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Report of the SBT gene-tagging program 2020

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Report to the Commission for the Conservation of Southern Bluefin Tuna

Contents

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Tissue collection during harvest: Seatec, Australian SBT Industry Association (ASBTIA) and members, and processing managers and staff of Australian Fishing Enterprises and Tony's Tuna International.

Diversity Arrays Technology Pty Ltd.

The CSIRO SBT research team and research support staff.

1 Abstract

The CCSBT gene-tagging program is designed to provide an estimate of the absolute abundance of the age-2 cohort in the year they were tagged (Preece et al., 2015). The process involves "tagging" fish by taking a very small tissue sample from a large number of 2-year-old SBT, releasing the fish alive, allowing 12 months for mixing with untagged SBT, and then taking tissue samples from the catch of 3-year-old fish at time of harvest. The two sets of samples are genotyped and then compared in order to find the samples with matching DNA; a match indicates that a tagged and released fish was recaptured. The abundance estimate is calculated from the number of samples in the release and harvest sets and the number of matches found.

In 2020 we report on the calculation of an absolute abundance estimate from the 2018 gene-tagging program which is the third full cycle of the CCSBT gene-tagging program. The analysis found 66 matches from 75.4 million comparisons across the tagging and harvest data sets. The abundance estimate for the age 2 cohort in 2018 is 1.143 million fish (C.V. 0.123). This abundance estimate is close to half of the estimate of age 2 fish in 2016 but is not as low as estimates for the age 2 cohorts from the years of very low recruitment in the stock assessment models (1999-2002). The next estimate of abundance (age-2 cohort in 2019) is on track to be provided in early 2021 with tagging and harvest sampling components completed. The 2020 tagging field work team had difficulties finding fish, and weather conditions were not ideal. The CSIRO field team were urgently recalled back to Hobart after 9 days of the 20-day field trip because of COVID-19 risks and border closure uncertainties at that time. Too few fish were sampled which means that the 2020 gene-tagging program will not deliver an estimate of abundance in 2022.

The completed data sets and abundance estimate has been provided to the CCSBT scientific data exchange in April 2020. The 2016-2018 abundance estimates will be used for the first time in the 2020 stock assessment, and in the new Cape Town Procedure for recommending the total global allowable catch.

2 Introduction

This report provides information on completion of the 2018 gene-tagging program, progress in the 2019 program, the 2020 tagging field work and Research Mortality Allowance (RMA) use, and the request for RMA in 2021.

Gene-tagging SBT involves "tagging" fish by taking a very small tissue sample from a large number of 2-year-old SBT, releasing the fish alive, allowing 12 months for mixing with untagged SBT, and then taking tissue samples from the catch of 3-year-old fish at time of harvest. The two sets of samples are genotyped and then compared in order to find the samples with matching DNA; a match indicates that a tagged and released fish was recaptured. The abundance estimate is calculated from the number of samples in the release and harvest sets and the number of matches found. The design study examined sample sizes, potential sources of bias, costs and precision of estimates and integration of data in stock assessment and management procedure models (Preece et al., 2015).

The calculation of an absolute abundance estimate, from the 2018 gene-tagging program, completed the third full cycle of the CCSBT gene-tagging program. The estimates of juvenile abundance from gene-tagging (2016-2018) will be used in the SBT stock assessment in 2020 and in the new management procedure (Hillary et al., 2019).

3 The 2018 gene-tagging program

The 2018 gene-tagging program followed the specifications for the pilot study as recommended in the design study. Twenty days at sea was considered the minimum viable period that would allow for bad weather and poor fishing days, based on previous experience with conventional SBT tagging projects. The design study recommended tagging and releasing 5,000 fish and harvest sampling 10,000 fish.

The project involved the following steps:

1. Tag and release: vessel charter and at-sea collection of tissue samples from age 2 fish in the Great Australian Bight during the summer of year 1 (2018).
2. Harvest sampling: collection of tissue samples from age 3 fish in winter of year 2 (2019), during harvest of fish in farms, which were caught by the Australian surface fishery.
3. DNA extraction and genotyping of tissue samples, using CSIRO-developed SNP markers.
4. Data analysis and calculation of an abundance estimate, and provision of results to the Extended Scientific Committee for use in stock assessment models in 2020 and the new management procedure.

The design study noted potential extensions to the basic design adopted, which include: tagging and resampling fish from regions outside the Great Australian Bight, tagging and resampling multiple age classes, and collection of otoliths to address uncertainties in age classes of the fish sampled. Direct ageing of otoliths and vertebrae has been used to revise length classes chosen to target 2-year-olds and 3-year-olds (Preece et al., 2019).

4 2018 gene-tagging results and discussion

4.1 Tag and release - tissue collection 2018

The 2018 tagging program was very successful, with nearly 8200 fish tagged and released during the vessel charter from 24th February to 16th March 2018. Results from the tagging component of the project were reported to the Extended Scientific Committee in 2018 (Preece and Bradford, 2018). During the 2018 tagging program there were two modes in the length frequency of the fish tagged (Figure 1), indicating that the length range being targeted for tagging (70-85cm) may potentially cover more than one age class in that year. These peaks in the length frequency were not observed in any of the other three tagging seasons (2016, 2017, 2019).

