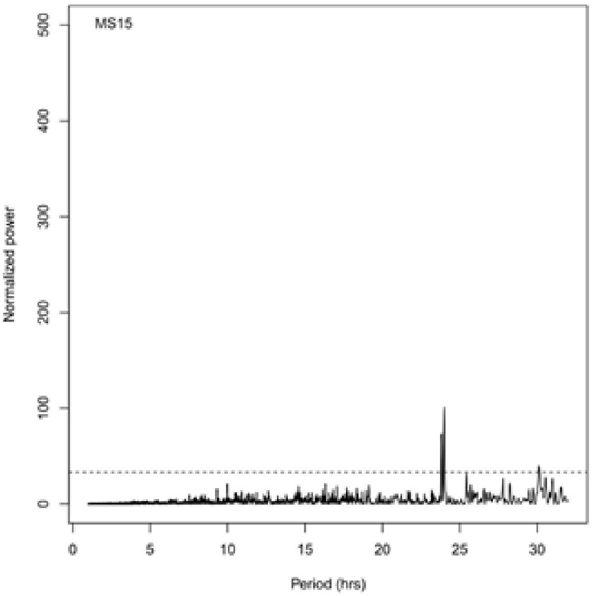
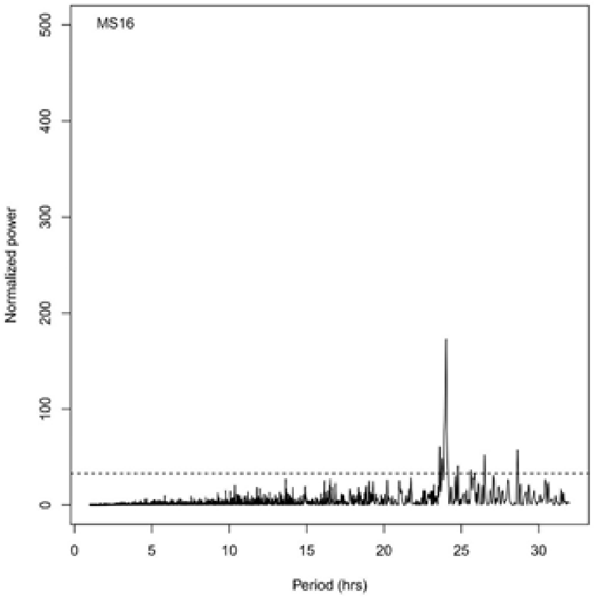
Appendix 7: Harmonic analysis of depth for tagged Maugean Skate showing long term residency within the study area

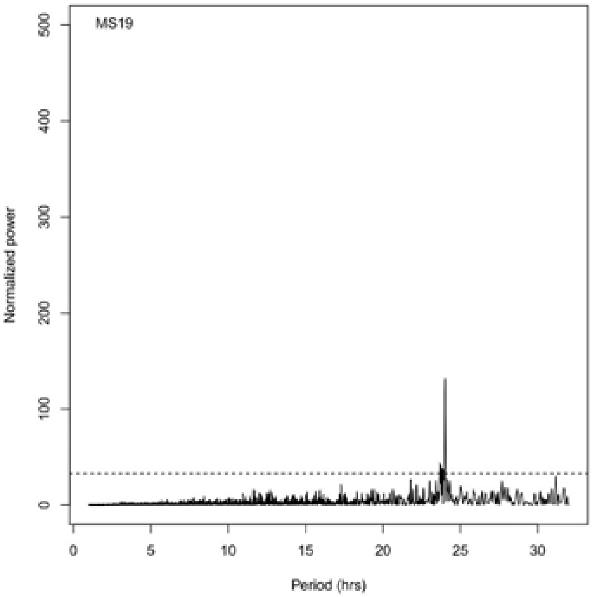


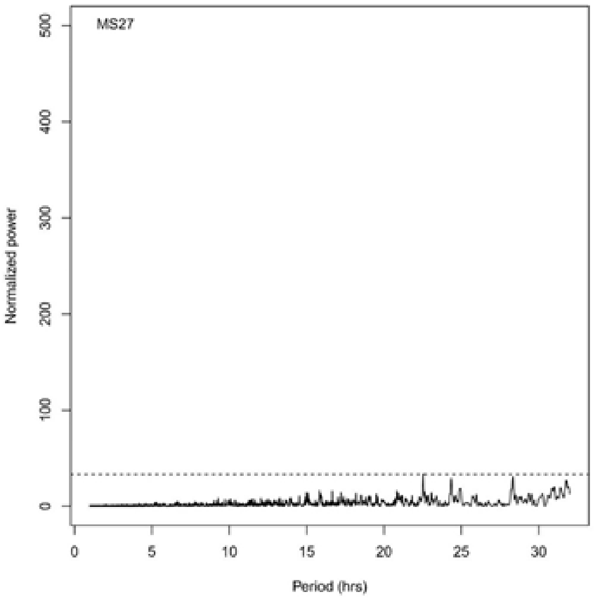







MS16
Normalized power
0
100
200
300
400
500
Period (hrs)
0
5
10
15
20
25
30


MS19
Normalized power
0
100
200
300
400
500
Period (hrs)
0
5
10
15
20
25
30


MS27
Normalized power
0
100
200
300
400
500
Period (hrs)
0
5
10
15
20
25
30