Environmental risk factors that may contribute to *Vibrio* outbreaks: A South Australian case study

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Abbreviations

ASQAAC	Australian Shellfish Quality Assurance Advisory Committee
ASQAP	Australian Shellfish Quality Assurance Program
BOM	Bureau of Meteorology
CDCB	Communicable Disease Control Branch, SA Health
CDC	Centres for Disease Control and Prevention, US Department of Health and Human Services
EHO	Environmental Health Officer
ENSO	El Niño–Southern Oscillation
FDA	US Food and Drug Administration
FSRB	Food Safety and Regulation Branch (formerly Food and Controlled Drugs Branch), SA Health
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specifications for Foods
IMOS	Integrated Marine Observing System
ISSC	Interstate Shellfish Sanitation Conference
MJO	Multi-jurisdictional outbreak
MLST	Multi-locus sequence typing
MPN	Most Probable Number
NOAA	National Oceanic and Atmospheric Administration
NRE	Department of Natural Resources and Environment, Tasmania
NSSP	US National Shellfish Sanitation Program
PIRSA	Department of Primary Industry and Regions South Australia
SAOGA	South Australian Oyster Growers Association
SAORC	South Australian Oyster Research Council
SASQAP	South Australian Shellfish Quality Assurance Program
SSChl-*a*	Sea surface chlorophyll-*a*
SST	Sea surface temperature
ST	Sequence Type
TDH	Thermostable direct haemolysin
TRH	TDH-related hemolysin

Executive Summary

Background

Vibrios are naturally occurring bacteria that are ubiquitous in fresh, estuarine and marine environments. Many *Vibrio* species are non-pathogenic, but some can cause disease in animals, and others are pathogenic to humans. People can contract vibriosis by consuming raw, undercooked or cross-contaminated seafood (predominantly oysters, crabs and shrimp) or exposing a wound to seawater. Bivalve molluscs, such as oysters, are a known vector for pathogenic bacteria as they are often consumed raw, and their filter feeding action concentrates bacteria within their tissues. Historically *Vibrio parahaemolyticus* has been rarely implicated in illnesses attributed to the consumption of Australian seafood. However, several recent outbreaks of gastroenteritis caused by *V. parahaemolyticus* in oysters have occurred in Australia.

In March 2021, SA Health commenced an investigation into an increased number of locally acquired cases of *V. parahaemolyticus*. A total of 21 vibriosis cases were reported, and trace-back identified a common source of Pacific Oysters (*Magallana gigas*) from a South Australian growing region. A second outbreak commenced in September 2021 with 268 cases reported between 7 September 2021 and 18 February 2022.

An improved understanding of the environmental determinants was warranted to assist in future risk management considerations and food safety requirements. This report describes the results of the study undertaken which recorded the available and relevant environmental information and considered known risk factors relevant to *V. parahaemolyticus* that could be used for future investigations and to help underpin risk management considerations. The identification of data gaps and tools that could be used to identify and assess potential *Vibrio* risk factors may help guide where additional effort is required to assist future understanding of this complex and emerging food safety issue in Australia.

Objectives

1. Collate and record all available environmental conditions (pre- and post-harvest) associated with the 2021-2022 *Vibrio* outbreaks related to oysters produced in South Australia
2. Review the environmental conditions which may have been risk factors contributing to the 2021-2022 outbreaks related to South Australian oysters
3. Review available tools that could be used to identify and assess potential *Vibrio* risk factors and any approaches for improved surveillance
4. Make recommendations on data and information collection deficiencies related to the South Australian situation.

Methodology

The study reviewed the published and grey literature on key pathogenic *Vibrio* species, their ecology, environmental risk factors, mitigation strategies and collated available environmental data surrounding the time and location of the outbreaks. Environmental data including sea-surface temperatures, salinity, phytoplankton/chlorophyll-*a* and weather observations were obtained from industry, Bureau of Meteorological and satellite data.

Results

Vibrios are part of the normal microbiota of many oysters and are ubiquitous in many other aquatic products. Vibrios multiply in oyster tissues at temperature-dependent rates before, during and after harvest. Across the two outbreaks, three sequence types (ST36, ST50 and ST417) were identified from clinical isolates and only one sequence type (ST417) was isolated from oysters as part of investigations following the second outbreak. The environmental conditions, notably sea surface temperature, oyster basket temperature and salinity, during the onset periods of the two *Vibrio* outbreaks (February 2021 and September 2021) were conducive to the growth of *V. parahaemolyticus*. However, there were no evident climatological anomalies in the collated data sets that help to substantiate why these *Vibrio* outbreaks occurred in South Australia at these times given that there had not been any significant changes in oyster production, harvest and post-harvest practices.

This project has also highlighted several data gaps. Poor traceability through supply chain hampered traceback investigations and the identification of the unique harvest date, harvest location, and subsequent production, harvest and post-harvest conditions was limited. There is no information publicly available on the levels of detection of *V. parahaemolyticus* in the implicated oysters. The occurrence of these two and similar recent *Vibrio* outbreaks in Australia demonstrates that vibrios are a risk that requires effective control mechanisms. A range of tools and approaches are available that could be used to identify and assess potential risk factors and improved surveillance. These tools include in-situ data collection, remote sensing of the environment, microbiological sampling and molecular diagnostics.

Implications

Environmental and biological factors and modifying harvest practices are continually being studied in Australia and overseas to find relationships between risk factors and the prevalence and concentration of *V. parahaemolyticus* in bivalve shellfish, as well as determining *Vibrio* growth in oysters. There is evidence internationally that vibrios are constantly evolving, creating more resilient and virulent strains. Although there were no clear climatological anomalies in the environmental data sets investigated as part of this project that would help to explain why these *Vibrio* outbreaks occurred during this period, the environmental conditions, notably sea surface temperature and salinity at the time of both outbreaks would have been conducive to the growth of *V. parahaemolyticus*.

The 2021 outbreaks were the largest vibriosis outbreaks on record associated with Australian product and resulted in substantial costs for industry, both economically and reputationally. The magnitude and severity of the September 2021 outbreak was likely compounded by several factors, including post-harvest temperature controls, timely reporting of illnesses, and poor traceability along the supply chain, which impacted traceback and the timing of growing area closure. Pre- and post-harvest control measures are critical to manage the risk of vibrios and accredited operators should pay special attention to their approved Food Safety Arrangements. Since the two *Vibrio* outbreaks the control measures in South Australia have been strengthened to prevent/help limit the occurrence and severity of any future *Vibrio* outbreaks.

Recommendations

1. In-situ environmental monitoring is improved through use of loggers in more growing and harvest areas.
2. Further work needs to be undertaken within the supply chain to ensure that legislated responsibilities on labelling, traceability and control of co-mingling are adhered to.
3. *Vibrio parahaemolyticus* isolates should be collected during vibriosis events (clinical and oyster) and an Australian isolate collection curated and maintained.
4. A review and refresh of growers recall plans is necessary and growers should participate in simulation training of recall events to improve the practices supporting speedy recalls.
5. Open lines of communication between regulators and industry should be maintained to determine what type of data can be shared and when.
6. Authorities should implement timely closure of growing areas following multiple illnesses in line with ASQAP guidelines.
7. Food Safety Management plans should be reviewed and closely adhered to, especially if there are any future outbreaks.
8. Regulators should hold a post event review that includes industry and research representatives to strengthen working relationships and improve joint outcomes.

Keywords

Vibrio parahaemolyticus, Pacific oyster, *Magallana gigas*, illness, outbreak, climatological, climate change, food safety, environmental factors, post-harvest

Introduction

Vibrios are a group of Gram-negative, rod-shaped bacteria that are widely distributed in fresh, estuarine and marine environments worldwide. They can be planktonic, free-living organisms, but are frequently associated with plankton or aquatic animals (Jones, 2014). *Vibrio* spp. are also able to form biofilms on a range of organic and inorganic substrates, which support colonisation and increase persistence (Kirstein *et al.*, 2016, Leighton *et al.*, 2022). Although many *Vibrio* species are non-pathogenic, some can cause disease in animals, and others have been associated with human illness. Human illness caused by pathogenic *Vibrio* species can be grouped into cholera and vibriosis (non-choleragenic) infections. People can contract vibriosis by consuming raw, undercooked or cross-contaminated seafood (predominantly oysters, crabs and shrimp) or exposing a wound to seawater. The most common symptoms are mild and self-limiting gastroenteritis from the consumption of contaminated seafood, but *Vibrio* bacteria can also cause a skin and tissue infection through an open wound. Severe illness is rare and typically occurs in people with a weakened immune system. Whilst more than 20 *Vibrio* species can cause vibriosis, three species - *Vibrio parahaemolyticus, Vibrio vulnificus*, and *Vibrio alginolyticus* - are responsible for most of these illnesses (Centres for Disease Control and Prevention (CDC), 2023). On the other hand, cholera is an acute diarrheal illness caused by the ingestion of food or water contaminated with *Vibrio cholerae* (serogroups O1 or O139), and if left untreated 25-50% of severe cholera cases can be fatal (Centres for Disease Control and Prevention (CDC), 2022). The occurrence of toxigenic *V. cholerae* in Australia is rare, but sporadic non-O1 and non-O139 infections with or without travel history have been reported (Bhandari *et al.*, 2023).

V. parahaemolyticus is the leading cause of bacterial gastroenteritis associated with the consumption of seafood products (FAO and WHO, 2021). *V. parahaemolyticus* was first identified following a large food poisoning outbreak that occurred in Japan in 1950 (Fujino *et al.*, 1953) and by the late 1960s and 1970s *V. parahaemolyticus* was recognised as a cause of gastroenteritis worldwide (Sumner and Pointon, 2007). Globally, the prevalence of vibriosis is linked to the effects of climate change, aging populations, dietary changes and improved detection methods (FAO and WHO, 2021). Vibrios are also constantly evolving, and potentially developing more resilient and virulent strains (Brumfield *et al.*, 2021, DePaola, 2019). *V. parahaemolyticus* infections were first reported in Australia in 1984 (Hall, 1993). By 2005, the median number of foodborne *V. parahaemolyticus* illnesses in Australia (circa 2000) was estimated at 740 cases per annum (Hall *et al.*, 2005).

Oysters naturally accumulate and depurate *V. parahaemolyticus* through filter-feeding. However, once oysters are no longer underwater depuration can no longer occur and *V. parahaemolyticus* levels increase quickly unless the oysters are held at less than 10°C. The US National Shellfish Sanitisation Program (NSSP) considers 10°C a temperature too low to enable *Vibrio* growth, whereas growth rates increase above 15°C, while temperatures approaching 20°C support higher growth rates (Ellett *et al.*, 2022). Contamination of oysters with *V. parahaemolyticus* can also occur during handling, processing or by cross-contamination through contact between oysters and other contaminated seafood products or seawater (NdrahaWong and Hsiao, 2020). *V. parahaemolyticus* can live in sediments and waters year-round and generally proliferates in the water column when water temperatures are greater than 14°C (Su and Liu, 2007). Higher concentrations of *V. parahaemolyticus* are usually present in warm waters of moderate salinity (Bell and Bott, 2021). In the United States most *Vibrio* infections follow a seasonal trend and occur when water temperatures are warmer (i.e. summer and autumn) (U.S. Food and Drug Administration, 2019). *V. parahaemolyticus* is rarely isolated when the seawater temperature is under 13-15°C (NdrahaWong and Hsiao, 2020).

V. parahaemolyticus is a genetically diverse bacterial species represented by multiple sequence types (STs), with some strains pathogenic to humans. The thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), encoded by *tdh* and *trh* genes respectively, are considered major virulence factors in *V. parahaemolyticus*. However, some isolates lacking *tdh* and/or *trh* are also highly cytotoxic to human gastrointestinal cells and about 10% of clinical strains do not contain those genes (Raghunath, 2015). Global warming has also been linked to an increasing geographical range and frequency of *V. parahaemolyticus* infections (Wang *et al.*, 2022). In recent decades, incidences of vibriosis have been occurring in regions with cooler climates (where there had previously been no reported cases) as well as upward trends in case numbers in affected jurisdictions (Baker-Austin *et al.*, 2016b, McLaughlin *et al.*, 2005). Aside from temperature, other environmental factors including salinity, chlorophyll and turbidity have also been linked to *Vibrio* prevalence and levels, but with inconsistent relationships (Johnson *et al.*, 2010, Martinez-Urtaza *et al.*, 2008, Parveen *et al.*, 2008, TakemuraChien and Polz, 2014).

Since 2002 there have been seven vibriosis outbreaks recorded in Australia (see Table 1). A number of other locally acquired sporadic foodborne cases have also occurred in Australia (HarlockQuinn and Turnbull, 2022). In Australia, vibriosis cases are likely to be under reported as it is not a nationally notifiable disease and many people with a foodborne disease do not visit a doctor. *Vibrio* infections are currently only a notifiable disease within the Northern Territory, South Australia, Tasmania and Western Australia; other jurisdictions only need to notify if cases are identified as part of a foodborne outbreak. In South Australia, *V. parahaemolyticus* infections became a notifiable condition on the 18 February 2016 and a number of locally acquired sporadic cases have been recorded (see Table 2).

Table 1: Outbreaks of vibriosis recorded in Australia since 2002.[a]

Year	Jurisdiction reporting outbreak	Cases (number confirmed)	*Vibrio* species	Suspected vehicle	Source jurisdiction
2002	New South Wales	2(1)	*V. parahaemolyticus*	Unknown	Unknown
2005	Tasmania	2(1)	*V. parahaemolyticus*	Unknown	Unknown
2016	Tasmania [b]	11 (8)	*V. parahaemolyticus*	Oysters	Tasmania
2016	Western Australia	9 (9)	*V. parahaemolyticus*	Oysters	South Australia
2017	New South Wales	3 (1)	*V. albensis*	Oysters	Tasmania
2021	South Australia	21	*V. parahaemolyticus*	Oysters	South Australia
2021	Multi-jurisdictional outbreak (MJO)	268	*V. parahaemolyticus*	Oysters	South Australia

[a] Adapted from Department for Health and Wellbeing (2021), Department for Health and Wellbeing (2022) and **HarlockQuinn and Turnbull (2022)**.