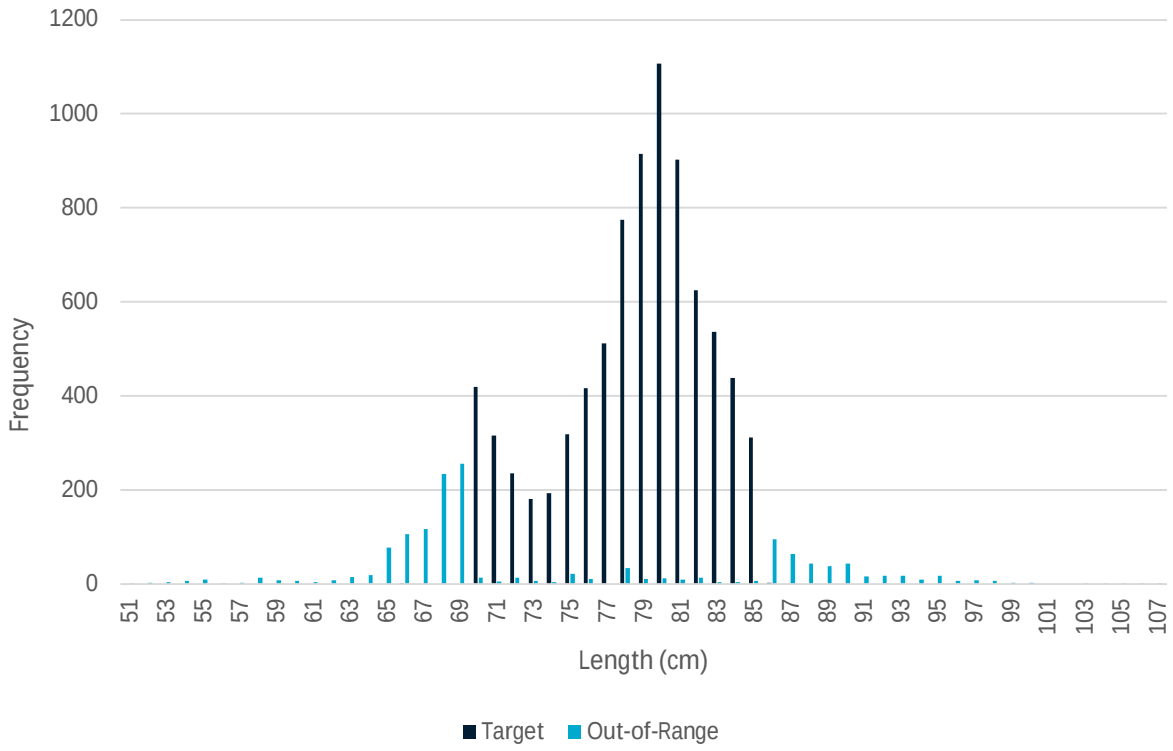


Figure 1 Length frequency of SBT tagged (in target range, black) and discarded (out of range, blue) in 2018.

To refine the length ranges for 2-year-olds (at time of tagging) and 3-year-olds (at time of harvest), CSIRO examined fish lengths at age from direct ageing of otoliths and vertebrae (Clear et al., 2019). Age from otoliths and vertebrae were compared (where we had both from the same fish) to verify the vertebrae ageing method (Gunn et al., 2008) was reliable and increments readable. In

total 100 vertebrae were aged (Figure 2). These results indicated that we should only include tagged fish in the length range 75-85 cm to best ensure that only 2-year-olds are being included in the estimation of abundance. The harvesting length range remained unchanged (98-109 cm, corresponding to 3-year-olds). This revised age-2 length range was used for all years for the age-2 abundance estimates and were provided to the CCSBT in 2019 (Preece et al., 2019).

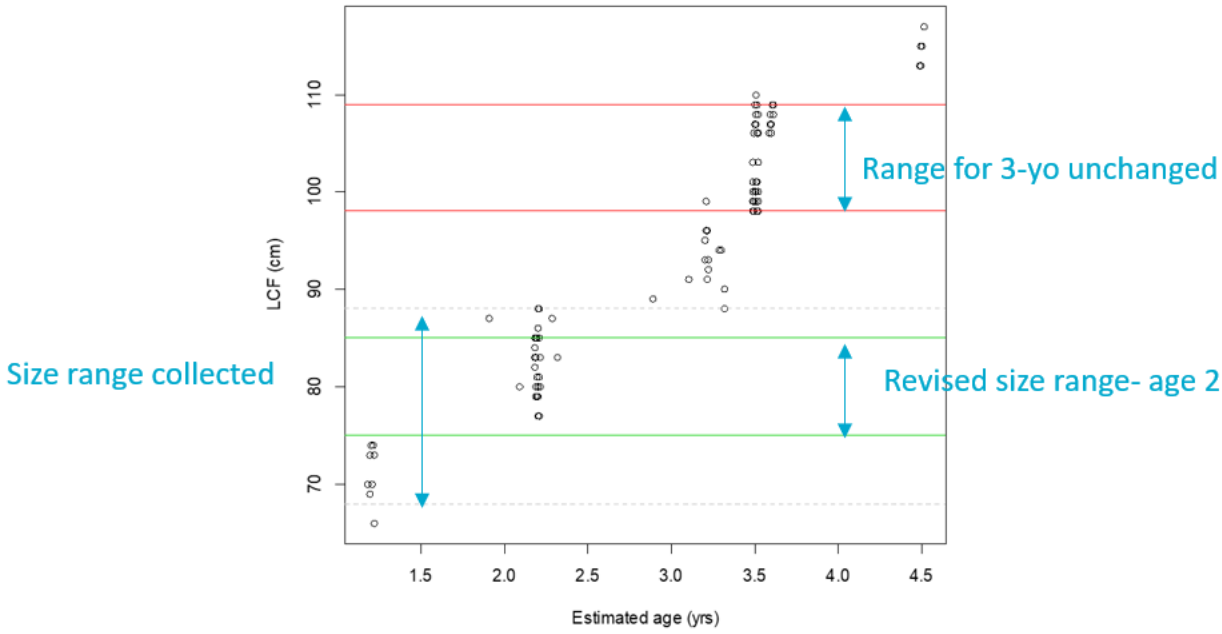


Figure 2 Vertebrae decimal-age estimate versus length. The green horizontal lines indicate the revised 75-85 cm length range used for tagging 2-year-old fish (2.25 years old in February/March). The size range of fish sampled in the pilot study was 68-88 cm as indicated by the grey dashed lines. The red horizontal lines indicate the unchanged 98-109 cm length range used for selecting 3-year-old fish during harvesting (3.5 years old in July-August).

