Ocean warming threatens key trophic interactions supporting a commercial fishery in a climate change hotspot

Owen J. Holland, Mary A. Young, Craig D. H. Sherman, Mun Hua Tan, Harry Gorfine, Ty Matthews, Adam D. Miller

1 Deakin University, Geelong, School of Life and Environmental Sciences, Centre for Integrative Ecology, Victoria, Australia
2 Deakin Genomics Centre, Deakin University, Geelong, Australia
3 School of Biosciences, University of Melbourne, Melbourne, Australia
4 Department of Microbiology and Immunology, University of Melbourne, Melbourne, Australia

ORCID IDs: Holland (0000-0002-2244-9373), Young (0000-0001-7426-2343), Sherman (0000-0003-2099-0462), Tan (0000-0003-3396-8213), Gorfine (0000-0001-6933-3389), Matthews (0000-0002-0606-5433), Miller (0000-0002-1632-7206)

* Corresponding author: Adam D. Miller, Tel: +61-355633171; E-mail: a.miller@deakin.edu.au

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ABSTRACT

Worldwide, rising ocean temperatures are causing declines and range shifts in marine species. The direct effects of climate change on the biology of marine organisms are often well documented, yet knowledge on the indirect effects, particularly through trophic interactions, is largely lacking. We provide evidence of ocean warming decoupling critical trophic interactions supporting a commercially important mollusc in a climate change hotspot. Dietary assessments of the Australian blacklip abalone (*Haliotis rubra*) indicate primary dependency on a widespread macroalgal species (*Phyllospora comosa*) which we show to be in state of decline due to ocean warming, resulting in abalone biomass reductions. Niche models suggest further declines in *P. comosa* over the coming decades and ongoing risks to *H. rubra*. This study highlights the importance of studies from climate change hotspots and understanding the interplay between climate and trophic interactions when determining the likely response of marine species to environmental changes.

KEYWORDS

Climate change, marine trophic interactions, *Haliotis rubra*, *Phyllospora comosa*, DNA metabarcoding, long-term marine monitoring

1 | INTRODUCTION
Changes to the physical ocean climate are threatening the health and function of marine ecosystems at a global scale (Smale et al. 2019; Brito-Morales et al. 2020). Rising ocean temperatures, increasing acidification, reduced oxygen solubility, and changing ocean currents are contributing to declines and range shifts in many marine species (Hoegh-Guldberg & Bruno 2010; Poloczanska et al. 2016; Pauly & Cheung 2018). The impacts of climate change are complex, involving direct effects that push individual species beyond their physiological limits (Hoffmann & Sgro 2011), and indirect effects that disrupt trophic interactions that are important regulators of marine biodiversity and ecosystem function (Ockendon et al. 2014; Durant et al. 2019). Understanding the interplay between climate and trophic interactions is essential for anticipating how marine species and ecosystems will respond to climate change. However, trophic interactions are often poorly understood (Alexander et al. 2016), particularly in the world’s marine climate change hotspot regions where physical and ecological changes are most pronounced (Hobday & Pecl 2014; Cameron et al. 2019).

Concerningly, many functionally important marine species are showing signs of climate stress, compromising the structure and function of marine ecosystems around the world through cascading ecological effects (Maynard et al. 2015; Chivers et al. 2017; Babcock et al. 2019; Cavanaugh et al. 2019a). Notably, macroalgal communities are suffering significant declines in the world’s temperate oceans as a result of warming (Filbee-Dexter et al. 2016; Beas-Luna et al. 2020; Smale 2020) and heatwave events (Wernberg et al. 2016; Rogers-Bennett & Catton 2019; Thomsen et al. 2019). Macroalgal forests are among the most productive ecosystems on our planet, providing critical habitat and contributing to detrital food webs, nutrient cycling, primary productivity, and carbon storage (Steneck & Johnson 2014; Teagle et al. 2017). Macroalgae also form the dietary basis of many marine herbivores (Steneck et al. 2017), highlighting the importance of understanding trophic interactions when assessing the effects of macroalgae loss at species, community and ecosystem scales (Verges et al. 2016; Smith et al. 2021).

Macroalgal community losses around the world are pronounced in climate change hotspots (Filbee-Dexter et al. 2016; Wernberg et al. 2016), such as south-eastern Australia, a region prone to extreme heatwave events (Oliver et al. 2017, 2018) and where sea surface temperatures are warming at approximately 4 times the global average (Frusher et al. 2014; Hobday & Pecl 2014). Here major dieback in macroalgal species and transformations of benthic reef communities have been linked to changes in physical ocean climate over the last two decades (Johnson et al. 2011; Verges et al. 2016; Butler et al. 2020; Davis et al. 2020).
The region is also home to the world’s largest wild abalone fishery, targeting the blacklip abalone (*Haliotis rubra*), a benthic herbivorous gastropod mollusc (FAO 2019). Evidence suggests climate change has the potential to directly compromise both juvenile and adult abalone life stages (Vilchis et al. 2005; Byrne et al. 2011), and cause mass mortalities and disease outbreaks in abalone fisheries (Lafferty & Kuris 1993; Moore et al. 2000; Ben-Horin et al. 2013; Oliver et al. 2017). However, climate change induced declines in macroalgal communities are also expected to affect key trophic interactions that support abalone fisheries. Indeed, collapses and depletions in some abalone fisheries in Australia and abroad have already been linked to declining macroalgal communities (Johnson et al. 2011; Strain & Johnson 2013). However, our understanding of the key macroalgal species that support the abalone’s herbivorous diet, and the trajectory of these key dietary species under climate change remain uncertain, hindering conservation and fisheries management.