[b] Reported by indicated jurisdiction, but multi-jurisdictional outbreak.

Table 2: Locally acquired cases of *V. parahaemolyticus* infections recorded in South Australia between 2016 and 2021.[a]

Year	Risk factor	
	Consumption of raw oysters	Contact with seawater
2016	1	2
2017	1	1
2018	3	2
2019	3	0
2020	0	0
2021	81	0

[a] Source: SA Health: Disease Surveillance and Investigation Annual Reports.

Given that vibrios can be naturally occurring in seafood products, mitigation measures in Australia have traditionally been primarily focused on post-harvest temperature control. *V. parahaemolyticus* can rapidly multiply and, if not controlled, can form an infectious dose in shellfish before consumption. The Tasmanian outbreak in 2016 occurred during a marine heat wave lasting 251 days where surface water temperatures were up to 2.9°C above climatology (HarlockQuinn and Turnbull, 2022). The Australian Shellfish Quality Assurance Program Operations Manual (ASQAP Manual) sets post-harvest temperature controls to limit the growth of bacterial pathogens (ASQAAC, 2022). The ASQAP Manual does not currently include a section on vibrios pre-harvest. However, since the recent occurrence of *Vibrio* outbreaks in Australia, Vibrio Control Plans have been developed and must be followed by bivalve mollusc producers in some harvest areas in Tasmania (namely Big Bay, Moulting Bay, Great Swanport and Pipeclay Lagoon) (Department of Natural Resources and Environment Tasmania (NRE), 2019)[1]. Torok *et al.* (2023) reported that water temperature was a major driver of *V. parahaemolyticus* levels in Tasmanian oysters at harvest, and air temperature was a major driver of *V. parahaemolyticus* growth/decline post-harvest. Torok *et al.* (2023) also noted that other environmental factors may be important to the level of *V. parahaemolyticus* and recommended any water temperature triggers and alerts may be growing area specific. *Vibrio* risk in South Australia is managed through the Food Safety Arrangement and subsequent HACCP plan (Department of Primary Industries and Regions (PIRSA), 2023a).

This research project aims to help manage the risk of vibriosis by providing a summary of the potential risk factors and the environmental conditions surrounding the recent 2021 and 2021/22 *Vibrio* outbreaks attributed to Pacific Oysters (*Magallana gigas*) harvested from South Australia and detailing tools that could be used to identify and assess potential *Vibrio* risk factors.

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[1] Department of Natural Resources and Environment Tasmania (NRE) (2019) recommends that all other bivalve growing areas should consider implementing the Vibrio Control Plan due to increasing risk across the state in warmer months.

Objectives

The specific objectives of this project were to:

- Collate and record all available environmental conditions (pre- and post-harvest) associated with the 2021-2022 *Vibrio* outbreaks related to oysters produced in South Australia.
- Review the environmental conditions which may have been risk factors contributing to the 2021-2022 outbreaks related to South Australian oysters.
- Review available tools that could be used to identify and assess potential *Vibrio* risk factors and any approaches for improved surveillance.
- Provide recommendations on data and information collection deficiencies related to the South Australian situation.

Method

A review of scientific and grey literature was undertaken to identify biotic and abiotic risk factors that could potentially be linked to the two *Vibrio* outbreaks in South Australia (February 2021 and September 2021). The project team identified the health and environmental information that could be important, as well as the key custodians and contacts of this information. This included data from industry, the South Australian Shellfish Quality Assurance Program (SASQAP), freely available and commercial weather, oceanic and environmental data. The study also explored if there were any climatological anomalies within the obtained data that may have contributed to the outbreaks. SA Health and PIRSA led the traceback investigations, and a summary of these findings have been included for context.

A search of the available tools that could be used to identify and assess potential *Vibrio* risk factors and any approaches for improved surveillance was completed by reviewing tools that are used in other domestic and international jurisdictions.

Results and Discussion

V. parahaemolyticus risk factors

Knowledge of seasonal and geographical distribution, and the effects of environmental parameters on the growth of *V. parahaemolyticus* are essential for creating science-based strategies for control and risk mitigation (De Souza Costa Sobrinho *et al.*, 2010, Flynn *et al.*, 2019). An overview of the limits for *V. parahaemolyticus* growth is reported in Table 3and temperature specific growth rates are reported in Appendix 3. Investigating the occurrence and abundance of *V. parahaemolyticus* is complex due to the ecological relationships (such as competition, growth, and survival) of *V. parahaemolyticus* strains in the environment and in oyster tissue matrices (Flynn *et al.*, 2019). In-situ sampling is also a challenge, since vibrios can form blooms of short duration in water (TakemuraChien and Polz, 2014).

Table 3: Limits for growth of *V. parahaemolyticus*. Adapted from International Commission on Microbiological Specifications for Foods (ICMSF) (1996).

	Optimal	Range
Temperature (°C)	37.0	5.0-43.0
pH	7.8-8.6	4.8-11.0
Water activity (a_w)	0.981	0.940-0.996
Atmosphere (respiration)	Aerobic	Aerobic-anaerobic
Salt (%)	3.0	0.5-10.0

Research into the biotic and abiotic factors influencing the prevalence and concentration of *V. parahaemolyticus* in oysters, as well as impacts of modifying production and harvest practices, is occurring internationally. The biotic and abiotic factors that can affect the risk of *V. parahaemolyticus* infections associated with oyster consumption are reported in Table 4. Given *V. parahaemolyticus* are mildly halophilic, mesophilic microorganisms (salt and temperature tolerant), a significant effort has been spent exploring the effects of seawater temperature and salinity, since they generally provide the strongest environmental determinants for abundance in aquatic environments (see Figure 1). However, other factors such as pH, dissolved oxygen, precipitation, cloudiness, wind speed, wind gusts or chlorophyll-*a* have occasionally been found to correlate to the concentration of *V. parahaemolyticus*. These other factors have been incorporated into some predictive models and can improve accuracy, but are often considered as secondary factors (Ndraha *et al.*, 2022).

Although many *Vibrio* spp. are endemic to coastal waters the abundance of pathogenic *V. parahaemolyticus* is a critical parameter to estimate the risk of infections (FAO and WHO, 2021). The NSSP stipulates that water temperature, air temperature and tidal stage can increase the risk of *V. parahaemolyticus* at the time of harvest (US Food and Drug Administration, 2019).

Table 4: Biotic and abiotic factors affecting the concentration of *V. parahaemolyticus* in oysters. Adapted from Brumfield *et al.* (2023), EilerJohansson and Bertilsson (2006), NdrahaWong and Hsiao (2020) and Ndraha et al. (2022).

Category	Risk factor
Culture area	- Human sewage - Fresh water inputs - Water circulation - Competing microbiota
Cultivation method	- On-bottom or off-bottom culture - Aquaculture practices (tumbling, desiccation)
Climatic variations	- Seawater temperature - Salinity - Turbidity - Dissolved oxygen - pH - Water depth
Extreme natural events	- Hurricane - Floods - Heat waves
Handling and processing	- Cross-contamination - Cooking practices
Cold chain control	- Temperature abuse

A summary of the predominant factors from the literature influencing *V. parahaemolyticus* occurrence, abundance and growth are discussed below. Many studies including Fernandez-Piquer *et al.* (2011), Kim *et al.* (2013), Parveen *et al.* (2013) and Yoon *et al.* (2008) have also attempted to develop predictive models describing the effects of environmental conditions on the growth rate of *V. parahaemolyticus* in oysters. The relationships between the environmental parameters, prevalence and concentration of *V. parahaemolyticus* are varied and is generally considered to be highly site specific. An example of the influence of selected environmental variables on total *Vibrio* abundance from a meta-analysis study is shown in Figure 1, whilst specific cases studies are provided in more detail below.

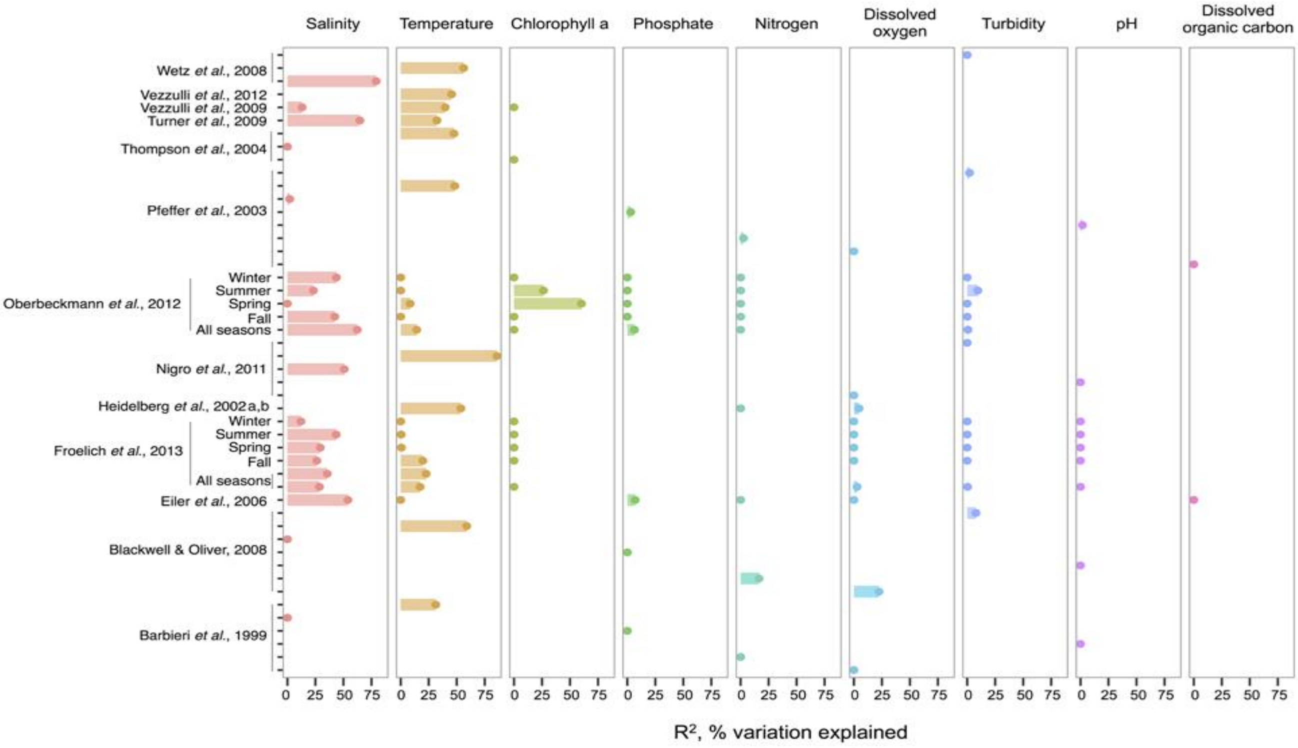

Figure 1: Influence of selected environmental variables on *Vibrio* abundance. Reproduced from TakemuraChien and Polz (2014).

Temperature and salinity

Temperature is one of the principle environmental factors responsible for increasing the abundance of *V. parahaemolyticus* in many areas of the world. Whilst the optimal temperature for *V. parahaemolyticus* growth is 35 to 37°C (NdrahaWong and Hsiao, 2020), the bacteria can grow over a much wider range (5 to 43°C) (Desmarchelier, 1997). During cooler periods, *V. parahaemolyticus* can survive in sediments and then move back into the water column once water temperatures rise to between 14–19°C (European Commission, 2001). Several outbreaks associated with climatic anomalies, such as those observed in the Northeast United States of America, Spain, Chile and Northern Europe during heatwave events, have outlined the importance of temperature (Baker-Austin *et al.*, 2016a, FAO and WHO, 2021). A Canadian outbreak in 2015 and the Tasmanian outbreak in 2016 occurred when sea surface temperatures (SST) were also above historical levels (GalanisOtterstatter and Taylor, 2020, HarlockQuinn and Turnbull, 2022). FAO and WHO (2020) recently summarised that the distribution of *V. parahaemolyticus* in relation to variations in seawater temperature and salinity follows the following pattern:

- Areas of moderate salinity (from 1 to 25 ppt) and temperate or warm waters (e.g. Gulf of Mexico, Chesapeake Bay, the United States of America): seawater temperature is the major factor influencing the abundance.
- Areas with salinity close to oceanic waters (from 25 to 35 ppt) and temperate waters (e.g. Atlantic coasts of Europe): *V. parahaemolyticus* is detected in areas and periods of lowest salinity, whereas seawater temperature influences the concentration.
- Tropical areas with minor changes in seawater temperature (e.g. India), no influence of salinity and temperature has been reported.

The following examples help to demonstrate some of the relationships between temperature, salinity and *V. parahaemolyticus* prevalence and/or concentration. It can be difficult to compare between studies as some authors focused on SST, whilst others use ocean temperature, shore temperature or mean monthly temperatures. Flynn *et al.* (2019) recommended that SST be collected, where possible, during *V. parahaemolyticus* monitoring programs due to superior model fits.

The relationship between seawater temperature and densities of *V. parahaemolyticus* in U.S. Gulf Coast oysters from a study reported by DePaola *et al.* (2003) is shown in Figure 2. The authors reported that the abundance was more affected by seawater temperature than by salinity. However, levels of *V. parahaemolyticus* also fluctuated independently of temperature and salinity (DePaola *et al.*, 2003). The unattributed fluctuation may indicate the importance of other biotic and abiotic factors.