In addition to the 8,200 fish tagged and released in 2018, another 1,429 fish were caught during tagging operations and released un-tagged (e.g. because they were outside the target length range, in poor condition etc). There were 39 mortalities from which biological samples were collected. Compared to 2017 there was a more restricted spatial spread to the tagging in 2018, with most fish tagged in the eastern GAB area. Further west, fish were generally too small, and further east fish tended to be too big. Most of the commercial fishing operations had been completed prior to the tagging work commencing, and the few remaining commercial sets occurred away from the tagging areas outside of the GAB.

The gene-tagging tool provided sufficient DNA from the tissue biopsy, even though the sample was smaller than a grain of rice. The tool allowed for efficient sampling, with fish out of the water for around 20 seconds or less; quicker than conventional tagging methods, and considerably faster and less invasive than archival tagging methods. The DNA samples have been safely archived at CSIRO, Hobart. Tagging data are managed in a database and are provided to the CCSBT data exchange.

4.2 Tissue collection during harvest in 2019

The method for collection of tissue samples during the commercial harvest from farms in Port Lincoln, South Australia, was developed in consultation with Industry representatives. This involves:

- Collection of tail stalks, which are removed as part of normal processing, and marked for later identification. Date of harvest, length, collector and other details are recorded. The tail stalks are frozen for later tissue biopsy.
- Collection of tissue samples from the tail stalks. Frozen tail stalks are thawed slightly, and a tissue biopsy taken. Tissue is collected through the skin using the gene-tag tool and loaded into individually labelled vials. Additional data are recorded (e.g. date, collector, sample number).

All participating factories were visited during the harvesting season. Additional biological samples were also collected. We thank the managers and staff in the processing factories for allowing CSIRO and Seatec access to their facilities, and for their assistance with the project.

Over 11,500 tissue samples were collected from fish in the target length range, well in excess of the design study target of 10,000 samples, and over 11,300 have been processed to extract DNA.

4.3 DNA extraction and sequencing, using CSIRO SNP markers

Nearly 20,000 tissue samples (release and harvest) have been processed using protocols established for tissue digestion, robotic DNA extraction and quality control. Data on processing, tracking, DNA quality and archiving were collected. As part of the quality control process Nano drop tests and gel electrophoresis are used to measure the quantity and quality of DNA extracted, prior to sending the plate of extracted DNA to Diversity Arrays Technology Pty Ltd (DArT) for sequencing. Two control wells in the plate are used as a unique plate identifier, using known DNA for quality/quantity checks. An archive plate of DNA is stored at CSIRO in the -80 degree ultra-freezer. Data are recorded during all stages of the processing, to note unusual samples or results, errors or changes from original storage box and position to a new plate and position.

The extracted DNA is sent to DArT for sequencing using specifically designed SNP markers. Each plate holds 92 gene-tagging samples, plus two control samples. A small percentage of samples had poor quality or quantity of DNA and, therefore, were not successfully sequenced, although the overall success rate was very high (>96%).