In this study we investigated the impacts of climate change on key trophic interactions that support south-eastern Australian *H. rubra* fisheries. Specifically, we characterised the dietary composition of *H. rubra* via genomic assessments of abalone stomach content samples collected from across the region. From these analyses, we identified the primary dietary macroalgal species and investigated trends in macroalgal canopy cover and relationships with abalone biomass over the last two decades using a unique long-term monitoring dataset. These analyses are complemented by niche suitability models that investigate changes in suitable macroalgal habitat in future decades. Our study suggests climate change is decoupling critical trophic interactions that support *H. rubra* fisheries. It also provides an important contribution to the paucity of literature on marine trophic interactions from the world’s marine climate change hotspots and highlights the complexity of climate change effects in marine ecosystems.

2 | METHODS

2.1 | Dietary analysis

2.1.1 | Sample collection

We explored *H. rubra*’s diet composition through genetic identification of macroalgal taxa in the stomach contents of 397 individual adult abalone sampled from 28 sites spanning 700 km of the Victorian coastline in south-eastern Australia (Table S1). Genetic analyses were performed on approximately 14 individual abalone from each site (a sample size of 10 – 13 individuals identified as being sufficient for capturing ≥ 95% of the species diversity at the
site level; Fig S1), including both males and females and a range of size classes (mature
animals recorded between 89 – 145 mm), and sites differing in habitat type (differing in
exposure to wave energy and reef geology), some of which were sampled at two seasonal
time points (Table S1). All sampling was performed between October 2018 and May 2019
with the assistance of commercial fishing operators. Following harvest, animals were
transported live to commercial processing facilities, where post-processed animals (shell and
stomach) from different harvest sites were bagged individually, labelled, and transported on
ice to the laboratory where they were stored at -20 °C until required for genetic analysis.

2.1.2 | DNA extraction
Total genomic DNA was extracted from 50 mg of stomach content using a modified CTAB
method (Clarke 2009). Stomach content samples were added to a 2 mL screw cap tube with 3
mm glass beads for macroalgal cell disruption. Five hundred µL of CTAB [2% CTAB (w/v),
100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0)], along with 500 µL of PBS 1X buffer was
added and the solution was homogenised using a Qiagen Tissue Lyser II at maximum speed
for 10 minutes (30 repetitions per second). Post homogenisation, 10 µL of Proteinase K (20
mg/mL) was added and samples were incubated at 55 °C for a minimum of 3 hours with
frequent inversion. Post incubation, 700 µL of the lysate was added to a 1.5 mL
microcentrifuge tube along with an equal volume of chloroform:isoamyl alcohol (24:1). The
solution was vortexed and centrifuged for 10 mins at 14,000 g. Post centrifugation, a 200 µL
aliquot of the aqueous phase (top phase) was pipetted into a fresh 1.5 mL microcentrifuge
tube along with 20 µL sodium acetate (3M, pH 5.2) and 400 µL absolute ethanol. The
solution was gently inverted and stored at -20 °C for at least 15 minutes to facilitate DNA
precipitation. Samples were centrifuged for 10 mins at 14,000 g to pellet DNA. Post
centrifugation, the supernatant was discarded while keeping the DNA pellet. 700 µL of 70%
ethanol was added, pipetting up and down to rinse the pellet off the bottom of the tube.
Rinsing was repeated a second time with centrifugation for 2 mins at 10,000 g in between
rinses. Post final rinse, the supernatant was discarded, the tube was centrifuged for 30 sec at
10,000 g and all remaining ethanol was removed from the tube and allowed to evaporate for
~5 mins. The solution was resuspended in 100 µL of 1X TE buffer (Tris-EDTA) and stored at
-20 °C until required for amplification.

2.1.3 | DNA metabarcoding and sequencing
Polymerase Chain Reactions (PCR) targeted a 390 base pair (bp) amplicon of the plastid 23S rRNA gene region using the algal universal primer pair p23SrV_F and p23SrV_R (Sherwood & Presting 2007). Primers were synthesized to contain partial Illumina adaptor sequence at their 5’ ends (TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG and GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G for forward and reverse primers, respectively) to enable the addition of Illumina dual index barcodes. First round PCRs were performed in 30 µL reaction matrices containing 1.2 µL forward and reverse primers (10 µM), 15 µL Bioline MyTaq™ Red Mix, 6.6 µL ddH₂O and 6 µL template DNA, with conditions consisting of an initial 3 min denaturing step at 95 °C, followed by 40 cycles of 95 °C for 20 s, 50 °C for 30 s and 72 °C for 1 min, and a final extension step of 72 °C for 10 min. Amplicons were visualised by electrophoresis on a 1% agarose gel. PCR products were then purified using 1.8 x volume of AmpureBead XP buffer (Beckman Coulter, Danvers, MA) and then used as the template for indexing. Index PCRs were performed in 15 µL reaction matrices containing 1.5 µL forward and reverse index primers, 7.5 µL Bioline MyTaq™ Red Mix and 4.5 µL purified template PCR product. Forward and reverse index primers provided dual indices in unique combinations, allowing demultiplexing of pooled products, and Illumina sequencing adapters. Index PCR conditions consisted of 95 °C for 3 minutes, followed by 8 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds, and a final extension step of 72 °C for 5 minutes. PCR products were purified again using 1.8 x volume of AmpureBead XP (Beckman Coulter, Danvers, MA) and quantified using Qubit dsDNA BR Assay Kit (Invitrogen, Santa Clara, CA). Indexed PCR products were normalised and pooled, creating 28 pooled libraries for sequencing. Libraries were denatured and sequenced on the MiSeq platform using the MiSeqV2 kit (2 x 300 bp paired end), allowing for ~30,000 DNA sequence reads per sample.