A similar coastal study but from the southern coast of the Sao Paulo state, Brazil concluded that total *V. parahaemolyticus* concentration in oysters was significantly correlated to seawater temperature but not salinity (De Souza Costa Sobrinho *et al.*, 2010). The relationship between the mean concentration of *V. parahaemolyticus* in the oysters and seawater temperature is shown in Figure 3. There was a plateau above 24°C and below 20°C in which the concentration was not significantly influenced by temperature.

In another study, the presence of *V. parahaemolyticus* in water, plankton and sediment samples from field sites in the north Adriatic Sea was significantly and positively correlated with SST; there was an increased probability of isolating organisms when the SST was greater than 19°C (Caburlotto *et al.*, 2010). There was also statistically significant probability of isolating *V. parahaemolyticus* when the salinity was <32 PSU, turbidity >3.97 and chlorophyll >3.07 µg mL^{-1}.

A Taiwanese study of oysters, seawater and sediments for a predictive model found that the concentration of *V. parahaemolyticus* in the oysters and sediment was influenced by the variation of SST up to 15 days before sample collection, while the abundance was influenced by the variation of SST up to 30 days before sample collection (Ndraha *et al.*, 2021). The authors also reported that the concentration of *V. parahaemolyticus* in oysters decreased substantially when the pH of the seawater was above 7.9, salinity greater than 30 ppt and wind-speed above 4m s^{-1}. The higher wind-speeds typically occurred from Autumn to Winter when the SST and pH values were decreasing (Ndraha *et al.*, 2021).

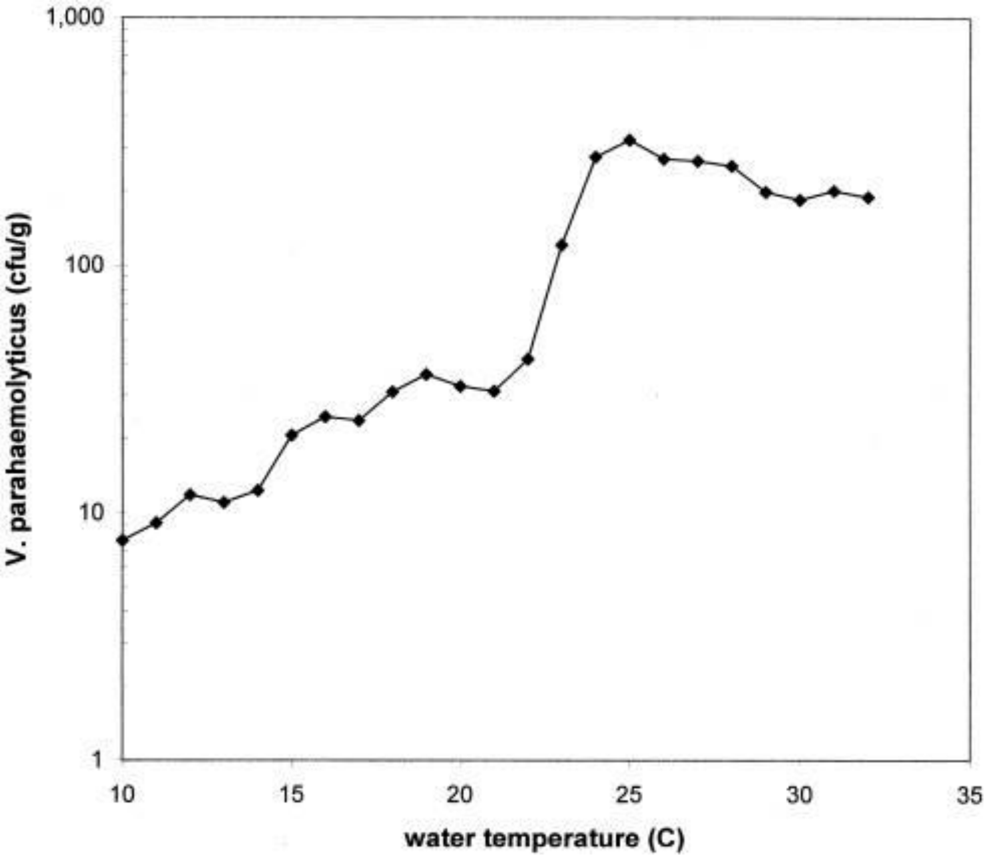

Figure 2: The impact of water temperature on *V. parahaemolyticus* density in U.S. Gulf Coast oysters harvested biweekly between March 1999 and September 2000. Mean salinities ranged between 17.6 and 27.5 ppt. Reproduced from DePaola et al. (2003).

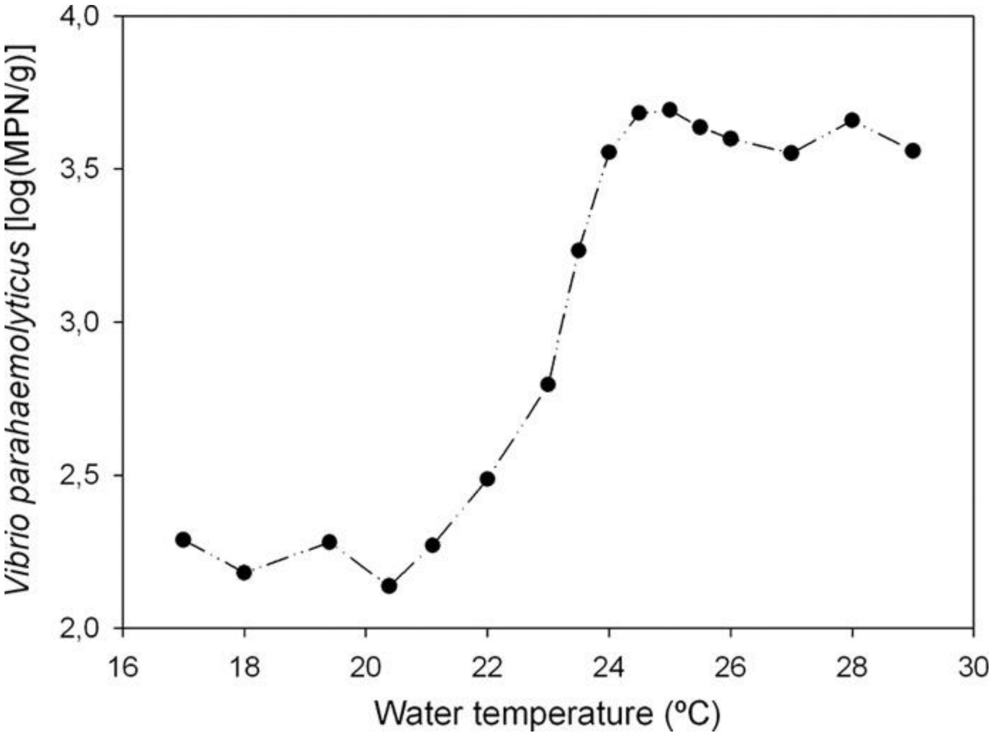

Figure 3: The impact of seawater temperature on *V. parahaemolyticus* density in oysters cultivated in the southern coast of Sao Paulo state, Brazil. Oysters were harvested between May 2004 and June 2005. Mean salinity varied between 17.3 and 24.2 ppt. Reproduced from De Souza Costa Sobrinho et al. (2010).

In some cases, higher salinity has been found to have a negative correlation with *V. parahaemolyticus* abundance (Caburlotto *et al.*, 2010, DePaola *et al.*, 2003, Chen *et al.*, 2011, Reyes Velazquez *et al.*, 2010, Johnson *et al.*, 2010, Cole *et al.*, 2015). Several studies conclude that this relationship is a secondary contributing factor (De Souza Costa Sobrinho *et al.*, 2010, DeepanjaliKumar and Karunasagar, 2005), with water temperature as the primary factor (Flynn *et al.*, 2019). However, in a Spanish study conducted over 3 years, where seawater temperature ranged from 11.7 to 20.8°C and salinity ranged from 30.9 to 36.2 ppt, the authors reported that salinity was the primary factor and seawater temperature was a secondary factor governing the bacteria abundance (Martinez-Urtaza *et al.*, 2008). The authors also reported that an increment of one ppt unit in salinity reduced the probability of detection of *V. parahaemolyticus* by more than half (Martinez-Urtaza *et al.*, 2008). Several studies have shown that relaying oysters to a high salinity environment can reduce *V. parahaemolyticus* concentrations (Parveen *et al.*, 2017, Taylor *et al.*, 2018, Walton *et al.*, 2013a). However, a recent systematic review of intervention strategies found that relaying to high salinity environments was often ineffective and recommended that additional studies be completed (Spaur *et al.*, 2020). Finally a New Zealand Risk Profile of *V. vulnificus* in Pacific Oysters concluded that the high-salinity relaying in New Zealand would be of limited benefit as the oysters are already harvested from high-salinity (>30‰) waters (KingMcCoubrey and Cressey, 2018).

Chlorophyll and plankton abundance

The association of vibrios with planktonic organisms has been suggested as an important component of *Vibrio* ecology as the high nutrients associated with plankton and plankton habitats may also favour vibrios (Diner *et al.*, 2021, Turner *et al.*, 2009). In the Chesapeake Bay, Marylands USA, chlorophyll-*a* levels (range 5-25 µg/L) were associated with increased abundance of *V. parahaemolyticus* (Brumfield *et al.*, 2023). Similarly, in New Hampshire and North Carolina, USA, higher *Vibrio* concentrations were observed in water samples with chlorophyll-*a* levels between 16 and 25 µg/L (Namadi and Deng, 2023). However, in an 18 month study in waters along the coastal area of the north Adriatic Sea chlorophyll-*a* values >1,000 µg/L (>1 µg/mL) provided a favourable condition for *V. parahaemolyticus* (Caburlotto *et al.*, 2010). The occurrence of *V. parahaemolyticus* may be highly correlated with zooplankton blooms (Turner *et al.*, 2014).

Turbidity, dissolved oxygen and pH

The turbidity of the water column can serve as an indicator of the organic content, sediment resuspension and plankton blooms (Johnson, 2015). Whilst the influence of turbidity on *Vibrio* levels in oysters is currently poorly defined (FAO and WHO, 2020), vibrios are frequently attached to the suspended matter, and polluted and turbid waters often present high nutrient levels for bacterial growth. Increasing turbidity can have a positive correlation with *V. parahaemolyticus* abundance in water (Blackwell and Oliver, 2008, Davis *et al.*, 2017, Johnson *et al.*, 2012, Johnson *et al.*, 2010, Parveen *et al.*, 2008, Zimmerman *et al.*, 2007), whereas other authors have reported a small but significant negative correlation (Froelich *et al.*, 2019). High wind speeds and currents, plus human interactions, can cause the resuspension of sediments and any sediment-dwelling bacteria into the water column (Davis *et al.*, 2017, Potdukhe *et al.*, 2021).

The influence of dissolved oxygen on *Vibrio* levels in oysters is currently poorly defined (FAO and WHO, 2020). Dissolved oxygen has been negatively and positively correlated to *Vibrio* spp. abundance in water samples (Blackwell and Oliver, 2008, Froelich *et al.*, 2019, Parveen *et al.*, 2008). Vibrios are a facultative anaerobes (able to grow with or without free oxygen) (Youngren-GrimesGrimes and Colwell, 1988) and consequently oxygen levels are unlikely to play a direct role in the abundance of *V. parahaemolyticus*.

Vibrios can tolerate and grow in a wide range of pH levels (pH 4.8 to 11), but prefer slightly alkaline conditions (pH 7.8-8.6) (Desmarchelier, 1997). Froelich *et al.* (2019) reported no significant association between *V. parahaemolyticus* concentrations and the pH of the water in an estuarine environment in North Carolina, USA, whereas López-Hernández *et al.* (2015) concluded that the pH (range 7.8-8.4) impacted abundance of *V. parahaemolyticus* in oysters harvested from the Gulf Coast of Mexico. López-Hernández *et al.* (2015) also determined that the higher pH values were associated with increases in turbidity. Whilst open oceanic waters have more stable pH values, more pronounced shifts are observed in estuarine, near-shore and in coastal upwelling locations (Duarte *et al.*, 2013).

Bacteriophages

Bacteriophages are viruses that specifically infect and replicate within bacterial cells. Whilst some bacteriophages have been shown to lyse different strains of *V. parahaemolyticus*, they are highly specific to their hosts (Bastías *et al.*, 2010, García *et al.*, 2013). Rong *et al.* (2014) demonstrated the effectiveness of a bacteriophage for reducing *V. parahaemolyticus* in oysters during depuration. Several recent in-vitro studies (Zhang *et al.* (2018), Yang *et al.* (2020) Tan *et al.* (2021) and Chen *et al.* (2023)) have also shown promising results of using bacteriophages as a biocontrol agent against *V. parahaemolyticus*.

Production and post-harvest practices

The interactions of production practices on *Vibrio* levels in oysters at the time of harvest is complex to assess and the published findings from many investigations are mixed. Production practices such as suspension of the oysters off-the-bottom of the seafloor have been found to reduce *V. parahaemolyticus* concentration in oysters (Cole *et al.*, 2015). One preliminary study determined that the concentration of *V. parahaemolyticus* in intertidal oysters from three Washington State growing areas can be dependent on the type of oceanic floor (Paranjpye *et al.*, 2020). The level of *V. parahaemolyticus* was highest in oysters harvested on gravel, followed by mud and a mixture of sand and mud substrates, whilst *tdh*+ strains were more prevalent in oysters harvested from a mixture of sand and mud substrates. South Australian oysters are grown in intertidal and subtidal waters using several methods including traditional rack and rail, longline and hybrid systems. Growing systems are different in each area to allow the oysters the greatest access to food, and to suit the environmental conditions and growers' operational preference.