4.4 Data analysis and calculation of an abundance estimate

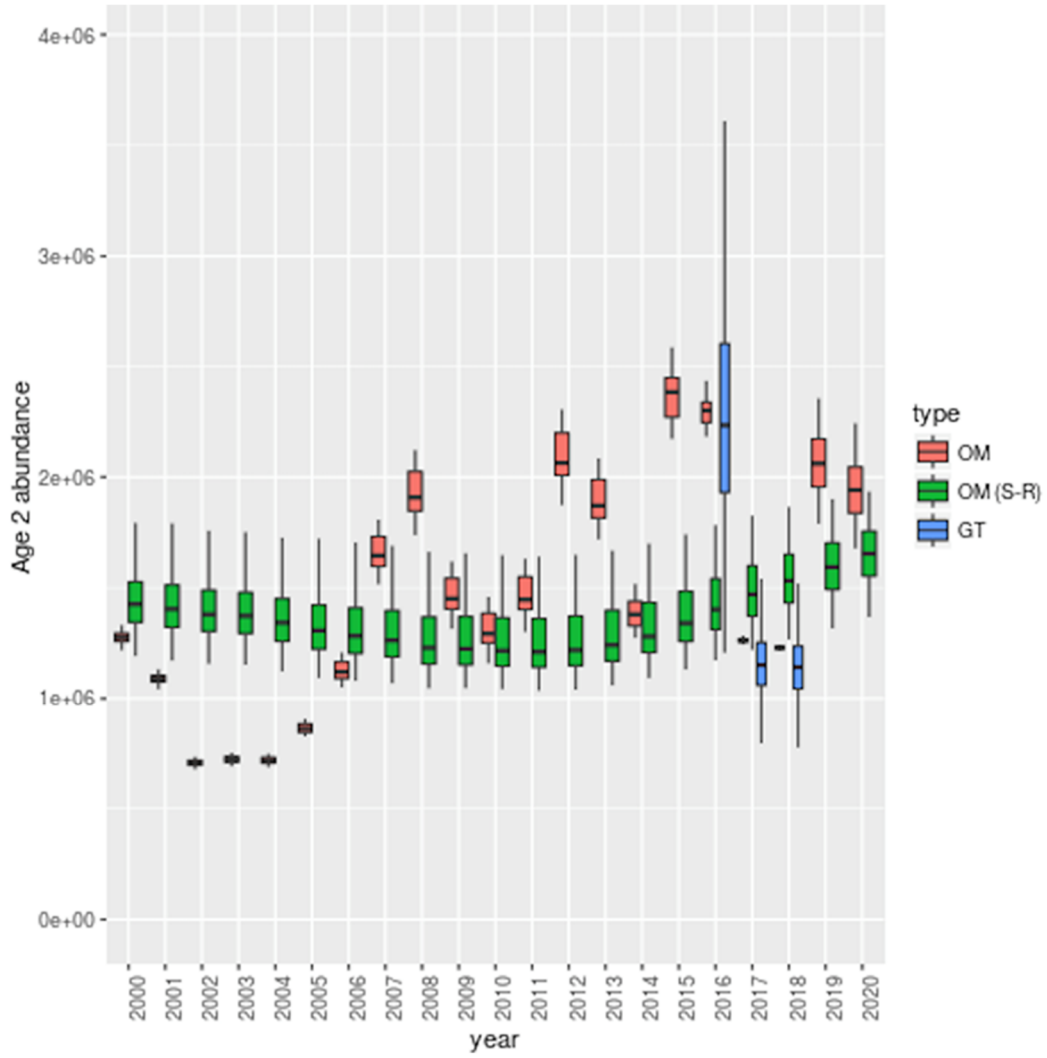
The data returned from DArT were analysed to determine whether the same fish was in the tagged set and the harvest set (using the unique DNA fingerprint of each individual). This involved first filtering the data to exclude fish with incomplete or poor genotype information (too few SNP markers with good sequencing results). Any fish outside the target release and harvest length ranges were also excluded. A fish was determined to have been recaptured if there was a fish with a matching set of markers in both the release and harvest sample sets. The analysis of releases against harvest samples involves approximately 75.4 million comparisons. The analysis has

identified 66 matches (recaptures). The estimate of abundance of age 2 fish in 2018 is 1.143 million with coefficient of variation (CV) of 0.123, which is within the range initially considered during the design project and similar to the 2017 estimate (1.15 million fish age 2, CV 0.122, Table 1). This abundance estimate is close to half of the gene-tagging estimate of age 2 fish in 2016, but is not as low as estimates for the age 2 cohorts (2002-2004) from the years of very low recruitment in the stock assessment models (2000-2002) Figure 3). Data were provided to the CCSBT scientific data exchange.

Table 1 The results of the gene-tagging programs 2016-2018 which provide the absolute abundance estimate for the age-2 cohort in the year of tagging.

YEAR	COHORT AGE	N RELEASES	N HARVEST	N MATCHES	ABUNDANCE ESTIMATE (MILLIONS)	CV
2016	2	2952	15389	20	2.27	0.224
2017	2	6480	11932	67	1.15	0.122
2018	2	6295	11980	66	1.14	0.123

Figure 3 Comparison of gene-tagging age-2 abundance estimates (blue) and recent age-2 estimates from the 2020 preliminary reconditioning of the OM (red) and those predicted from the stock-recruitment function (OM-(S-R)) (green).



The gene-tagging data used in the stock assessment models are the year of tag and release, age of fish at time of release, year of harvest sampling, the number of releases, number of harvest samples, and the number of matches. The gene-tagging data used in the Cape Town Procedure are the year that estimate applies to, abundance estimate and number of matches.

5 Progress in the 2019 gene-tagging program

The fourth cycle of the gene-tagging program commenced with tagging field work in 2019, when approximately 4,800 tissue samples were collected from fish in the age-2 size class (Preece et al., 2019). These fish were tagged at sea and released to mix with the population. The next stage involved collection of tissue samples during the 2020 harvest season. Over 12,000 tissue samples have been collected from fish in the age-3 size class, thanks to support from Seatec and the fishing Industry members in Port Lincoln who assisted with access to fish and navigated the restrictions related to COVID-19. These tissue samples will now be processed, and the extracted DNA will be genotyped. The abundance estimate for the age 2 cohort in 2019 will be available in early 2021.

6 The 2020 gene-tagging program

In 2020, the fifth cycle of gene-tagging commenced with at-sea tagging in March. The field team had difficulties finding fish, and weather conditions were not ideal. The CSIRO field team were urgently recalled back to Hobart after only 9 days of the 20-day field trip because of COVID-19 risks and border closure uncertainties at that time. Too few fish were sampled in the limited time at sea, which means that the gene-tagging program will not deliver an estimate of abundance in 2022. A detailed trip report is provided in Appendix A: Field trip report. Timing and logistics of the field work are being reviewed to minimise risks of finding too few fish in the 2021 field work, and to make contingency plans in case there are travel or other restrictions in place in relation to COVID-19.

7 Research Mortality Allowance

None of the 2020 RMA was used during the shortened field work. Details are provided in the field trip report (Appendix A: Field trip report).

The request for RMA for the 2021 field trip is 2t. This is expected to be an over-estimate of the requirements, that allows for unusual and unforeseen conditions.