2.1.4 | Bioinformatic analysis

A reference DNA sequence library was constructed by searching for sequences publicly available from the National Center for Biotechnology Information’s (NCBI) nucleotide database. The search query was limited to the 23S gene region, as well as the order Phaeophyceae (brown algae), and the phyla Rhodophyta (red algae) and Chlorophyta (green algae), resulting in 6,872 23S rRNA haplotype reference sequences representing 1,541 species, 548 genera, 176 families, and 76 orders. This list was supplemented with DNA sequence data from the 23S rRNA gene from 24 additional macrophyte species native to south-eastern Australia and representing common and abundant Phaeophyceae, Rhodophyta...
and Chlorophyta taxa. Specimens were collected from freshly deposited wrack from beaches in Lady Bay, Warrnambool, Victoria, which were morphologically identified to the species or genus level. Total genomic DNA was extracted from each specimen using the NucleoSpin® 96 Plant II protocol (Macherey-Nagel Inc.), and quantified was using the QuantiFluor® dsDNA System (Promega Inc). Unmodified universal primer pair p23SrV_F and p23SrV_R (Sherwood & Presting, 2006) was used to amplify an ~ 390 bp fragment of the plastid 23S rRNA gene region following the first round PCR conditions outlined above. Amplicons were sequenced on an ABI 3730XL genetic analyser (Applied Biosystems). DNA sequences for each species were trimmed to remove low quality base calls using Geneious Prime 2019.2.3 (https://www.geneious.com) and appended to the reference sequences extracted from the NCBI database.

Demultiplexed paired end sequence reads in FASTQ format were imported into the R package DADA2 v1.14 (Callahan et al. 2016). Data filtering was performed using the filterAndTrim function with forward and reverse truncation set to retain reads with a Phred score >20, followed by calculation of error rates using learnErrors. Data dereplication and ASV (amplicon sequence variant) inference was performed using the derepFastq and dada2 functions respectively, followed by paired end read merging using mergePairs. Chimeras were removed using the removeBimeraDenovo function, and non-chimeric ASVs were written to FASTA and sequence table formats including read counts. Samples with less than 20 reads were omitted to reduce non-informative OTU inclusion.

FASTA derived ASV sequences were clustered to OTUs (operational taxonomic units) using VSEARCH following a 99% similarity threshold. OTUs were compared and aligned with reference sequences from the custom 23S rDNA database using BLASTn v2.6.0 (Altschul et al. 1990), restricting hits to a 75% minimum query length cover, and minimum 99% identity match for family level taxonomic assignment. The minimum identity threshold was determined based on comparative distance matrices between macroalgal 23S rDNA sequences, identifying taxon within families to share haplotype sequence similarity between 98% and 99% at this locus. Additionally, OTU derived sequences were compared and aligned using BLAST against the reference DNA sequence library, and familiar taxonomies were compared to their percent ID score. Alignments were imported into MEtaGenome ANalyser (MEGAN6; (Huson et al. 2016) where taxonomic assignment to the family level or higher was validated using the default LCA (lowest common ancestor) parameters. Taxonomic assignments were referred back to the DADA2 derived sequence table and sample read counts were summarised for each taxon. OTU sequences of predominant taxa were manually
checked against the NCBI nucleotide database for verification purposes. Given that we applied stringent parameter settings for taxonomic assignment, it was expected that a number of unassigned “no hit” OTUs would be encountered. We therefore applied more relaxed parameter settings (97% identity match) to determine coarse taxonomic resolution (i.e. phylum level) for unassigned OTUs using the SINA alignment tool and the SILVA LSU database (Quast et al. 2013). We also assessed relative read abundances (RRA) and frequencies of occurrence (FoO) for all unassigned OTUs to ensure potentially important taxa were not overlooked in our analyses.

A graphical representation of dietary macroalgal family FoOs and RRAs was generated from the MEGAN6 genealogical output and subsequently modified in FigTree (http://tree.bio.ed.ac.uk/software/figtree/), while plots demonstrating overall numbers of OTUs, and FoOs and RRAs for dietary macroalgal groups were generated using the ggplot2 package in R (R Core Development Team 2011; Wickham 2011).

2.1.5 | Statistical analyses

Initial analyses were performed on 36 individuals from 2 sample sites to identify the optimal sample size needed to capture ~95% of macroalgal species diversity in the abalone diet at a given location. Following genetic processing and DNA sequencing, rarefaction curves were generated using the specaccum function in the R package vegan (Oksanen et al. 2008) generating species richness vs sample size curves using the “rarefaction” method. The optimal sample size was applied to subsequent analysis.