Vibrios can also rapidly proliferate once oysters are removed from the water and exposed to the ambient air conditions, such as during intertidal periods, physical handling, or transport. When oysters are exposed to ambient air during intertidal periods, the temperature of the oysters can rise considerably, and enable *V. parahaemolyticus* concentrations to rapidly increase (Flynn *et al.*, 2019, Grodeska *et al.*, 2017, Grodeska *et al.*, 2019, Jones, 2014). Following intertidal exposure, the concentration of *V. parahaemolyticus* in oysters generally returns to background levels after one tidal cycle (Jones *et al.*, 2016, Nordstrom *et al.*, 2004). Madigan *et al.* (2007) also identified extended intertidal exposure as a practice that results in the increased number of vibrios in oysters. Proliferation may also be impacted by specific genes. For example, Ben-Horin *et al.* (2022) reported that whilst total *V. parahaemolyticus* concentrations did not significantly differ between intertidal and subtidal cultured oysters, the concentration of *tdh*+ or *trh*+ increased 1.5 times when exposed to low tide. However, a New Zealand study of two geographically different commercial oyster growing areas found no clear evidence that different farming methods (floating, subtidal or intertidal at different depths) affect *Vibrio* populations (Cruz *et al.*, 2020). Several studies have reported that oyster ploidy does not significantly impact the abundance of *V. parahaemolyticus* in oysters (JonesLydon and Walton, 2020, Walton *et al.*, 2013b).

Both the South Australian and Tasmanian *Vibrio* guides recommend that following land-based oyster activities (i.e. sorting, rumbling and grading), the oysters are returned to the growing area for at least two tidal cycles before harvesting for human consumption (Oysters South Australia, 2023, Oysters Tasmania, 2019). However, several international studies have reported it can take up to 7-14 days for *V. parahaemolyticus* levels to return to background levels after routine handling (involving product out of water for 24-26 hours) (Kinsey *et al.*, 2015, Pruente *et al.*, 2022, Pruente *et al.*, 2020, PruenteWalton and Jones, 2021). The existing Codex guideline states that whilst pre-harvest controls can be used, harvest is the most critical stage for controlling *V. parahaemolyticus* infections, as it is from this point onwards that control measures can usually be implemented (CODEX, 2010). There are no known methods to reduce the levels of *V. parahaemolyticus* in the water. The supply chains of oysters are complex with shellstock moving from growers, sometimes through brokers or wholesalers, to processes, retailers, food service or directly to the public. Post-harvest temperature abuse at any part of the supply chain can allow for growth of pathogenic *Vibrio* spp. and increase the risk of vibriosis. Whilst the ASQAP Manual does not contain any pre-harvest *Vibrio* management strategies, it has established post-harvest temperature controls to limit the growth of bacterial pathogens (ASQAAC, 2022). Internationally, a range of pre-harvest, harvest and post-harvest mitigation techniques are used in regions where *V. parahaemolyticus* infections have occurred (see Table 5 for examples).

Table 5: *V. parahaemolyticus* mitigation techniques that have been used in various jurisdictions. Adapted from Dorothy-Jean & Associates Ltd (2018).

Control point in production chain	Process
Pre-harvest controls	- Deep water suspension of cultures. - Relaying. - Re-submerging.
Harvesting controls	- Cease harvesting for raw product market during the high risk *V. parahaemolyticus* period. - Suspend intertidal harvesting for raw product market. - Harvesting curfews based on tidal conditions or time conditions. - Shading of shellstock on harvesting vessels.
Post-harvest controls	- Divert product for shucking (cooked product) market. - Rapid cooling of oysters using ice and ice slurries on board vessels (rapidly cools products to <10°C in 20 minutes and maintains the cold chain (4°C)). - Other cooling systems on the harvest vessel. - Establishing time/temperature controls for harvested product. - Adequate refrigeration at the distribution, retail and food service levels. Monitoring of temperature on shipments of oysters upon arrival at these various stages. - Cooling after landing. Maintaining seafood temperature at or below 10°C during distribution and storage.
Processing conditions	- Process oysters in an environment which is temperature controlled. - Use disinfected or artificial seawater, or potable water, for washing and processing seafood. - Processing companies operate under a HACCP plan which includes Critical Points for incoming product.
End-product microbiological limits	- Japanese microbiological standards: ≤100 MPN *V. parahaemolyticus*/g for seafood intended for raw consumption, not detected/25 g for ready-to-eat boiled seafood. (n=5, c=0, m=100 *V. parahaemolyticus* MPN/g). - Canada summer microbiological criterion for live oysters in the shell and intended for the raw market in Canada: (n=5, c=0, m=100 *V. parahaemolyticus* MPN/g).
Education	- US FDA and ISSC have put significant resources into educating the public and medical practitioners about the risks of vibriosis (See https://www.fda.gov/food/populartopics/ucm341987.htm and https://www.issc.org/vibrio-specific-information).
Consumer advisories [a]	- US FDA requires that advisory health warnings are provided when selling shellfish.

[a] The advisory is meant to inform consumers, especially susceptible populations (i.e. elderly, children, pregnant mothers, immunocompromised), about the increased risk of foodborne illness from eating raw or undercooked animal foods. The intent is to have the advisory conveniently displayed for consumer awareness. Therefore, the statement shall be displayed on brochures, deli cases, menus, stickers, table tents, placards, or other effective written means. An example warning advisory is:
"Consuming raw or undercooked meats, poultry, seafood, shellfish, or eggs, may increase your risk of foodborne illness, especially if you have certain medical conditions."

No microbiological limits are set for vibrios in the Australia New Zealand Food Standards Code or by the Codex Alimentarius Commission. However, the Food Standards Australia New Zealand's (FSANZ) Compendium of Microbiological Criteria for Food provides guidance on interpreting *V. parahaemolyticus* concentrations in ready-to-eat foods (see Table 6). *Vibrio* control strategies and/or microbiological limits and sampling plans have been established by several other countries (such as the USA, Canada, Japan, China and Singapore). These can involve mandatory testing of shellfish for *V. parahaemolyticus* during high-risk periods, closures of harvest areas based on illness rates, high water temperatures, and struct post-harvest temperature control requirements. The cost for preventative efforts in Washington State (USA) was recently estimated to be an average of US$0.349 per dozen oysters (Freitag *et al.*, 2021).

Table 6: Interpreting results for *V. parahaemolyticus* in ready-to-eat foods. Reproduced from Food Standards Australia New Zealand (2022).

Hazard	Result (cfu/g)	Interpretation	Likely cause	Recommended action
Vibrio parahaemolyticus	$>10^4$	Potentially hazardous	Poor temperature control (rapid chilling and storage at <5°C), inadequate processing, cross-contamination or high contamination levels in harvested seafood	• Product disposition action to assess safety and determine if disposal or product recall is needed. May need confirmation to determine whether the genetic markers of virulence are present and the *V. parahaemolyticus* are able to cause disease. • An investigation should assess: – The source of raw product and potential for high levels of contamination (e.g. harvest water temperature and water salinity) – The adequacy of time and temperature controls (chilling and storage) implemented post-harvest – The adequacy of the processing used (e.g. adequate cooking) – Likelihood of cross-contamination • Confirmation of identity and typing may be required where cases of foodborne illness are suspected.
	$10^2 – 10^4$	Unsatisfactory	As above	• An investigation should be done, as above.
	$<3 – 10^2$	Marginal	Indication that temperature control or food handling controls are not fully achieved. It may be expected that naturally contaminated raw seafood may have low levels present (<100 cfu/g)	• Proactive investigation to ensure temperature and food handling controls are effectively implemented.
	<3	Satisfactory		

Potential distribution pathways of *V. parahaemolyticus*

Significant international efforts have been undertaken to understand, reduce, or mitigate the risk of vibriosis. *Vibrio* species have undergone a global expansion in recent decades. A number of mechanisms, including oceanic currents, transfer via migrating bird and fish species, or from human activity (e.g. disposal of contaminated seawater or ballast water) could be involved in the distribution of strains (Abanto *et al.*, 2020, Miller *et al.*, 2021, Trinanes and Martinez-Urtaza, 2021, Urmersbach *et al.*, 2014, Vezzulli, 2023). Pathogenic *V. parahaemolyticus* has been reported in oysters from Australia (Lewis *et al.*, 2003, Madigan *et* *al.*, 2007). Other studies have reported the presence of *V. parahaemolyticus* in Australian seafood, but the methods used could not identify the toxigenic strains (Bird *et al.*, 1992, Desmarchelier, 1978, Eyles and Davey, 1984, EylesDavey and Arnold, 1985). Various studies in New Zealand (1985, 2008-09, 2009-12 and 2013-15) have also concluded that *V. parahaemolyticus* is prevalent in their local marine environment and can be isolated from bivalve mollusc species (Dorothy-Jean & Associates Ltd, 2018).

Oyster growing regions in South Australia

Pacific oysters were introduced to the Coffin Bay area in the late 1960s and are currently commercially cultivated in a number of geographically separated growing regions shown in Figure 4. In 2021-22 there were over 350 licensed oyster growing sites covering approximately 975 hectares (Department of Primary Industries and Regions (PIRSA), 2023b). There are large spatial differences between the growing regions and the local growing environment between and within each harvest zone is unique and depending on proximity to the bay opening, water depth, tidal channels, sand banks, or enclosed embayments.

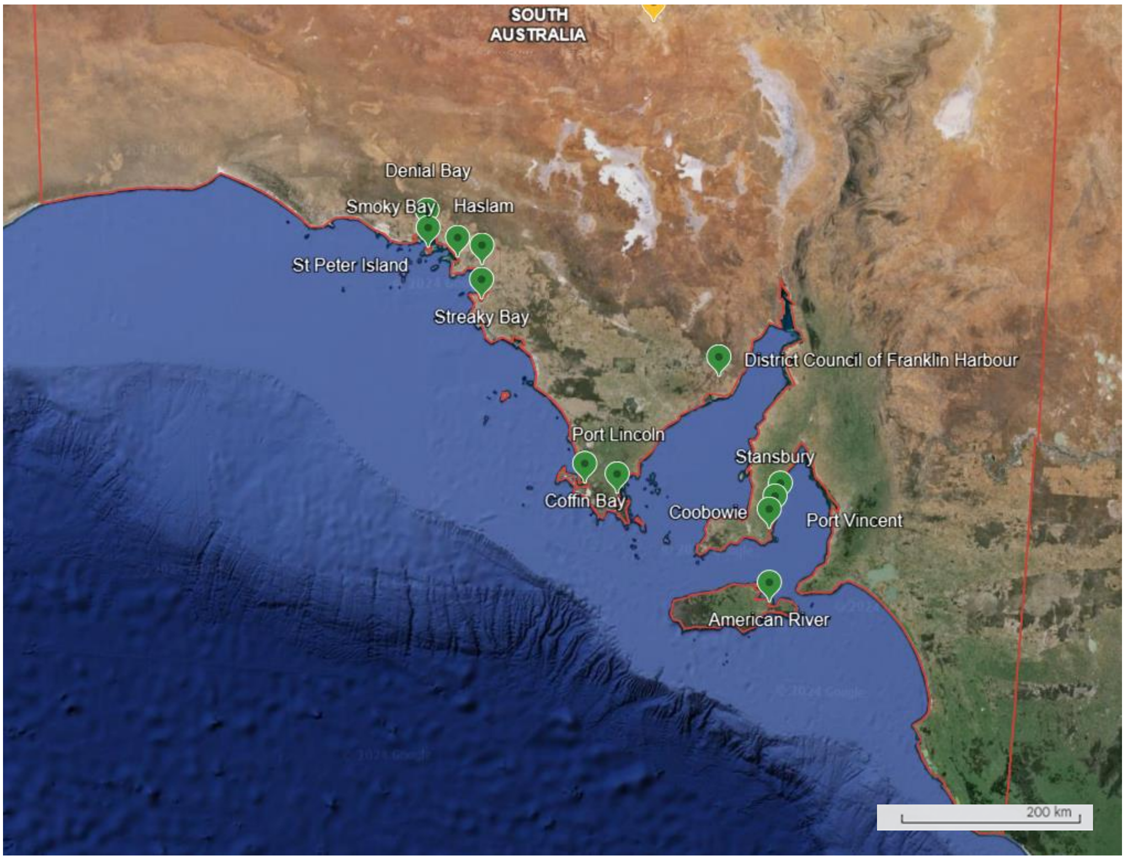

Figure 4: Pacific oyster growing regions in South Australia

Summary of the two 2021 *V. parahaemolyticus* outbreaks attributed to oysters harvested from South Australia

During the 2020/21 and 2021/22 reporting periods the South Australian Communicable Disease Control Branch (CDCB) and the Food Safety and Regulation Branch (FSRB) (formerly Food and Controlled Drugs Branch) collaborated with local councils and PIRSA to investigate two 2021 *V. parahaemolyticus* outbreaks attributed to oysters harvested from South Australia. The objectives of these investigations were to mitigate any risk to public health, establish the cause of the outbreak, ensure food businesses implement short-term and long-term corrective actions and to determine if an offence has been committed against the

Food Act 2001. Excerpt of the two outbreak investigations from SA Health Annual Reports are in Box 1 and Box 2. There were no closures of harvest areas or product recalls during the first outbreak.

Multi-locus sequence typing (MLST) is a DNA sequence-based typing method useful for molecular epidemiology and population genetic studies of bacteria. The determination of the ST type of *Vibrio* isolates can be used to identify pandemic or highly virulent strains. An awareness of the local isolates and the locations in which these have previously been found can provide insights into the environmental conditions where they may thrive. Across the two outbreaks, three sequence types (ST36, ST50 and ST417) were identified from clinical isolates and only one sequence type (ST417) was isolated from oysters as part of investigations following the second outbreak. There is no information publicly available on the levels of detection of *V. parahaemolyticus* in the implicated oysters.