8 Summary

The third full-cycle of the CCSBT gene-tagging program has successfully tagged over 8000 fish in 2018 and collected samples from over 11,500 fish during the harvest in 2019. DNA has been extracted from all suitable tissue samples. Quality control filtering of the sequencing data ensures only samples with good DNA are included in the analysis. The analysis found 66 matches from 75.4 million comparisons across the tagging and harvest data sets. The abundance estimate for the age 2 cohort in 2018 is 1.143 million fish (C.V. 0.123). The completed data sets and abundance estimate has been provided to the CCSBT scientific data exchange in April 2020. The 2016-2018 abundance estimates will be used for the first time in the 2020 stock assessment, and in the new management procedures for recommending the total global allowable catch.

The harvest sampling component of the 2019 gene-tagging program has been completed (during the 2020 harvest season). Processing of these samples is the next stage, and the analysis will provide an abundance estimate for the 2019 age-2 cohort in early 2021.

The 2020 tagging field work was not successful. Only a few fish were tagged, and the field work was cut short because of COVID-19 restrictions. Therefore, there will not be a gene-tagging program estimate of the 2020 age-2 cohort in 2022.

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Appendix A: Field trip report

CCSBT-CSIRO Southern Bluefin Tuna Gene Tagging – March 2020

CSIRO Personnel: Russell Bradford, Jason Hartog

Trip dates: 17 March 2020 to 25 March 2020.

The southern bluefin tuna gene tagging project aims, on an annual basis, to catch, obtain a tissue sample (gene tag), and then release 5,000 southern bluefin tuna (SBT) within the Great Australian Bight. The 2020 gene tagging trip was the 5th such trip to tag live SBT.

The gene tagging team departed Port Lincoln on the 17th March 2020, heading first to Rosalind Shoal where the spotter pilot had observed schools of SBT within the previous few days. The locations where fishing occurred (Figure A1) were guided by reports from the aerial spotter as well as from intelligence provided by the last commercial vessel still fishing for the season. The gene tagging team returned to Port Lincoln on 25 March 2020, having tagged a total of 66 SBT. Coronavirus (COVID-19) risks and Australian regulations limited options for the team to continue. The remainder of the charter was cancelled.

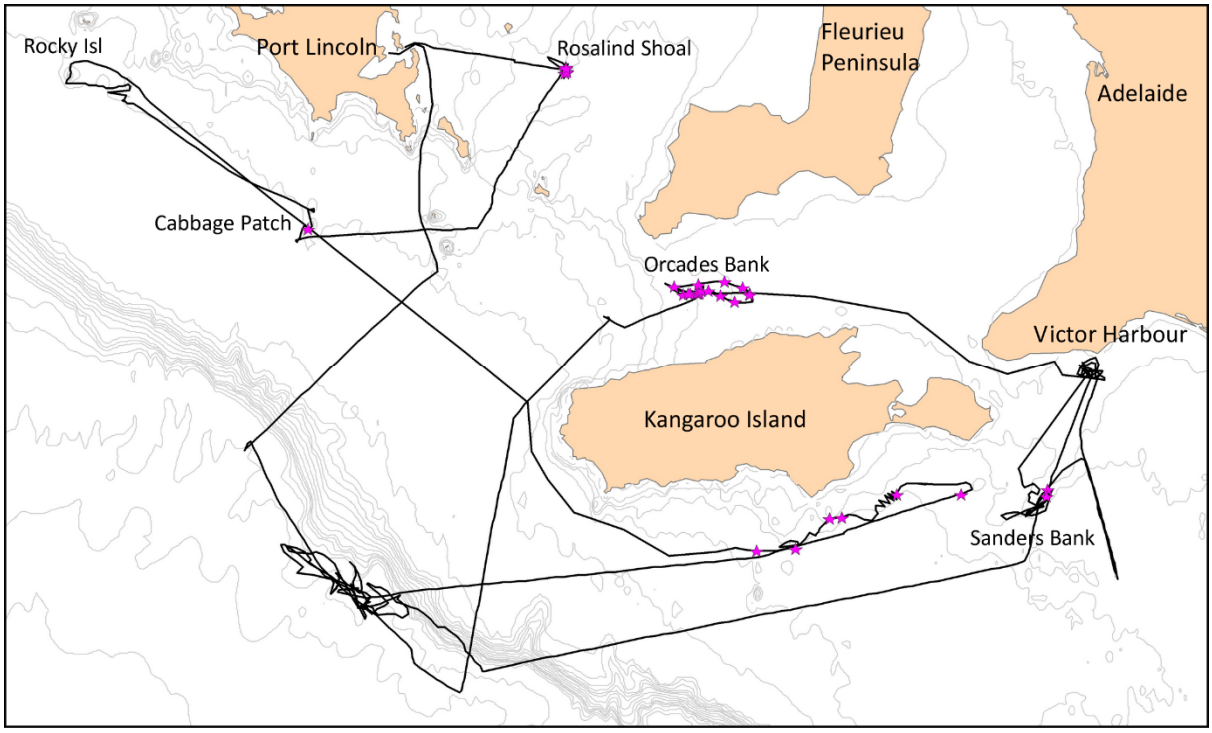

Figure A1. Approximated track of the 2020 gene tagging charter with location of troll strikes ( ) identified.

The charter covered a linear distance of approx. 2,800 km; fishing occurred both at locations identified as containing fish in the current season as well as locations that were highly successful in previous years. Fishing was conducted from dawn to dusk. The high level of effort indicated that SBT had largely departed the shelf regions; the SBT that were present were scattered and in small groups of <100 fish. The last of the Commercial fishing was occurring off the shelf with reports of several schools of appropriately sized SBT for gene tagging (9-13 kg). The gene tagging team found fish (two large schools) in this region. However, birds numbering in the thousands made fishing impossible on the first day. Fishing on the second day in this region was unsuccessful with no SBT strikes on the troll lines and no schools observed despite the number of birds dramatically falling.