PERmutational Multivariate Analyses Of Variance (PERMANOVA) were performed in Primer v7.0.13 using the PERMANOVA+ add on (Anderson 2005; Clarke & Gorley 2015) to explore the effects of habitat type, sex, size class, season and geography on abalone diet composition. Two dataset types were generated for statistical analysis: presence/absence and relative read abundance per OTU. Read count data were transformed to binary values for presence/absence data (“1” if present, “0” if absent) and standardised to continuous values for relative abundance data both in Primer v7.0.13 (Clarke & Gorley 2015). Diversity matrices were generated for the presence/absence and relative abundance data using the Jaccard and Bray-Curtis similarity measures, respectively. PERMANOVAs were subsequently performed using dietary composition as the response variable and wave energy, reef substratum type, sex, fishing zone, and sampling time as predictor variables (refer to Supporting Information for detailed outlines of each analysis). All models were run as Type III tests for sums of
squares with Monte Carlo tests, using unrestricted permutations of raw data, except for the model including shell length as a co-variate, which used permutations of residuals under a reduced model. Non-metric multidimensional scaling (nMDS) plots were used to provide a visual display of the similarity data for each of the four statistical tests and generated using the `vegdist` and `metaMDS` functions in the R package `vegan`. Analyses of SIMilarity PERcentages (SIMPER) were run to identify OTUs contributing to differences among significant effects based on Bray-Curtis similarity measures.

### 2.2 | Influence of trophic interactions on abalone biomass

#### 2.2.1 | *P. comosa* abundance and abalone biomass estimates

Surveys of *P. comosa* canopy cover and *H. rubra* biomass were conducted annually between 2003 and 2015 during summer at approximately 200 sites across 14 marine BioUnits (biogeographical units; Edmunds & Flynn (2018)) along the south-eastern Australia’s Victorian coastline. Sites were initially chosen haphazardly on macroalgal covered reefs that supported abundant *H. rubra* populations. Centroids of nine percent of the survey sites were shallower than 5 m and nine percent were deeper than 15 m, with the remaining 82% coinciding with the depth gradient at which *P. comosa* and *H. rubra* are most abundant in cooler temperate Australian waters (5-15m; Gorfine & Dixon 2001; Lucieer et al. 2009; Strain & Johnson 2010; Coleman & Wernberg 2017). Sites were then fixed over time for subsequent surveys which were conducted during the summer season of each year.

Abundance of *P. comosa* was estimated concurrently with *H. rubra* biomass during the same surveys.

At each monitoring site and time point, two research divers independently estimated the total canopy cover (% of area) of *P. comosa* and the total number of abalone. Surveys at each monitoring site were performed over an area of 90 m$^2$, with each diver assigned three 35m x 1m wide belt transects radiating outwards in randomly chosen cardinal directions separated by 30-degree arcs from a fixed vessel anchor point at the centroid of the site. This ensured that transects were as representative of the complex physiography of each site as practicable whilst avoiding overlapping and interdependence. The estimates of *P. comosa* cover was made visually by approximating the proportion in aggregate of the 105 x 1-m segments that was covered by the species along the transect lines. To improve the reliability of the estimates within the practical constraints imposed by visually observing whilst swimming underwater, it was assumed that there were roughly one hundred segments among
the three transects. Divers rounded their estimates of total cover abundance to the nearest 10%. Abundance of *H. rubra* was estimated concurrently with algal abundance during the same surveys. Divers counted all visible juvenile, sub-adult and mature abalone within each of the transects. Biomass was estimated by sampling the first 25 abalone encountered at the end of each transect, providing approximately 150 abalone at each site. Animals were subsequently brought aboard the research vessel and their maximum shell diameter measured using an electronic shellfish measuring board before being returned to the bottom and replaced by hand on the reef beyond the transect radius. The shell size distribution was used to fraction the abalone counts into 1 mm bins and each bin was converted from length to weight via the equation:

\[ W = aL^b \]

Where \( W \) = weight in g, \( L \) = shell length in mm, and \( a = 3.34 \times 10^{-4} \) and \( b = 2.857 \) are fixed parameters taken from Helidoniotis and Haddon (2014). Resultant weights were totalled to generate estimates of biomass for different length categories of interest e.g. mature spawners, and exploitable stock.

### 2.2.2 | Relationships between *P. comosa* abundance and *H. rubra* biomass

We modelled the relationship between *P. comosa* percent cover and abalone biomass using generalised additive models (GAMs), while also accounting for any variation associated with location or temperature. GAMs are widely used for modelling species distributions due to their ability to model nonlinear relationships (Guisan & Zimmermann 2000). Because of the known effects of temperature on the productivity of abalone (Young et al. 2020), we accounted for variation in temperature across the state using spatial maps of sea surface temperature (SST; data sourced from IMOS, 2017). Local SST was expected to reflect the temperature from most, if not all, of the depth range of abalone and *P. comosa* given the potential for vertical mixing due to significant wave activity and relatively few days of calm conditions throughout the year (Ierodiaconou et al. 2020; Young et al. 2020). Also, due to spatial and temporal correlation of the long-term dataset, we included biounits (biogeographical units; Edmunds & Flynn (2018)) and year of survey as random effects in the GAM. This allowed for any similarities across temporal timeframes and within a biounit (e.g., eastern biounits affected by a range expansion of urchins, which could impact *P. comosa* percent cover) to be accounted for.
Prior to running the model, data were split into training (70%) and evaluation (30%)
datasets to test the predictive performance of the model. After first verifying that the data
contained no outliers and the variables were not correlated (boxplots and Pearson correlation,
respectively), we used the ‘mgcv’ package in R (R Core Development Team 2011) to test for
significant relationships between abalone biomass, *P. comosa* percent cover, SST, biounit,
and year in a series of GAMs based on the following equation:

\[
gam(\text{Abalone Biomass} \sim s(\text{P. comosa Percent Cover}) + s(\text{SST}) + \text{biounit} + s(\text{Year, bs = “re”}) + s(\text{P. comosa Percent Cover, by = SST}))
\]

The “s” in the equation signifies those continuous variables that were smoothed using
a spline curve to account for the nonlinear relationships. Year was treated as a random effect
and the interaction between *P. comosa* percent cover and SST was included in the full model.
Terms were dropped from the model until a final “best” model, based on model performance,
which was measured using AIC, deviance explained, and the predictive performance, was
determined.

2.2.3 | Influence of climate change on the trajectory of *P. comosa*

Once we determined the significant effect of *P. comosa* percent cover on abalone biomass,
we explored future distributions of *P. comosa*. To do this, we again used GAMs to associate
the time series of *P. comosa* percent cover with SST, biounit, and year. We used similar
methods as above for data exploration and splitting the data into training and evaluation
datasets. The final GAM used the following equation:

\[
gam(\text{P. comosa Percent Cover} \sim s(\text{SST}) + \text{biounit} + s(\text{Year, bs = “re”}))
\]

A smoother (s) was applied to SST to account for the nonlinear relationship with
percent cover of *P. comosa*. This model was then used to predict current percent cover using
SST data from 2019 for 59% of the coastline where adequate data were available for
modelling. Several of the biounits, making up 41% of the coastline, did not contain any
monitoring sites and were excluded from the analysis. These biounits are largely sediment
dominated habitats not supporting abalone populations or macroalgal communities. Potential
future distributions of *P. comosa* along the Victorian coast in 2019 were also projected
adding 2.5 °C to the 2019 SST map and running a prediction for 2090 over the same area of the coastline as the 2019 prediction. These predictions were based on the relationships between percent cover, future SST projections, and biounit in the GAM. The current and future predictions were then used to determine which areas along the coast of Victoria are likely to experience increases or decreases in *P. comosa* percent cover based on changes in SST. Although modelling of future SST along the coast shows that the temperature increases will vary spatially (CSIRO and Bureau of Meteorology, 2018), we did not have access to spatial layers at the time of this study and, therefore, were not able to incorporate them into our projections. Future niche suitability models that capture the variation in temperature increases along the coast will provide added confidence in expected niche shifts.

3 | RESULTS

3.1 | Dietary specialisation in *H. rubra*

While the dietary differences between juvenile and adult abalone life stages are well established (Hahn 1988; Daume et al. 2000; Guest et al. 2008), the relative contributions of brown, red and green macroalgae to adult *H. rubra* diets, and how this varies among regions in south-eastern Australia, remain uncertain (Guest et al. 2008). Genetic analyses were performed on approximately 14 individual abalone from each site (a sample size of 10 – 13 individuals identified as being sufficient for capturing ≥ 95% of the species diversity at the site level; Fig S1), including both males and females and a range of size classes (mature animals recorded between 89 – 145 mm), and sites differing in habitat type (differing in exposure to wave energy and reef geology) sampled at two seasonal time points (October 2018 and May 2019).

Our analyses identified a total of 68 operational taxonomic units (OTUs) representing 33 families of brown macroalgae (Phaeophyceae), red macroalgae (Rhodophyta), green macro- and microalgae (Chlorophyta), and flowering plants (Magnoliophyta; seagrass) across all stomach samples (Figs 1 and 2a). However, we found a significant bias toward brown macroalgae (frequency of occurrence (FoO) of 96.9% and relative DNA sequence read abundance (RRA) of 93.4% across all samples), and an overwhelming dominance of single taxon, *Phyllospora comosa* (Seirococcaceae), one of the most common and widespread canopy forming seaweeds in south-eastern Australia (Figures 1 and 2). This taxon was genetically identified in the stomach contents of 85.9% of all animals, having had an RRA of...
66.3% across all samples. Comparatively, average FoOs and RRAs of the remaining 67 operational taxonomic units were 5.4% (not exceeding 26.6%) and 1% (not exceeding 12.0%), respectively.

Our analyses indicated no significant difference in diet composition between animals varying in sex or size class, or animals sampled across different habitat types or seasons. However, we identified significant \( P < 0.01 \) site differences both within and between geographical regions of our sampling distribution (western, central and eastern regions; Figs S2-7). Previous dietary analyses of the South African \( H. \textit{midae} \) and Western Australian \( H. \textit{roei} \) have also demonstrated differences in diet composition at both regional and local spatial scales (Barkai & Griffiths 1986; Wells & Keesing 1989). Spatial heterogeneity of canopy and understory macroalgae is well recognised both in Australia and overseas and influenced by both biotic and abiotic factors (England et al. 2008; Johnson et al. 2011; Martins et al. 2013; Verges et al. 2016). While spatial variation in diet composition could result from spatial variation in resource availability, other studies suggest that species interactions and selective feeding behaviours may also be contributing factors (Foale & Day 1992; Guest et al. 2008; Eisaguirre et al. 2020). Nevertheless, our analyses confirmed significant spatial variation in diet composition was largely driven by variation in the FoO and RRA of less frequently detected taxa, while \( P. \textit{comosa} \) remained the dominant taxon across all sites, sexes, habitat types, and seasons.