- ST36 was a common sequence type recovered from clinical isolates during the February 2021 outbreak. ST36 was historically endemic to the cooler waters of the US Pacific Northwest. In 2013, Turner *et al.* (2013) stated that ST36 appeared to be geographically restricted to the Pacific Coast of the Americas (Peru, Chile and/or USA). It has since been detected on the East Coast of the United States, and around Europe, South America and New Zealand (FAO and WHO, 2021). ST36 is the first pandemic strain that appears to be evolving into numerous variants with increased virulence. The increased virulence of ST36 means that a lower number of bacterial cells of this strain can be sufficient to cause an infection (Baker-Austin *et al.*, 2018, Martinez-Urtaza *et al.*, 2017).
- ST50 was recovered from clinical isolates during the September 2021 outbreak. This sequence type has also been recently identified from clinical and environmental isolates in New Zealand (ESR (New Zealand's Institute of Environmental Science and Research), 2023, Vasey *et al.*, 2023). ST50 is infrequently reported in the international literature (ESR (New Zealand's Institute of Environmental Science and Research), 2023). This sequence type has also been detected as a clinical isolate from other regions including North Carolina, USA (Miller *et al.*, 2021) and Canada (Banerjee *et al.*, 2014).
- ST417 was also recovered from clinical isolates during the September 2021 outbreak and was the only sequence type to be isolated from oyster samples. ST417 was first associated from cases of vibriosis from Washington State, USA, in 2006 (Turner *et al.*, 2013). At the time Turner *et al.* (2013) stated that this sequence type appeared to be geographically restricted to the Pacific Coast of the Americas (Peru, Chile and/or USA), but may be more widely distributed due to underrepresentation in the PubMLST database[2]. ST417 has also been detected from clinical isolates in Canada (Banerjee *et al.*, 2014).

Box 1: February 2021 *Vibrio parahaemolyticus* outbreak. Reproduced from Department for Health and Wellbeing (2021)

An investigation into an increased number of locally acquired cases of *Vibrio parahaemolyticus* was initiated in South Australia in March 2021. Cases were also identified in other jurisdictions, with a total of 21 cases reported in the outbreak with onsets between 1 February and 30 April 2021, including eight cases in South Australia, 12 cases in Victoria and one case in Western Australia. Nineteen cases (90 percent) reported eating oysters in their incubation period, including 16 cases that ate oysters uncooked. Oysters were consumed at a variety of commercial restaurants and purchased for consumption at home. Trace back identified a common source of oysters in a South Australian growing region. Retail samples of oysters were collected in South Australia and no *V. parahaemolyticus* was identified. A common sequence type (ST 36) was identified for four South Australian cases and 11 of the Victorian cases that were able to undergo whole genome sequencing, and phylogenetic analysis on the isolates found they were highly related and suggestive of a common source. SA Health distributed information regarding safe seafood handling via social media and a communication letter was distributed to South Australian food businesses including growers, processors, transporters, brokers and retail on controlling the risks in oysters and the importance of traceability.

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[2] The PubMLST database (https://pubmlst.org/) is a public database for molecular typing and microbial genome diversity.

Box 2: September 2021 *Vibrio parahaemolyticus* outbreak. Reproduced from Department for Health and Wellbeing (2022)

In September 2021, an increase in locally acquired *V. parahaemolyticus* was identified in South Australia (SA), with an investigation initiated. *V. parahaemolyticus* is notifiable in SA, Western Australia (WA), Tasmania (TAS) and the Northern Territory (NT), with laboratories providing ad hoc reporting to health departments in other jurisdictions when increases are noted. In October, cases were also reported in WA and Victoria. Cases increased in several jurisdictions and affected more jurisdictions in November when an MJOI was triggered. Cases included *V. parahaemolyticus* reported from faecal specimens collected between 7 September 2021 and 18 February 2022. A total of 268 cases were identified, including 143 that were further typed as multi-locus sequence type (ST) 417, 70 as ST 50 and 55 not able to be further typed. Cases were reported from residents in every jurisdiction; with most in SA (76 cases, 28 per cent), followed by Victoria (69 cases, 26 per cent), Queensland (59 cases, 22 per cent), WA (33 cases, 12 per cent), New South Wales (26 cases, 10 per cent), Australian Capital Territory (3 cases, 1 per cent), TAS (1 case, 0.4 per cent) and NT (1 case, 0.4 per cent). A total of 206 cases were able to be interviewed and 199 (97 per cent) reported consumption of oysters, 189 of which reported eating raw oysters (95 per cent).

Traceback was conducted by Environmental Health Officers (EHOs) and SA Health food regulators to determine the origin of oysters consumed by cases, with 173 oyster exposures traced back to South Australian oysters. Oyster samples were collected from retail, case households, brokers and as part of the South Australian Shellfish Quality Assurance Program (SASQAP). *V. parahaemolyticus* ST 417 was isolated from 14 oyster samples, all from the same growing region in SA. Implicated oyster bays were closed on 16 November 2021, and SA growers implemented a Vibrio control program including ensuring infrastructure was available for adequate post-harvest temperature control and improved traceability of oysters. A recall of Coffin Bay oysters occurred on 19 November 2021 via Emergency Orders under the *Food Act 2001*. Media alerts were distributed in several jurisdictions and a Public Health Alert issued in SA to doctors to encourage testing for *V. parahaemolyticus* for people reporting gastroenteritis after consumption of seafood. Case reports peaked in mid-November and declined in late November after the recall. SA Health worked with the Department of Primary Industry and Regions South Australia (PIRSA) officers to implement a clearance program for the affected growing area. PIRSA continues to monitor compliance with the Vibrio control programs.

The purchase location and number of businesses implicated during the outbreak, and from the cases reported only in SA, is summarised in Table 7.

Table 7: Purchase locations and number of implicated businesses from SA Health's traceback investigations from the September 2021 outbreak. SA case data only. No data was readily available from cases in other jurisdictions. Data provided by SA Health (pers. comm. 7 June 2023).

Purchase location [a]	Number of cases	Number of businesses implicated
Direct from farm	22	9
Food service	27	21
Retailer	22	22
Seafood processor	17	9
Distributor	2	1

[a] Direct from farm (direct from an oyster farm, farm gate sales, oyster farm tours); food service (café, restaurant, takeaway); retailer (supermarket, fish monger); seafood processor (seafood processor/wholesaler with attached retail store); distributor (direct from an oyster broker).

At the time of the closure and subsequent recall there were some data gaps, and the regulatory authorities were working off the best available information at the time. The epidemic curve (distribution of cases over time) for the second outbreak, by jurisdiction and the MLST, are shown in Figures 5 and 6, respectively. Cases were reported from every Australian state and territory and the number of reported infections rapidly declined after the closure and recall.

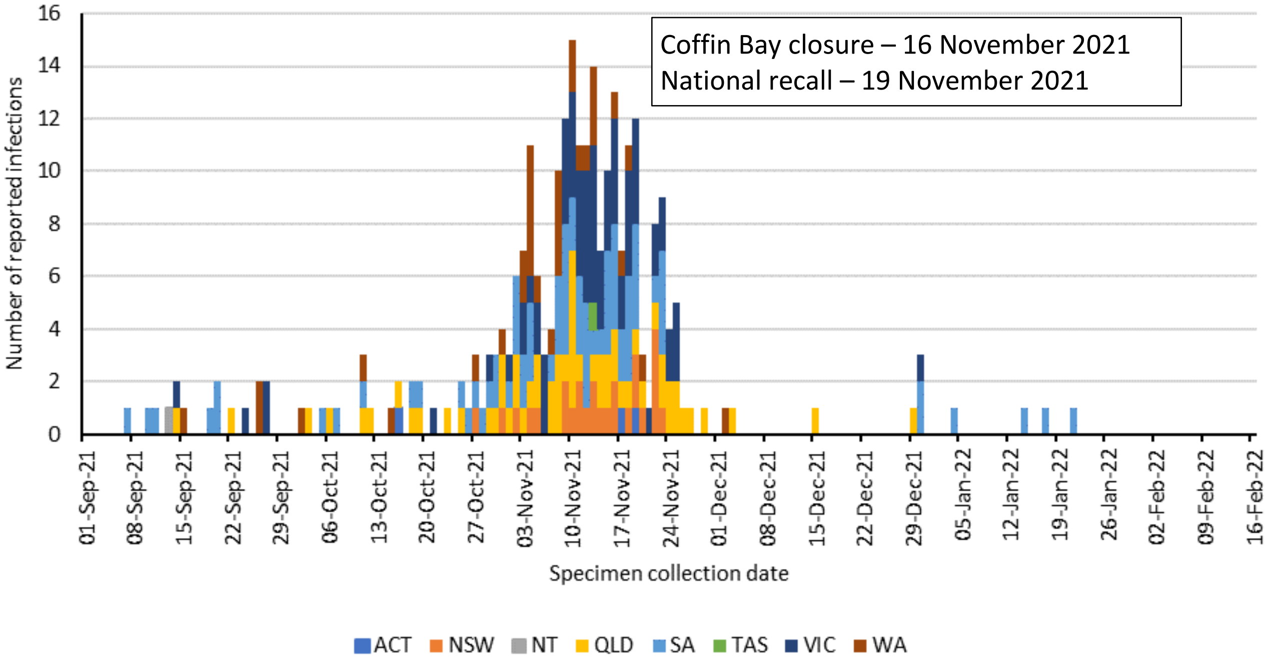

Figure 5: Epidemic curve of *V. parahaemolyticus* cases by specimen collection date and jurisdiction, 1 September 2021 to 18 February 2022. Reproduced from Fearnley et al. (2022).

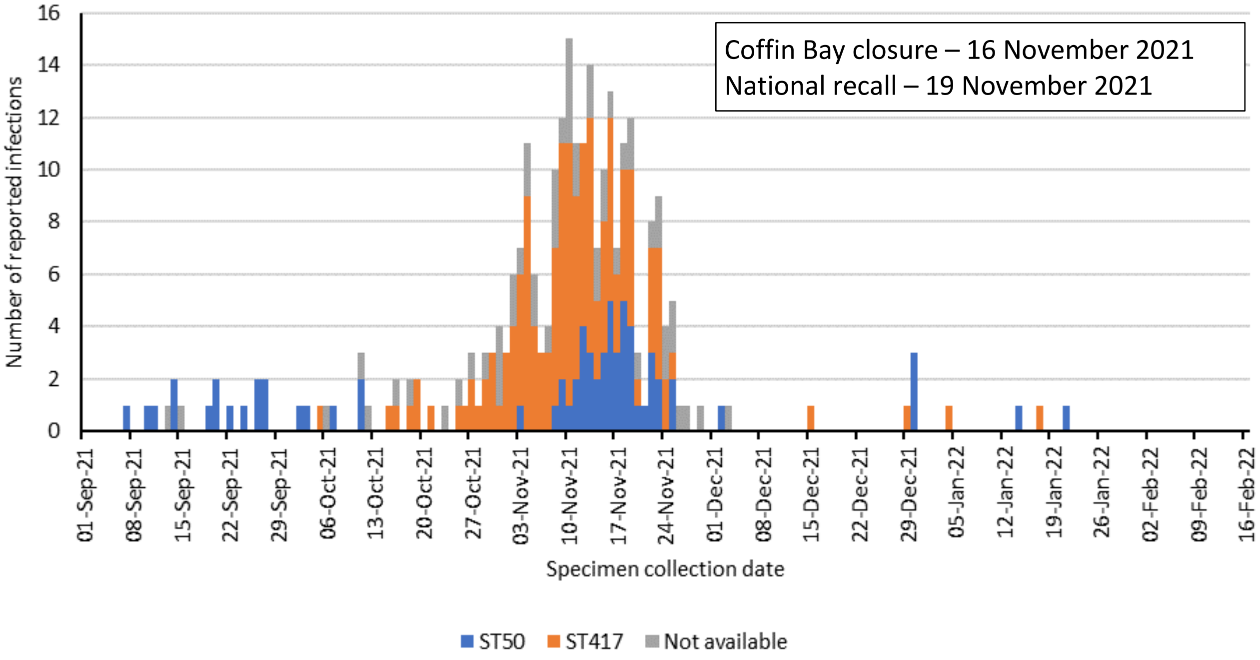

Figure 6: Epidemic curve of *V. parahaemolyticus* cases by specimen collection date and MLST type, 1 September 2021 to 18 February 2022. Reproduced from Fearnley et al. (2022).

PIRSA Biosecurity, in conjunction with SA Health, undertook traceback investigations of the implicated oysters through supply chain to grower and growing area. A summary of the traceback information including harvest dates, growing area and the MLST sequence type of the clinical isolate (where known) is reported in Table 8. SA Health and PIRSA Biosecurity have some additional information which was gathered during their investigations (such as purchase location, purchase dates, supply chain actors and growing/lease location) that due to the sensitive nature and privacy requirements the information, or redacted information, cannot be shared without a suitable human ethics approval.