In total, the gene tagging team were able to tag 66 SBT. A further nine SBT were poled on-board but not tagged because they were too big (6), too small (1), or were damaged in the process of poling (2). The troll lines caught a total of 39 SBT, all were released alive in good condition without being tagged. No SBT were killed; therefore, no biological samples were collected.

Sea surface temperatures and predicted habitat modelling were indicating highly favourable fishing conditions early in the year (Figures A3-A6). However, by the time the commercial fishery was coming to an end the conditions were rapidly changing with water temperatures declining. This appears to have been driven by the prevailing wind conditions and a strong Bonny upwelling in the SE, and further strong upwelling was observed between Coffin Bay and Elliston.

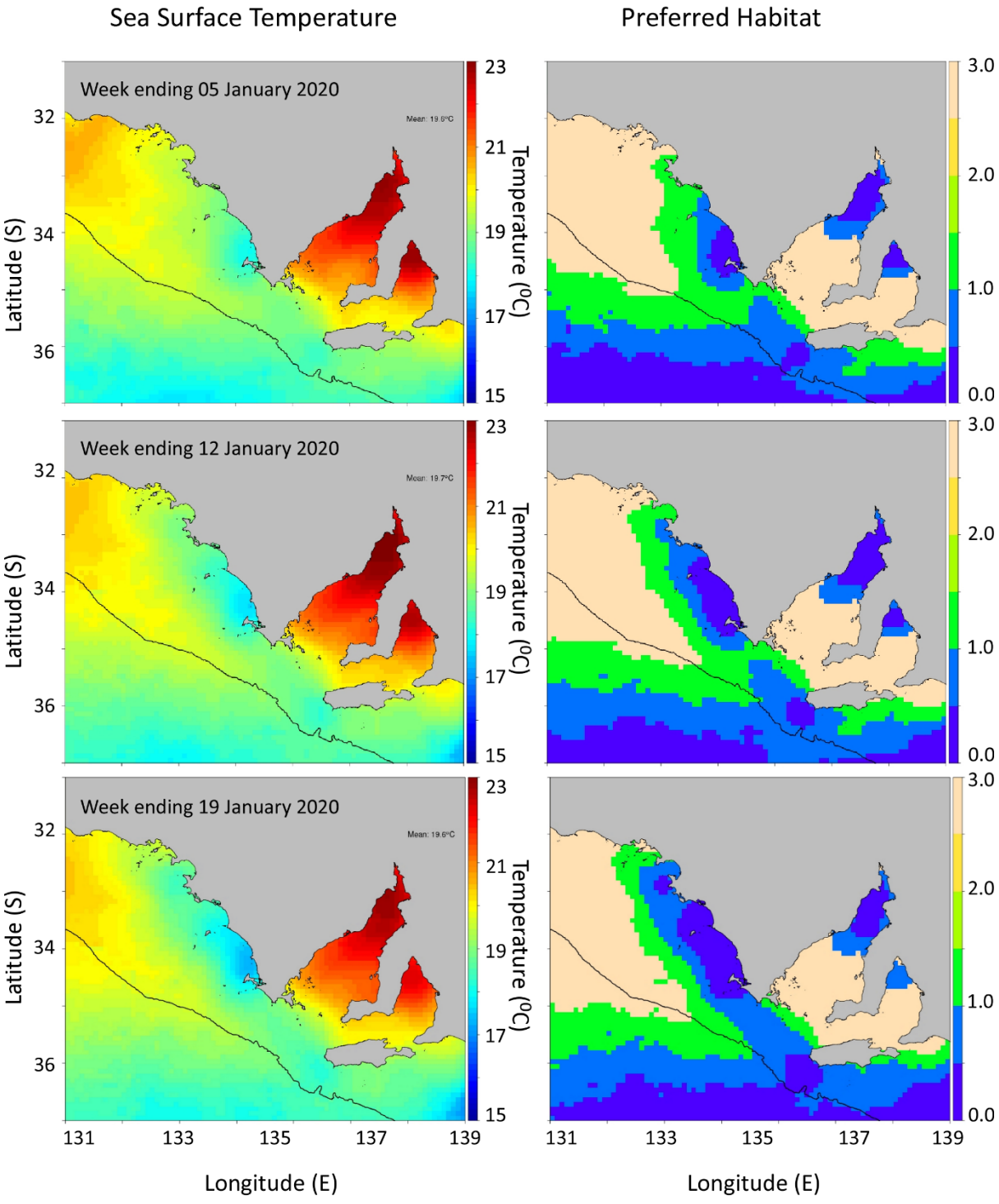

Figure A3. Compilation of weekly sea surface temperature maps (Bureau of Meteorology, Beggs et al 2011, Reynolds et al.,1994) and habitat prediction modelling from January through March 2020.