### 3.2 Effect of \( P. \textit{comosa} \) declines on abalone distribution and abundance

While the distributions of \( H. \textit{rubra} \) and \( P. \textit{comosa} \) are almost identical suggesting a strong reliance of \( H. \textit{rubra} \) on \( P. \textit{comosa} \) as its primary food source (Handlinger et al. 2006; Coleman & Wernberg 2017), we directly tested how abalone biomass is affected by the distribution and abundance of \( P. \textit{comosa} \). The best GAM (Table S2) shows \( H. \textit{rubra} \) abundance has a negative relationship with sea surface temperature (SST; Figure 3a), but a significant positive relationship with \( P. \textit{comosa} \) percent cover despite spatial variation in temperature along the coast (Fig 3b). Therefore, \( H. \textit{rubra} \) abundance is expected to decrease with decreases in \( P. \textit{comosa} \) cover (when cover falls below 20%), while also accounting for decreases associated with increasing SST. Patterns in abalone biomass were also explained by the spatial variation in populations along the coast with biounit serving as a significant factor in the final model.
3.3 | Trajectory of *P. comosa* under climate change

Generalised additive models (GAMs) indicate significant declines in *P. comosa* canopy cover across most parts of the Victorian coastline between 2003 and 2015 (Figs 4a and 5; Table S3), which has a significant relationship with increasing sea surface temperature (SST; Fig 4b). Our GAM models suggest little effect of SST on *P. comosa* percent cover until temperatures exceed 20°C, at which point a negative relationship with temperature occurs and dramatic declines begin around 22°C (Fig 4b).

Our climate niche suitability models highlight risks of ongoing decreases in *P. comosa* in future decades. Specifically, our models indicate substantial reductions of suitable habitat by 2090 with projected decreases in *P. comosa* across 48% of the Victorian coastal waters (Fig 5). Most of these declines are predicted to be moderate (32%), while the remaining 16% are expected to be medium to high losses. Only 11% of the current range is expected to see increases but these are predicted to be moderate increases. The projections also show that the patterns and severity of declines in *P. comosa* percentage cover are spatially variable with greater decreases in the east of the state and relatively persistent coverage in the west.

4 | DISCUSSION

Ocean temperatures are increasing around the world but at geographically variable rates (Hoegh-Guldberg & Bruno 2010; Hobday & Pecl 2014). Studies conducted in warming ‘hotspots’ provide opportunities to gain early insights into the effects of climate change and to inform future management of marine resources (Pecl et al. 2014). In particular, there is an urgent need for studies that help to tease apart the direct and indirect effects of ocean warming and improve our understanding of the additive effects of climate change. Here we provide evidence of ocean warming having an indirect negative effect on a commercially important mollusc in a climate change hotspot through the destabilisation of critical trophic interactions. Specifically, we demonstrate dietary dependency of *H. rubra* on a widespread macroalgal species, which we show to be in state of decline due to ocean warming, resulting in abalone biomass reductions. Niche models suggest further declines in *P. comosa* over the coming decades potentially leading to ongoing risks to local fisheries.

Many abalone fisheries around the world have collapsed in recent decades due to over exploitation, environmental change, and disease, with several Haliotid species now listed as endangered or considered ‘species of concern’ (Karpov et al. 2000; Stierhoff et al. 2012).
However, a growing body of literature points to climate change as an emerging threat to abalone fisheries, particularly those occurring in climate change hotspots such as south-eastern Australia and the west coast of North America (Johnson et al. 2011; Rogers-Bennett & Catton 2019). While in some areas *H. rubra* fisheries are currently considered sustainable, notable declines in regions of the fishery have occurred over the past two decades raising concerns for industry (Mayfield et al. 2012). Young et al. (2020) recently provided evidence of declining abalone fisheries in south-eastern Australia independent of fishing pressure. Instead, declining trends in abalone densities in *H. rubra* fisheries appear to be associated with changes in oceanographic conditions, with changes in SST being a significant contributor. While further research is needed to determine relative contributions of direct thermal stress to these fishery trends, our findings suggest that ocean warming is likely to be decoupling key trophic interactions supporting *H. rubra* fisheries and contributing to their decline. This highlights the need for further research to characterise and track the trajectory of key dietary species supporting other Haliotid fisheries around the world. While climate change is impacting many fisheries through direct thermal stress and the emergence of infectious diseases (Moore et al. 2000; Vilchis et al. 2005; Byrne et al. 2011; Oliver et al. 2017), the destabilisation of key trophic interactions could be an underappreciated factor contributing to their historical collapse and potential for recovery.