Table 8: Traceback of implicated oysters to harvest date and growing area from SA cases. In some cases, there were multiple exposures to oysters in the 7 days prior to illness onset; multiple exposures are documented on separate rows. Blank cells are where traceback data was missing, not collected or unable to be collected. Information provided by PIRSA Biosecurity (pers. comm. 26 July 2023)

Case	Harvest date (where known)	Growing area (where known)	MLST (clinical isolate)
01		Smoky Bay	50
	1/09/2021	Smoky Bay	
02		Streaky Bay	50
		Streaky Bay	
03	1/09/2021	Smoky Bay	50
04		Streaky Bay	50
05		Streaky Bay	50
06		Streaky Bay or Coffin Bay	50
07			50
08		Streaky Bay	50
09	6/10/2021	Coffin Bay	417
10	19/10/2021	Coffin Bay	417
11		Coffin Bay	417
12	19/10/2021	Coffin Bay	417
13	22/10/2021	Coffin Bay	417
14		Smoky Bay	417
	19/10/2021	Coffin Bay	
15	22/10/2021	Coffin Bay	417
16	23/10/2021	Coffin Bay	417
17	22/10/2021	Coffin Bay	417
18	22/10/2021	Coffin Bay	417
19	22/10/2021	Coffin Bay	417
	22/10/2021	Coffin Bay	
	25/10/2021	Coffin Bay	
20	25/10/2021	Coffin Bay	417

Case	Harvest date (where known)	Growing area (where known)	MLST (clinical isolate)
21		Coffin Bay	417
22		Coffin Bay	417
23	22/10/2021	Coffin Bay	417
		Coffin Bay	
	26/10/2021	Coffin Bay	
24		Coffin Bay or Kangaroo Island	417
25	25/10/2021	Coffin Bay	417
26	29/10/2021	Coffin Bay	417
27		Coffin Bay	417
28	27/10/2021	Coffin Bay or Smoky Bay	417
29		Coffin Bay	417
30		Coffin Bay	417
31		Smoky Bay	50
32	22/10/2021	Coffin Bay	417
33			417
34	1/11/2021	Coffin Bay	417
	29/10/2021	Coffin Bay	
	26/10/2021	Smoky Bay	
	26/10/2021	Smoky Bay	
35	3/11/2021	Coffin Bay	417
36		Coffin Bay	417
37	4/11/2021	Smoky Bay	
38	4/11/2021	Smoky Bay	
39		Coffin Bay, Smoky Bay or Kangaroo Island	
40		Coffin Bay	
41	1/11/2021	Coffin Bay	
42	26/10/2021	Smoky Bay	
	26/10/2021	Smoky Bay	
43	3/11/2021	Coffin Bay	
44	4/11/2021	Smoky Bay	
45	10/11/2021	Smoky Bay	
46	8/11/2021	Coffin Bay	
	5/10/2021	Smoky Bay	
	9/11/2021	Coffin Bay	

Case	Harvest date (where known)	Growing area (where known)	MLST (clinical isolate)
	11/11/2021	Coffin Bay	
	11/11/2021	Coffin Bay	
47	6/11/2021	Coffin Bay	
48	1/11/2021	Streaky Bay	
49	19/10/2021	Coffin Bay	
50	3/11/2021	Smoky Bay	
51	5/11/2021	Coffin Bay	
52			
53	10/11/2021	Smoky Bay	
54	6/11/2021	Coffin Bay	

From the confirmed vibriosis cases in SA, only 23 out of the 76 cases (approximately 30%) were traced to a unique growing area and harvest date. Traceback was confounded in situations where patients had reportedly consumed oysters from multiple outlets during the onset period, or where co-mingling (the act of combining different batches of shellfish, which is not permitted under Standard 4.2.1 of the Australia New Zealand Food Standards Code) could not be excluded. In addition, some harvest data (harvest date, lease number) was missing or was not able to be obtained from within parts of the supply chain. Tracebacks to individual growers ceased when the compliance order was issued as the focus was on ensuring all recalled product was removed from market. Of those confirmed cases that could be traced, there was a range of harvest dates different various purchase locations and businesses implicated. The spread, coupled with the different MLST types, indicates that *V. parahaemolyticus* involved in the outbreak originated from the marine environment, and not from cross-contamination.

Environmental and climatological conditions in South Australia surrounding the outbreaks

Oceanography in southern Australia and the Great Australian Bight

The oceanography of the Great Australian Bight (GAB) is characterised by its unique geophysical features, diverse ecosystem and dynamic oceanographic processes. The area is defined by the wide continental shelf, deep oceanic waters, and the presence of a significant upwelling system, which brings nutrient-rich waters to the surface, and supports a rich biodiversity. This region is also influenced by the Southern Ocean's large-scale circulation patterns. Two major boundary currents (see Figure 7) play a crucial role. The warm, surfaced intensified, southward-flowing Leeuwin Current and the cooler, eastward-flowing, deeper, Flinders Current interact in complex way. The Leeuwin Current sweeps down Australia's west coast, from about the North West Cape (Exmouth, WA) and can extend into the GAB and as far as the southwest of Tasmania. Although the Leeuwin Current flows all year round, it exhibits a strong seasonality, with the stronger flows into the GAB occurring during winter, and weaker flows during the summer months (Feng *et al.*, 2003, GodfreyVaudrey and Hahn, 1986, Oke *et al.*, 2018). During late summer months (February-March), the warm waters of the south-west coast of Eyre Peninsula (from Baird Bay to western Kangaroo Island), are subjected to localised, seasonal, cold, nutrient-rich coastal upwellings (Middleton *et al.*, 2007, Richardson *et al.*, 2020). The Leeuwin Current along the West Australian Coast is also stronger in La Niña years (Feng *et al.*, 2013). The contribution of the Leeuwin Current on the total flow along the southern Australian coast diminishes toward the east. Off the eastern Great Australian Bight, the Leeuwin Current only drives approximately 15% of the total flow, while wind forces (approximately 47%) and a pressure gradient term (approximately 38%) become more important (Cirano and Middleton, 2004).

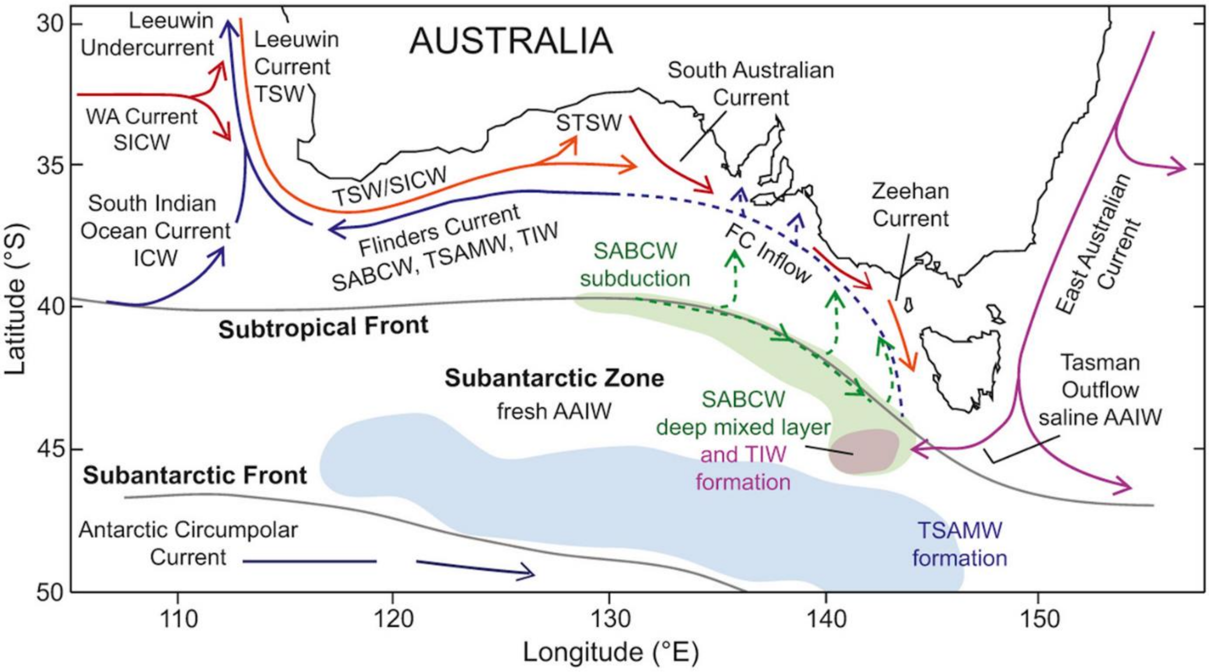

Figure 7: Major boundary currents influencing southern Australia. Surface currents - solid line, deeper currents - dashed line. Warm colours denote warmer, more saline water. AAIW: Antarctic Intermediate Water; FC: Flinders Current; ICW: Indian Central Water; SABCW: South Australian Basin Central Water; SICW: South Indian Central Water; STSW Subtropical Surface Water; TIW: Tasmanian Intermediate Water; TSAMW: Tasmanian Subantarctic Mode Water; TSW: Tropical Surface Water; WA Current: West Australian Current. Reproduced from Richardson *et al.* (2019).

The north-westward Flinders Current, affects the regional climate and marine ecosystems by transporting cool waters originated from the Southern Ocean, and contributes to the overall lower sea surface temperatures in the region (Duran *et al.*, 2020, Richardson *et al.*, 2019, Richardson *et al.*, 2020). Along the shelf, several water masses interact, creating, creating a complex and dynamic environment. The coastal upwelling leads to distinct variations in temperature, salinity, and nutrient levels from November to April. The cold water plumes are followed closely by high-chlorophyll concentrations (Nieblas *et al.*, 2009), particularly along the Bonney Coast, Kangaroo Island and the Eyre Peninsula (van RuthGanf and Ward, 2010, Kämpf, 2010). Along the sheltered waters of the SA Gulfs and Bays, including Coffin Bay, high water temperatures and strong evaporation are ubiquitous during summer. The development of high salinity (above 36PSU) is a common occurrence in the bays off Spencer Gulf, reaching as high as 43PSU in the northern sectors in Autumn (Nunes VazLennon and Bowers, 1990).

The sea surface temperatures (SST) at a given location may be influenced by the time of year, wind, currents and climate patterns. The NINO3.4 index[3] is one of several ENSO (El Niño–Southern Oscillation) indicators that is used by climatologists to provide an indication of the state of certain climate variables and climate drivers. The NINO3.4 index between 1993 and 2022 is shown in Figure 8, whilst the Australian Bureau of Meteorology's (BOM) monthly ENSO Outlook values are reported in Table 9. The BOM's National Climate Centre classifies a NINO3.4 temperature anomaly as "*warm*" if it exceeds +0.8°C and "*cool*" if it is less than -0.8°C (Bureau of Meteorology, 2021). The ENSO cycle loosely operates over timescales from one to eight years, and El Niño or La Niña phases typically last 9-12 months (Bureau of Meteorology, 2021). Different agencies have different criteria for when an El Niño or La Niña phase is considered active. The BOM will only declare an El Niño or La Niña phase when a minimum of three out of four criteria have been satisfied. These criteria are based on the SST anomaly within defined regions (NINO3 or NINO3.4) of the Pacific Ocean, trade wind strength in the western or central equatorial Pacific Ocean, the Southern

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[3] The NINO3.4 index is defined as the average SST anomalies over the central and eastern tropical Pacific Ocean (region 5°N – 5°S and 170°W – 120°W). The Australian Bureau of Meteorology's National Climate Centre uses the NINO3.4 index as it more closely related to Australian climate.

Oscillation Index (atmospheric pressure differences between Tahiti and Darwin) and climate model outputs. During the two South Australian *Vibrio* outbreaks in 2021/22 there was either an active La Niña episode or a La Niña Watch/Alert phase.