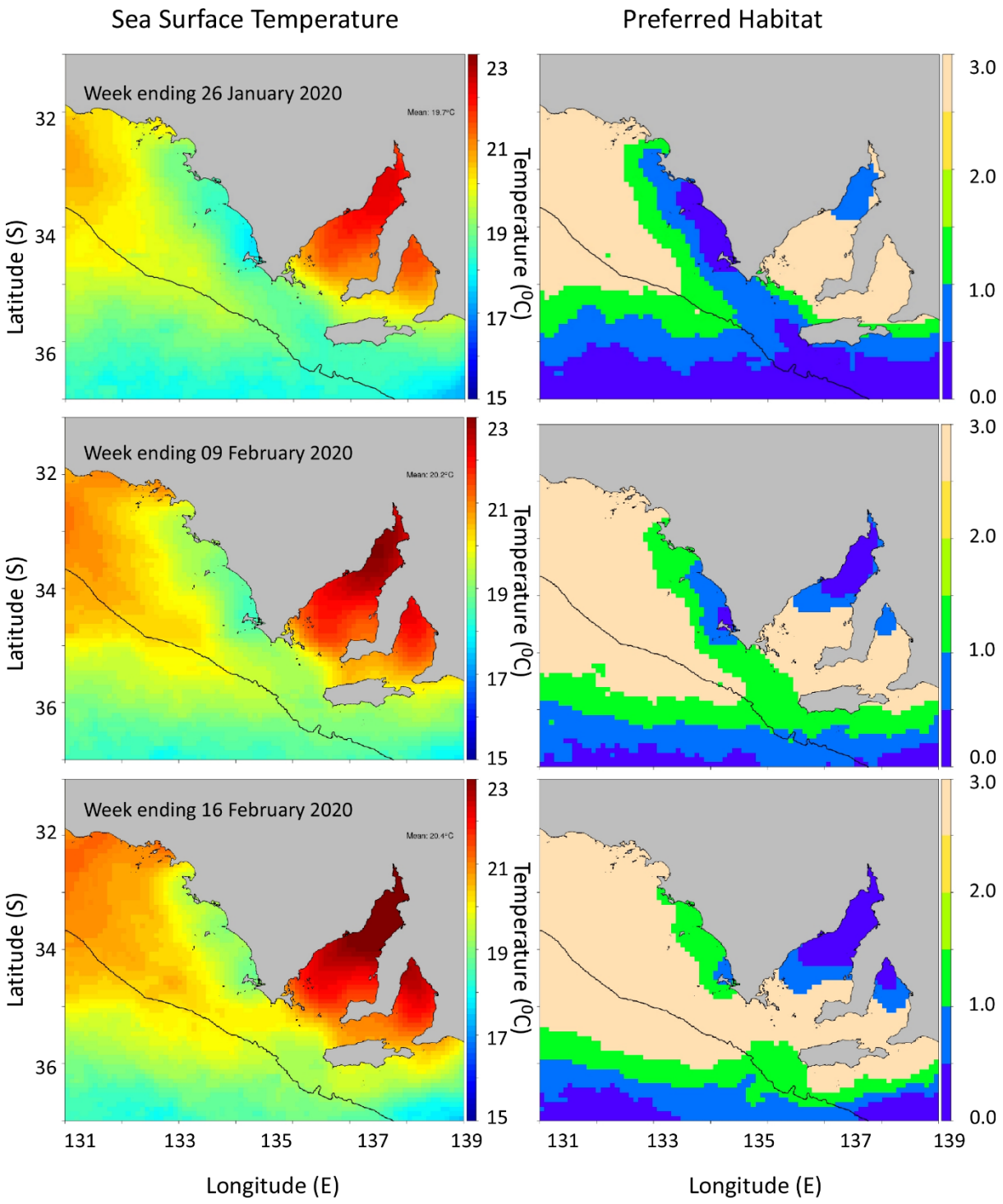

Figure A4. Compilation of weekly sea surface temperature maps and habitat prediction modelling from January through March 2020.