Declines in some abalone fisheries in south-eastern Australia have been previously attributed to macroalgal losses, primarily due to overgrazing by native and range extending urchins (Ling 2008; Johnson et al. 2011; Carnell & Keough 2019). The impacts of urchin overgrazing on abalone fisheries are constrained to the far east of the state as well as Port Phillip Bay and Tasmania, where urchin activity has effectively created ‘reef barrens’ (Jalali et al. 2018). However, our dataset indicates significant declines in *P. comosa* over the last two decades at a region-wide scale, and in non-urchin affected areas, due to ocean warming, resulting in abalone biomass reductions. Our models indicate a strong positive relationship between *P. comosa* and abalone abundance despite spatial variation in temperature along the coast, and when controlling for direct temperature effects. This suggests that declines in *P. comosa* are likely to be having a direct causal effect on abalone biomass, compounding already recognised risks associated with the direct influence of SST (Young et al. 2020). Specifically, our models suggest a minimum threshold of 20% *P. comosa* canopy cover is required to support local abalone populations, a threshold that *P. comosa* cover is expected to fall short of for a substantial portion of the coastline by 2090 based on our predictive models.
Ongoing risks will largely depend on the ability of *H. rubra* to accommodate shifts in resource availability through diet modification and *P. comosa*’s capability of adapting to future oceanographic conditions. Evidence from *in vitro* aquaria trials suggests that *H. rubra* will readily feed on both red and brown macroalgae and that both groups provide adequate nutritional value (McShane et al. 1994; Fleming 1995). However, these findings may not necessarily translate to wild environments. In the wild, adult abalone feed primarily upon drift macroalgae rather than actively grazing attached algae (Cornwall et al. 2009), and brown macroalgae (including *P. comosa*) is the dominant constituent of drift algae in southern Australian marine environments (Hill et al. 2015). Evidence also suggests *H. rubra* feeding appears to be more selective than opportunistic in the wild. Previous research on the dietary composition of Tasmanian *H. rubra* indicates brown macroalgae to be the dominant dietary taxon even when local habitats are dominated by red macroalgae (Guest et al. 2008). We corroborate these findings by demonstrating a preference for *P. comosa* despite south-eastern Australia having the world’s most speciose macroalgal communities and where many macroalgal species are widespread and freely available for consumption by benthic herbivores (Phillips 2001). Further, we provide evidence of declining abalone biomass following reductions in *P. comosa* canopy cover, while reef habitats remain inhabited by dense and speciose macroalgal communities consisting of other canopy and understory taxa (Ierodiaconou et al. 2020b). Collectively this evidence suggests diet modification in wild *H. rubra* populations might not be a simple transition.

However, there could be potential for *P. comosa* populations to evolve more thermally tolerant populations, given the species’ wide latitudinal distribution, which extends approximately ~1,300 km from 43 to 31 degrees south along the east coast of Australia (Coleman & Wernberg 2017). Species with distributions spanning major thermal gradients often show genetically based clines (Hoffmann & Sgro 2011; Jeffery et al. 2017; Halbritter et al. 2018). Indeed, Wood et al. (2021) recently reported evidence of genetic structuring among *P. comosa* populations spanning different thermal environments from eastern Australia. While these findings point to heritable genetic variation relating to temperature, evidence of strong population genetic structuring in *P. comosa* suggests that gene flow is unlikely to assist populations in adapting to warming SST via the migration of thermally adapted genotypes (Coleman & Kelaher 2009; Wood et al. 2021). Instead, a lack of gene flow will necessitate *in situ* adaptation dependent on local standing genetic variation (Hoffmann & Sgro 2011).
Our models indicate uneven spatial patterns of decline in *P. comosa* over the last two decades. These findings are consistent with other studies reporting declines of macroalgal communities in south-eastern Australia (Johnson et al. 2011; Verges et al. 2016; Carnell & Keough 2019; Butler et al. 2020) and other parts of Australia and overseas due to warming and heatwave events (Wernberg et al. 2016; Cavanaugh et al. 2019b; Thomsen et al. 2019; Smale 2020). In particular, Valentine and Johnson (2004) reported major diebacks of *P. comosa* on the east coast of Tasmania following above average SSTs during the summer/autumn period of 2001. These findings are also consistent with previous studies indicating heterogenous effects of ocean warming on early developmental stages in *P. comosa* from different regions (Flukes et al. 2015; Cumming et al. 2019; Britton et al. 2020). Evidence of uneven effects have also been reported in a range of northern and southern hemisphere macroalgal species (Smale 2020). While these patterns might be due to adaptive genetic differences among populations (Miller et al. 2020; Wood et al. 2021), other potential factors include differences in local climate velocities (Pinsky et al. 2013), habitat features contributing to different thermal environments (Ierodiaconou et al. 2018), and trophic interactions (Johnson et al. 2011; Eisaguirre et al. 2020). In fact, some cases of regional depletion of *P. comosa* populations have been linked directly to overgrazing by range shifting fish and aforementioned urchin species (Ling 2008; Johnson et al. 2011; Verges et al. 2016). Additional biotic and/or abiotic factors could also be contributing to the decline of *P. comosa* and potentially the spatial patchiness of dieback patterns, as evidenced by the unexplained variance in our GAMs. For example, ocean warming is contributing to macroalgal declines in other parts of the world by causing changes in herbivory pressure (Verges et al. 2016; Smith et al. 2021), risks of infectious diseases (Eggert et al. 2010), consumer-mediated nutrient cycling (Peters et al. 2019), and microbiome communities (Qiu et al. 2019). While further research is needed to determine the potential contribution of such factors to the decline on *P. comosa*, future declines are likely to remain uneven and create nonuniform pressures across abalone fisheries.