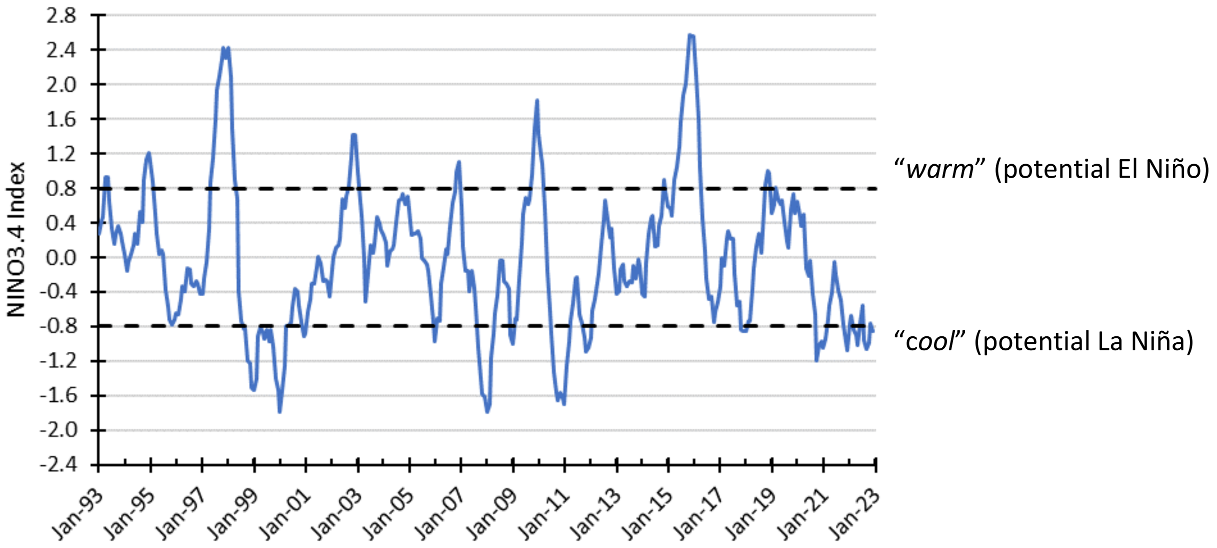
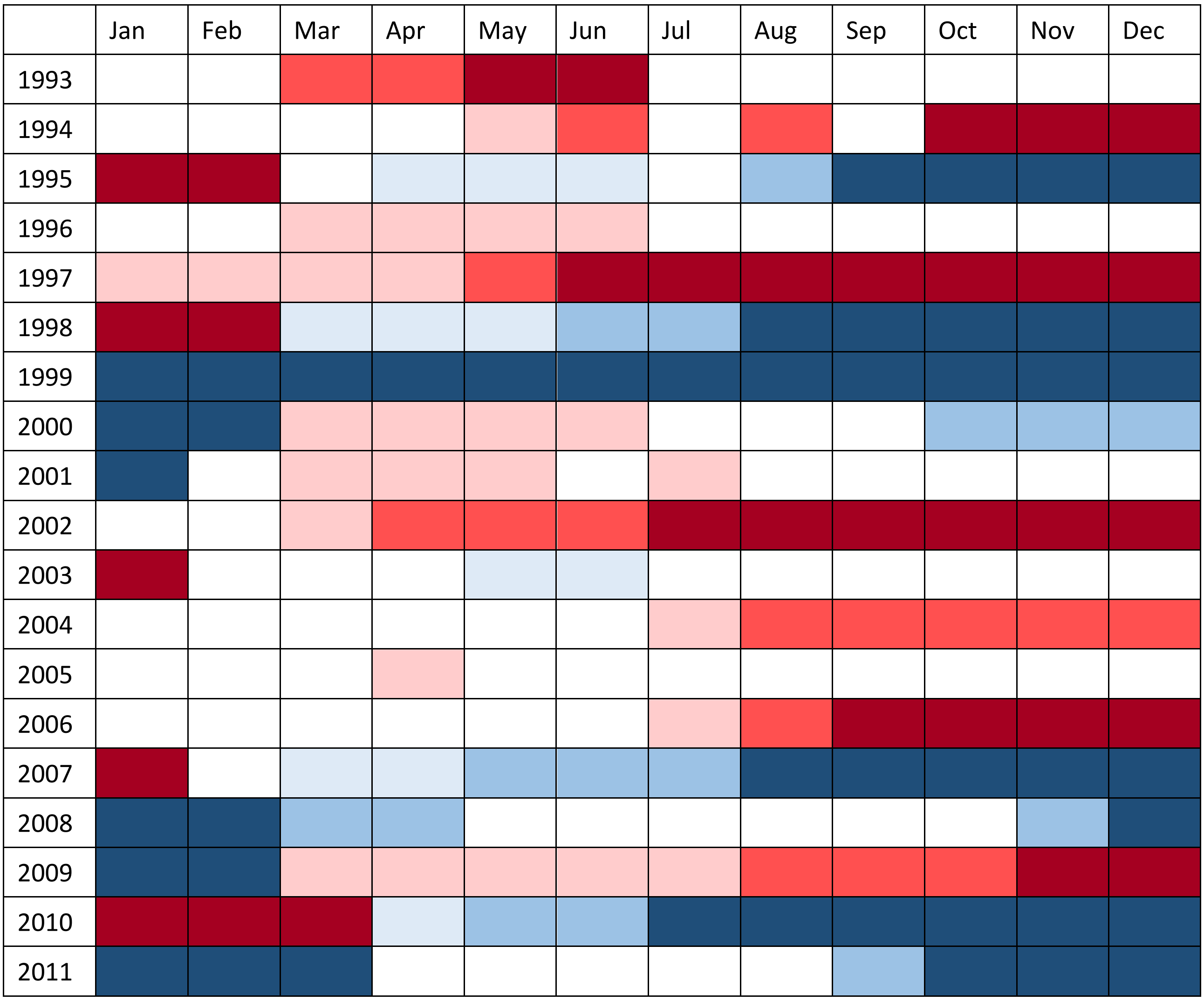
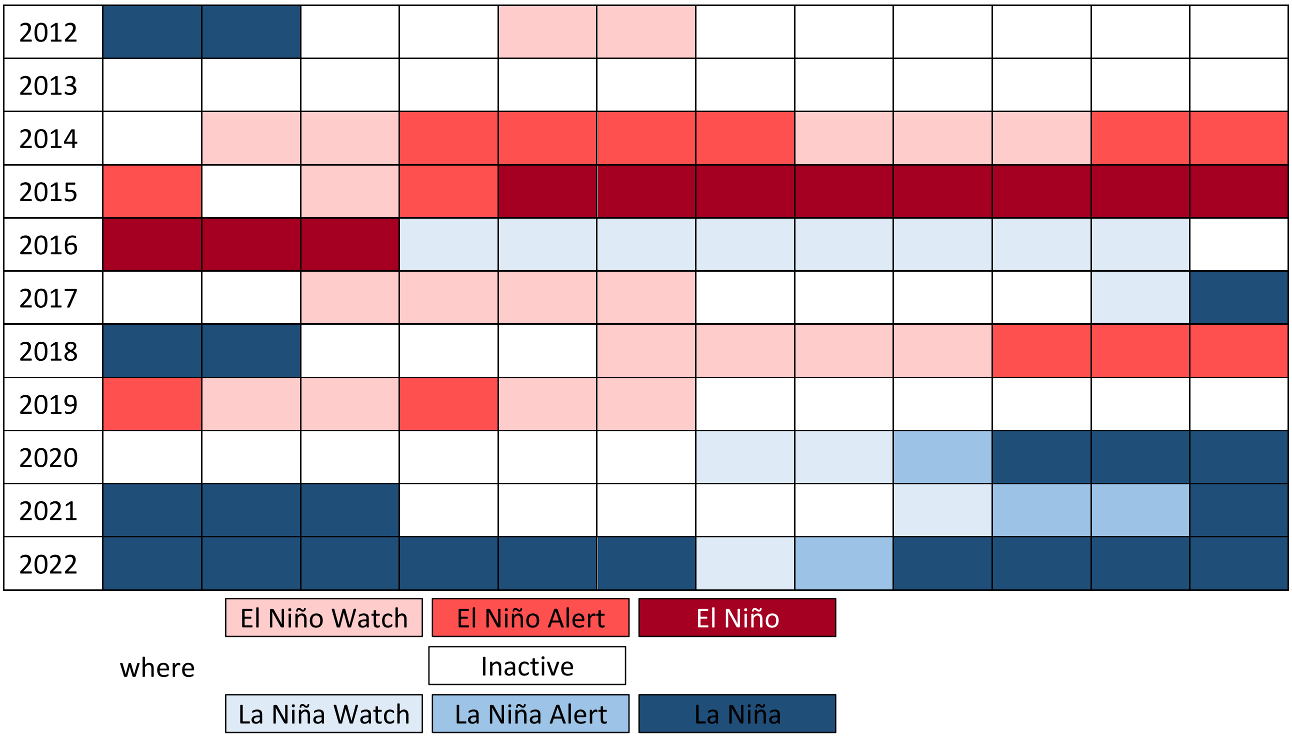


Figure 8: NINO3.4 Index from Jan 1993 until December 2022. Raw data obtained from https://psl.noaa.gov/gcos_wgsp/Timeseries/. Periods above/below the horizontal dashed lines at ±0.8 may correspond to BOM declared El Niño or La Niña phases.

Table 9: Monthly ENSO outlook values (shading of cells refer to La Niña and El Niño status based on the criteria specified by the BOM). Adapted from Bureau of Meteorology (2023).



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1993			🔴	🔴	🟥	🟥						
1994					🩷	🔴		🔴		🟥	🟥	🟥
1995	🟥	🟥		🩵	🩵	🩵		🔵	🟦	🟦	🟦	🟦
1996			🩷	🩷	🩷	🩷						
1997	🩷	🩷	🩷	🩷	🔴	🟥	🟥	🟥	🟥	🟥	🟥	🟥
1998	🟥	🟥	🩵	🩵	🩵	🔵	🔵	🟦	🟦	🟦	🟦	🟦
1999	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
2000	🟦	🟦	🩷	🩷	🩷	🩷				🔵	🔵	🔵
2001	🟦		🩷	🩷	🩷		🩷					
2002			🩷	🔴	🔴	🔴	🟥	🟥	🟥	🟥	🟥	🟥
2003	🟥				🩵	🩵						
2004							🩷	🔴	🔴	🔴	🔴	🔴
2005				🩷								
2006							🩷	🔴	🟥	🟥	🟥	🟥
2007	🟥		🩵	🩵	🔵	🔵	🔵	🟦	🟦	🟦	🟦	🟦
2008	🟦	🟦	🔵	🔵							🔵	🟦
2009	🟦	🟦	🩷	🩷	🩷	🩷	🩷	🔴	🔴	🔴	🟥	🟥
2010	🟥	🟥	🟥	🩵	🔵	🔵	🟦	🟦	🟦	🟦	🟦	🟦
2011	🟦	🟦	🟦						🔵	🟦	🟦	🟦



2012	●	●			░	░						
2013												
2014		░	░	▒	▒	▒	▒	░	░	░	▒	▒
2015	▒		░	▒	▓	▓	▓	▓	▓	▓	▓	▓
2016	▓	▓	▓	○	○	○	○	○	○	○	○	
2017			░	░	░	░					○	●
2018	●	●				░	░	░	░	▒	▒	▒
2019	▒	░	░	▒	░	░						
2020							○	○	◐	●	●	●
2021	●	●	●						○	◐	◐	●
2022	●	●	●	●	●	●	○	◐	●	●	●	●

░ El Niño Watch ▒ El Niño Alert ▓ El Niño

where ☐ Inactive

○ La Niña Watch ◐ La Niña Alert ● La Niña

The Australian Integrated Marine Observing System (IMOS) houses an archive of SST data from the United States National Oceanic and Atmospheric Administration (NOAA) Polar-Orbiting Environmental Satellites. The information that is publicly available varies by geographical region. Six-day SST composite and six-day SST anomaly composite images (one image per week centred around the specified date) for the Southern Australia region between January 2021 and February 2022 are shown in Appendix 4. From these images the 6-day composite SSTs in the oyster growing areas over the time periods when the *Vibrio* outbreaks occurred were generally above 15°C and in a range that would support the growth of *V. parahaemolyticus*.

The 6-day SST anomaly composites (shown in Appendix 4) were approximately 1.0-1.5°C above average for short periods of time around the middle of February 2021, late March through April, and again at the beginning of September 2021 but were at or below average for the remainder of the period. Some of these SST anomalies are also evident in Figure 9 which displays the monthly SST anomalies for the SA Gulfs region. The temperature anomalies from the SA Gulfs region shows that the monthly SST anomaly between March 2021 and May 2021 was above average, but the monthly SST anomalies during the two *Vibrio* outbreaks were generally at or below average. These multi-day composite images or monthly values do not consider instantaneous or shorter-term variability.