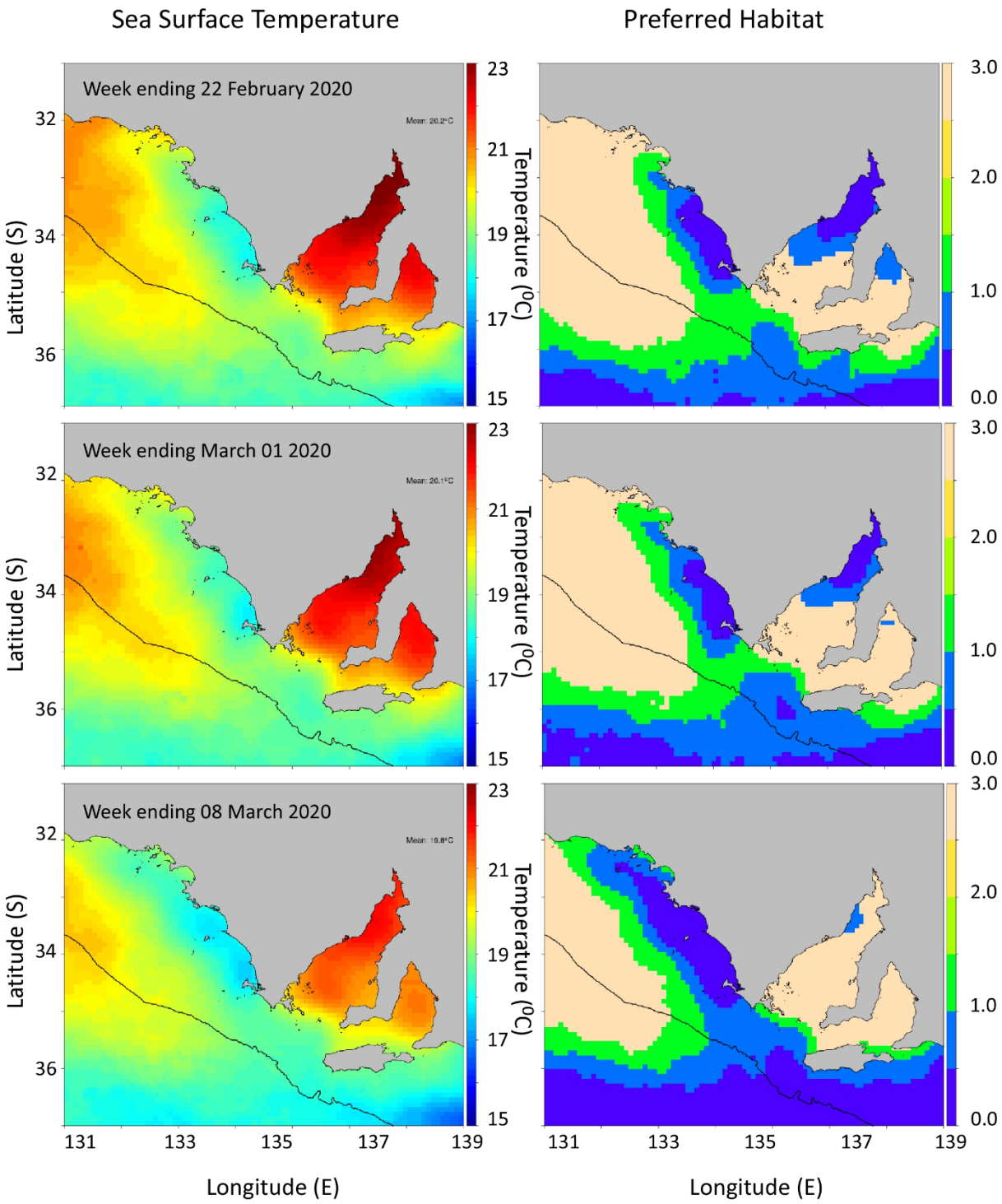

Figure A5. Compilation of weekly sea surface temperature maps and habitat prediction modelling from January through March 2020.

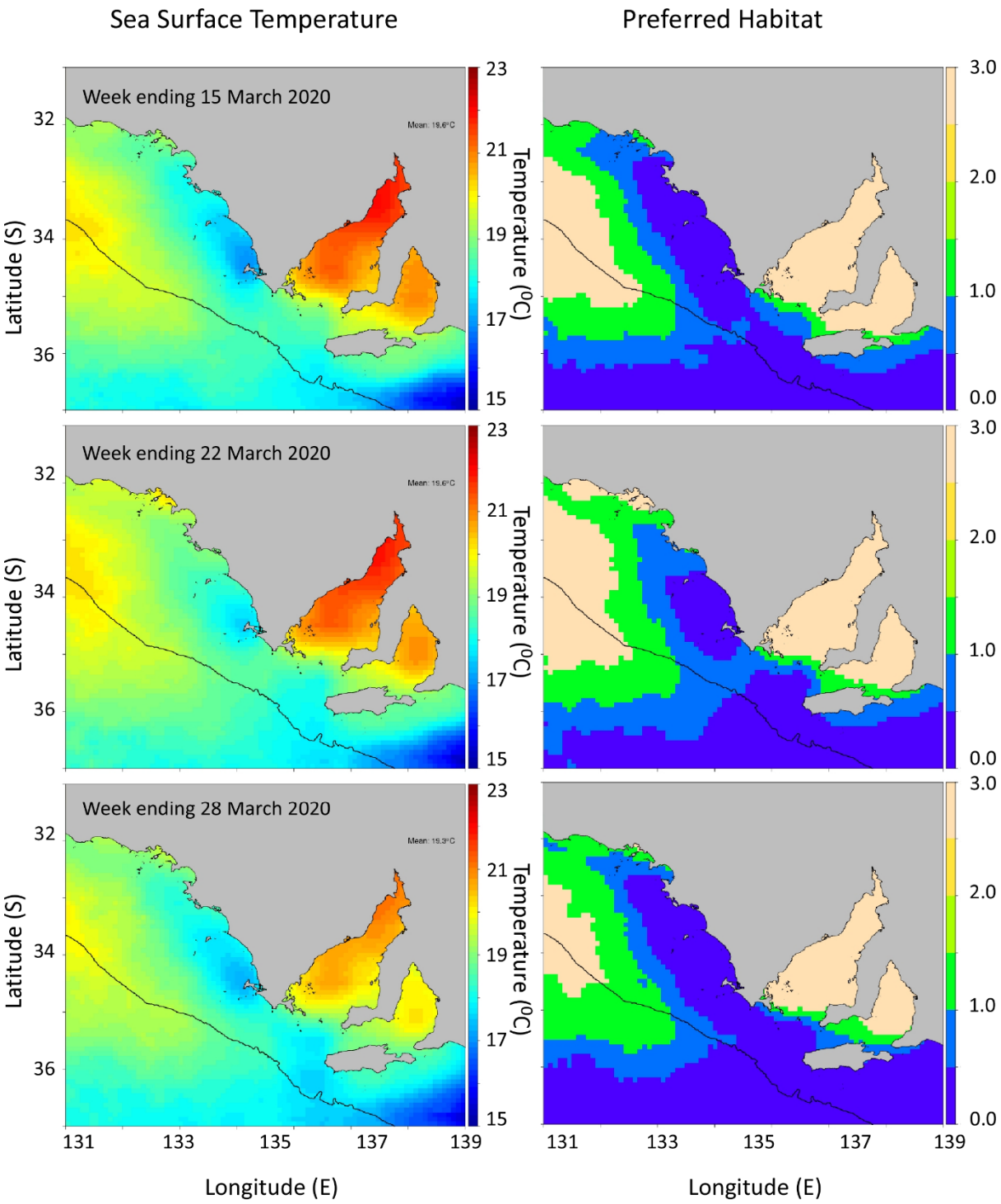

Figure A6. Compilation of weekly sea surface temperature maps and habitat prediction modelling from January through March 2020.

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