Ongoing monitoring programs in south-eastern Australia will be needed to identify and track *P. comosa* populations showing signs of climate stress. Such programs will help managers determine when and where interventions, such as macroalgal restoration programs, may be needed (Layton et al. 2020). Globally, few macroalgal restoration attempts have been made (Benayas et al. 2009), but restoration of depleted *P. comosa* populations in the Sydney region of south-eastern Australia have been successful and will help direct future restoration investments (Verges et al. 2020). Undertaking such activities at scales capable of supporting
commercial fisheries and wider ecosystem functioning will be a significant challenge. However, strategic provenancing approaches involving the introduction of thermally adapted genotypes may help local *P. comosa* populations overcome risks of maladaptation under future climates (Hoffmann et al. 2020; Wood et al. 2021).

This study highlights the potential of climate change to drastically alter trophic interactions in marine environments, compromising the health and function of marine ecosystems and commercial fisheries (Ockendon et al. 2014; Durant et al. 2019). Indeed, it has been acknowledged that biotic interactions are even more important than the direct abiotic impacts of climate change (Ockendon et al. 2014; Alexander et al. 2016). However, the existing literature associated with marine trophic interactions is heavily biased towards the northern hemisphere Atlantic Ocean, while few studies overlap with known marine climate change hotspots, including south-eastern Australia (Johnson et al. 2011; Hobday & Pecl 2014; Cameron et al. 2019). Our study demonstrates the importance of studying the interplay of climate and trophic interactions from oceanic regions experiencing above average rates of change in order to anticipate the likely response of marine species and ecosystems to future climate change across the world’s oceans.

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**AUTHOR CONTRIBUTIONS**

This project was conceptualised by A.D.M., C.D.H., and M.Y. Genotyping was undertaken by O.J.H. and A.D.M. with bioinformatic and statistical support from M.H.T., C.D.H. and T.M. Long-term monitoring of *P. comosa* was led by H.G., with M.Y. responsible for
producing GAMs and SDMs from the long-term monitoring datasets. Writing of the manuscript was led by A.D.M. and O.J.H. with assistance from all authors.

DATA AVAILABILITY STATEMENT
All data analysed in this paper is available upon request.

COMPETING INTERESTS
The authors declare no competing interest

FIGURE 1 Results from the genetic analysis of *H. rubra* stomach contents highlighting a dominance of brown macroalgae. a, Figure depicting the relative frequency of occurrences (FoO) of macro and micro-algal groups across all 397 samples. b, Figure depicting the relative DNA sequence read abundances (RRA) across all samples.

Figure 2 Summary of all macro and micro-algal families genetically identified across all abalone stomach samples. The total number of operational taxonomic units (OTUs) for each family, along with the relative frequencies of occurrence (FoO) and relative DNA sequence read abundances (RRA) for each family are also provided. Tree nodes labelled 1 and 2 represent taxa assigned to the level of class (Florideophyceae and Rhodymeniophycidae).

Figure 3 Smoothed curves obtained by the generalised additive model for abalone abundance (*Haliotis rubra*) including a. the effect of sea surface temperature (SST) on abalone abundance, along with the standard errors shaded in blue (95% confidence interval) and b. the effect of percent cover of *Phyllospora comosa* on abalone abundance. The horizontal axes shows the gradient in SST and *P. comosa* percent cover while the vertical axis is the contribution of the smoother to the model’s abalone abundance fitted values. The red dashed lines indicate thresholds in the data where notable effects become apparent (e.g.,
abalone abundance does not change as much after 20% cover of *P. comosa* and abalone abundance decreases faster (steeper slope) after 20 °C.

**Figure 4** Current trajectory of *Phyllospora comosa* inferred from a generalised additive model (GAM). a, Mapped depiction of significant changes in *P. comosa* percent cover across biounits with the warmer colours representing significant decreases and the cooler colours representing significant increases, based on the coefficients from the GAM and their relative significance. b, Partial effect plot showing the estimated smoother for change in percent cover with increasing SST along with the standard errors (95% confidence interval) in blue. The x-axis in this plot shows the SST while the y-axis represents contribution of the smoother to the model’s fitted values for percent cover of *P. comosa*. The pink dashed line indicates the temperature where the partial effect begins to decrease, and the red dashed line shows where it reduces beyond zero, signifying significant loss in *P. comosa*.

**Figure 5** Predicted climatic niche for *Phyllospora comosa* in south-eastern Australia under climate normal (top), in the 2090s (middle) based on RCP8.5 scenario (assuming a 2.5 °C increasing in SST; IPCC Global Climate Model), and total change in percentage cover between the two time periods (bottom).

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(a) Frequency of Occurrence

- Red Macroalgaes: 25%
- Brown Macroalgaes: 75%
- Green Macroalgaes: 5%
- Green Microalgaes: 5%
- Seagrass: 0%

(b) Relative DNA Sequence Read Abundance

- Brown Macroalgaes: 93.40%
- Red Macroalgaes: 4.09%
- Green Macroalgaes: 1.73%
- Seagrass: 0.21%
- Green Microalgaes: 0.58%