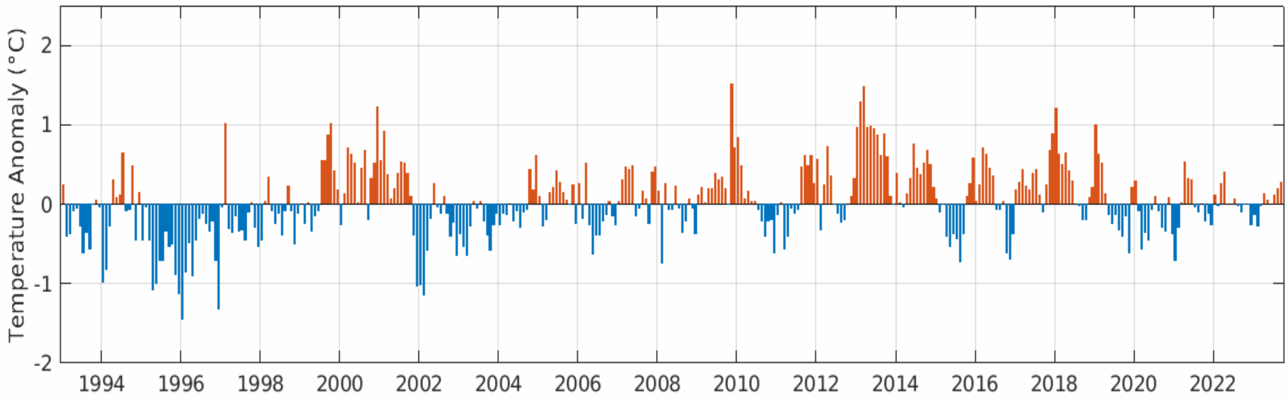
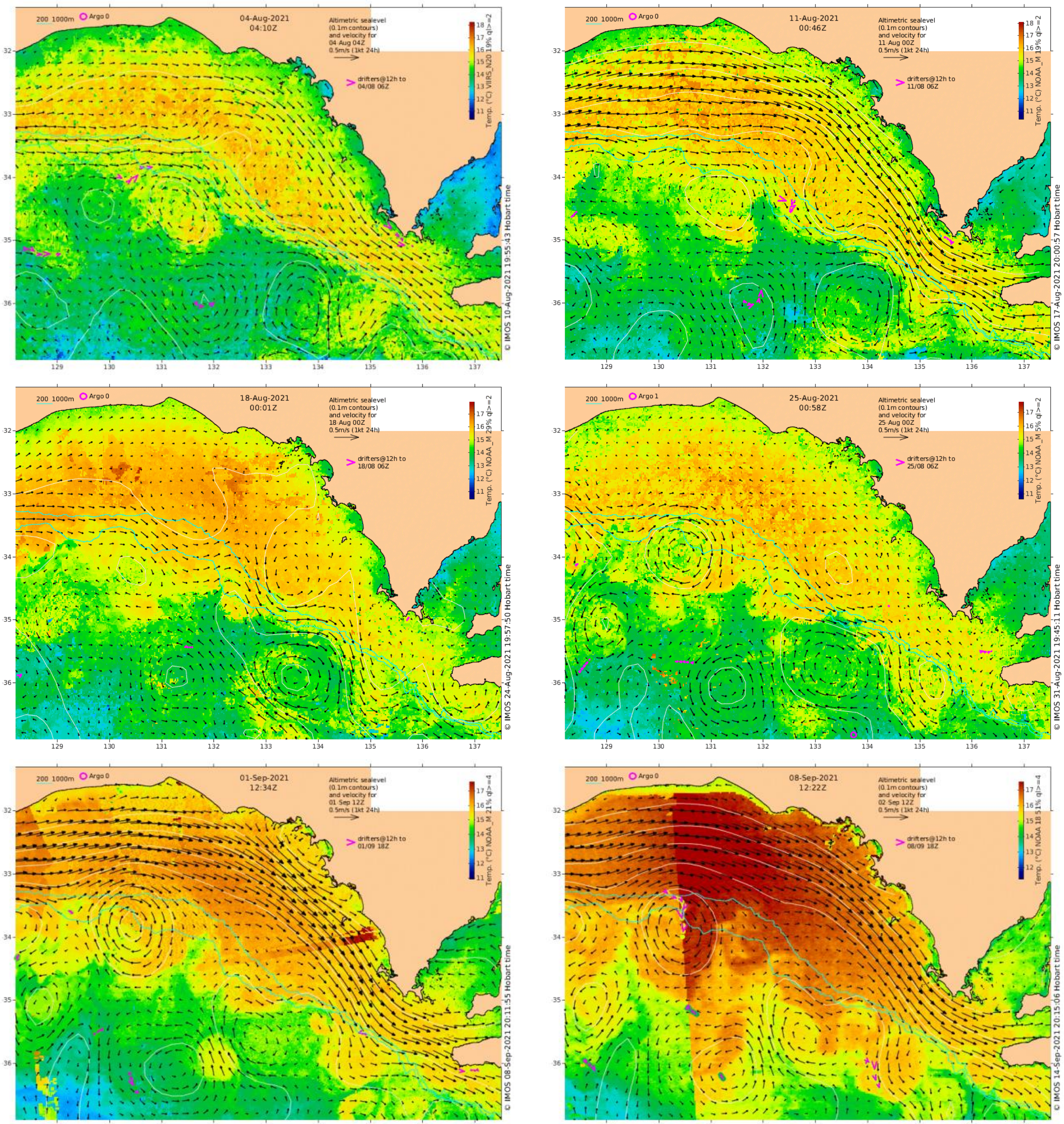
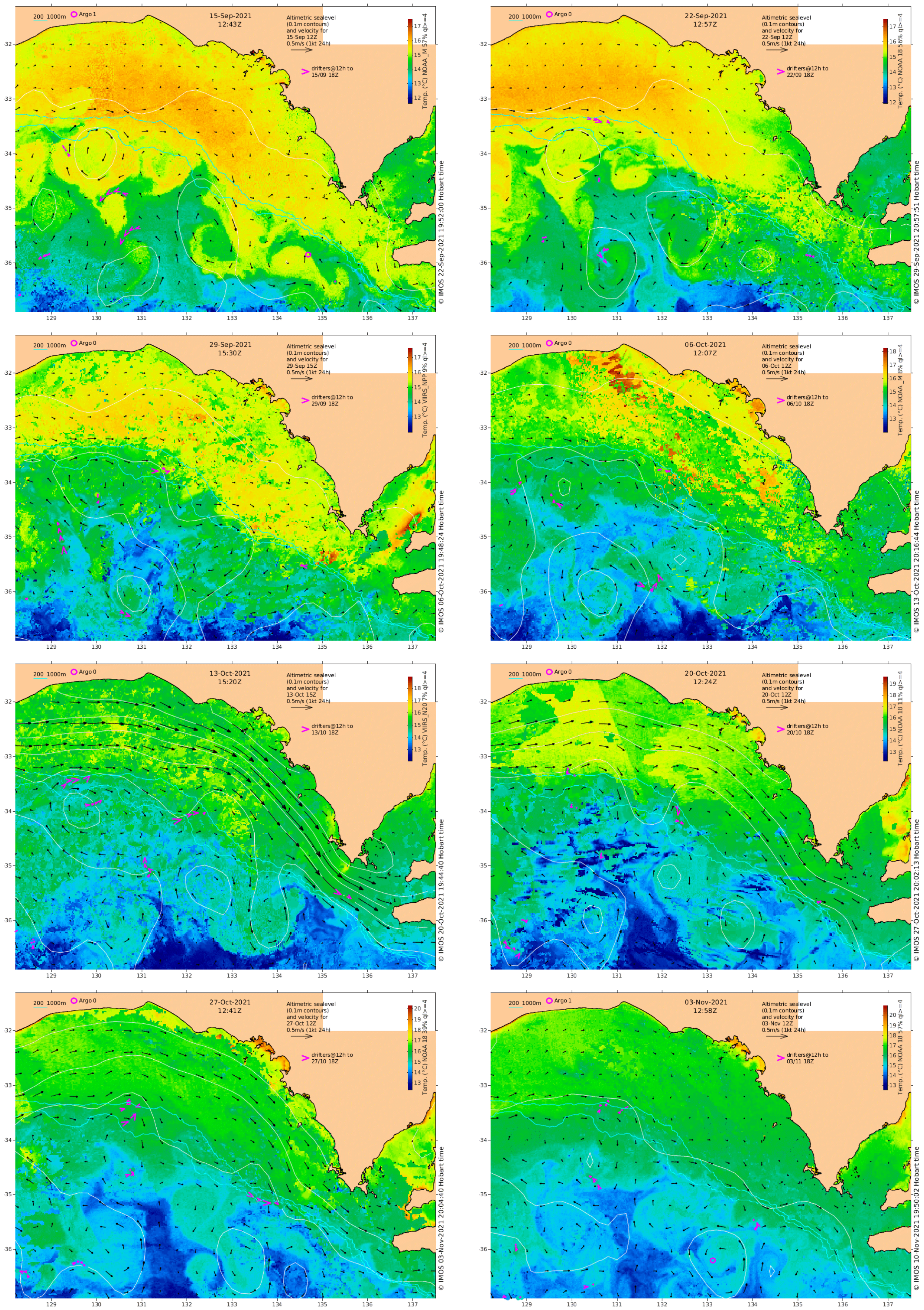


Figure 9: Monthly SST anomaly for the SA Gulfs region (Latitude: -40 to -34; Longitude: 134 to 141) between 1993 and 2022. Reproduced from IMOS (2023)

The IMOS-OceanCurrent website also provides a range of other data including snapshot SST. Snapshot SST can provide greater detail as there is no time-based averaging. Snapshot SST images for the Eastern Great Australian Bight (GAB) region (one image per week) between August 2021 and November 2021 are shown in Figure 10. However, Snapshot SST anomalies are not yet readily available for this region. Between August 2021 and November 2021 there were several short bursts/bands of warmer SST lasting 2-3 days that approached 17°C. These bands moved into the GAB in an easterly direction in early and mid-September 2021.


Snapshot SST for the Eastern GAB region

Snapshot SST for the Eastern GAB region



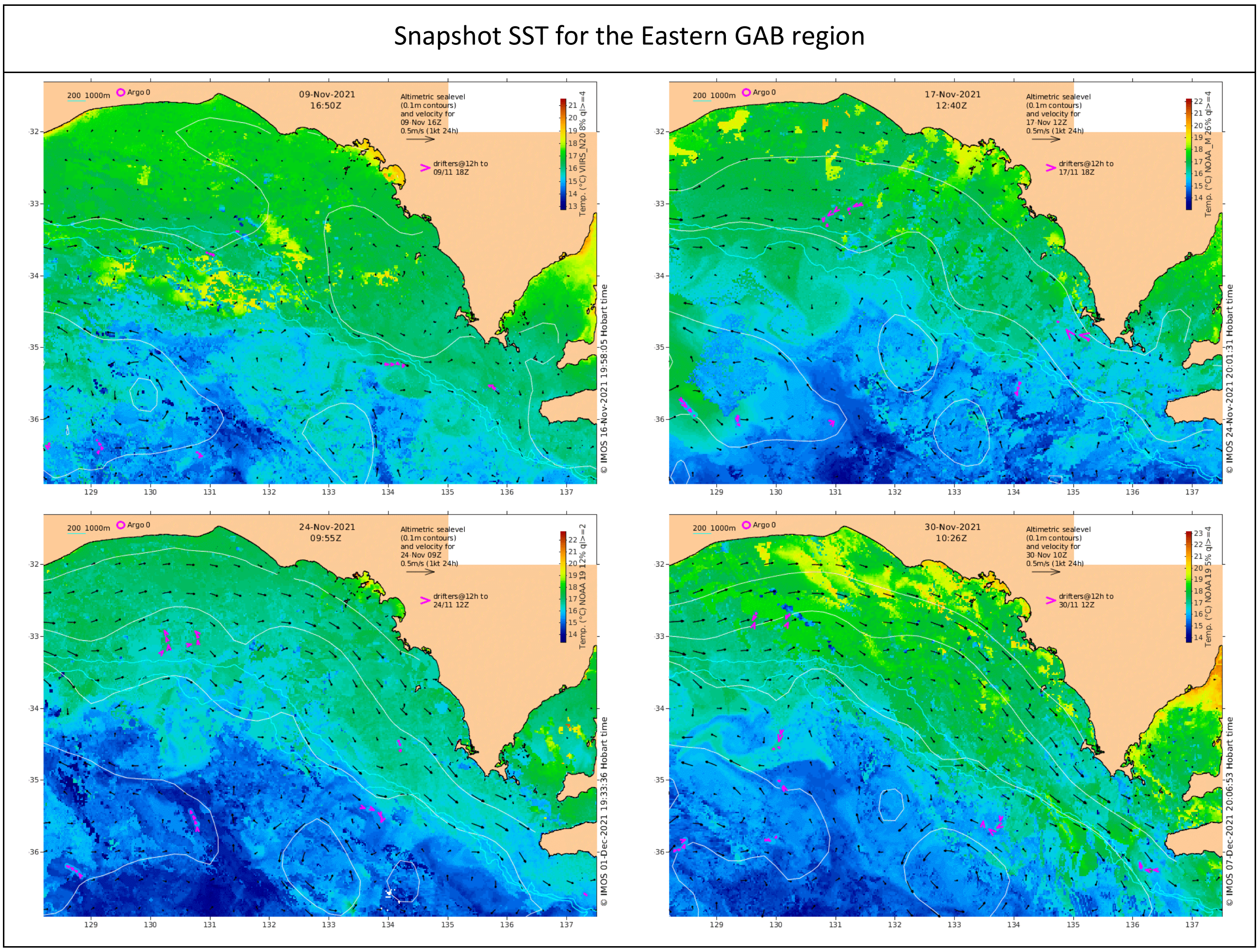


Figure 10: Instantaneous SST for the Eastern GAB region from August 2021 to November 2021. Reproduced from IMOS (2023). Note the colour scale varies from image to image.

Site specific SASQAP monitoring data

The South Australian Shellfish Quality Assurance Program (SASQAP) monitors the water quality in shellfish harvesting areas of the state and was established in 1994. The location of specific algal monitoring sites for Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay harvest areas are reported in Table 10 and shown in Appendix 5.

Table 10: Oyster growing regions and coordinates used to obtain representative measures of SST and SSChl-a from satellite observations.

Harvest Area	Latitude [DD]	Longitude [DD]
Franklin Harbour	-33.7519	136.9047
Port Douglas (Coffin Bay)	-34.5488	135.3783
Smoky Bay	-32.3758	133.8883
Streaky Bay	-32.7183	134.2013

In-situ sea surface temperatures (SST) and salinity levels collected during 2021 and 2022 and compared to a longer-term average from the specific algal monitoring sites are shown in Figure 11, whilst total phytoplankton counts are shown in Figure 12. Total phytoplankton counts do not correlate to productivity or the chlorophyll-*a* level in the water column due to size, nutritional differences and variability between phytoplankton species.

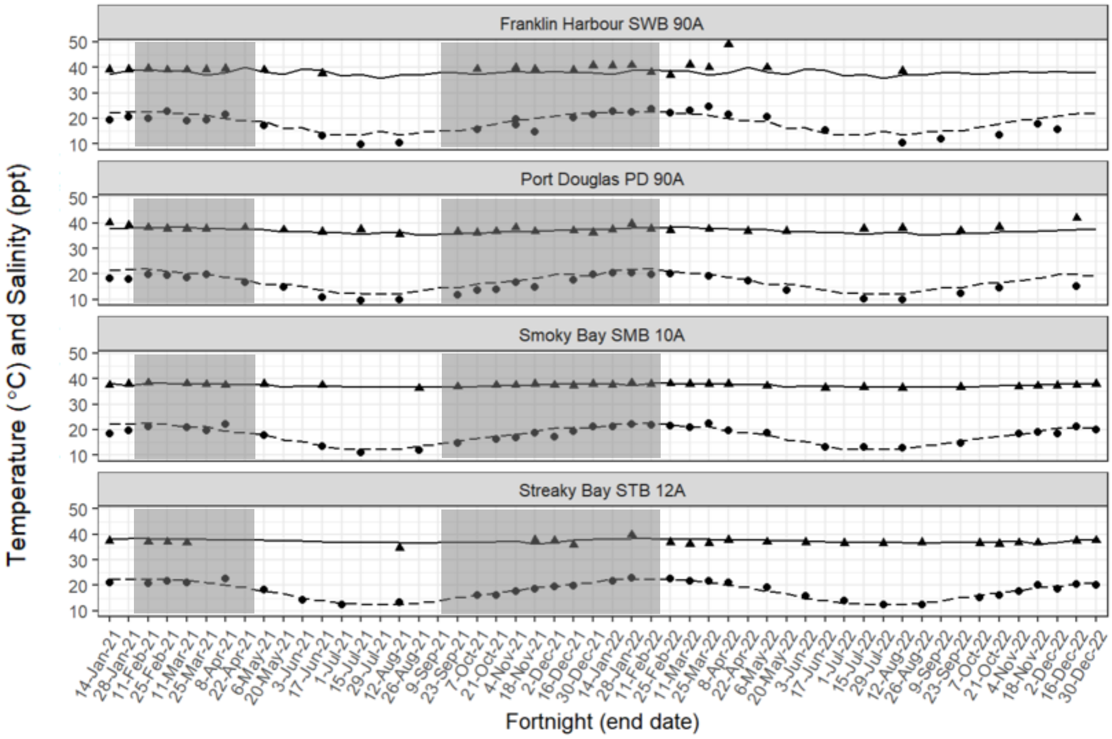


Figure 11: Sea surface temperature and salinity from specific SASQAP algal monitoring sites in Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay. Solid line is a 19-year salinity average (data from 2002 to 2020), dashed line is the 19-year SST average (data from 2002 to 2020), triangles are discrete salinity readings in 2021 and 2022, circles are discrete SST readings in 2021 and 2022. Water samples collected 1-5m below surface depending on location and date. Any recorded salinity values less than 34.0 ppt were excluded. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

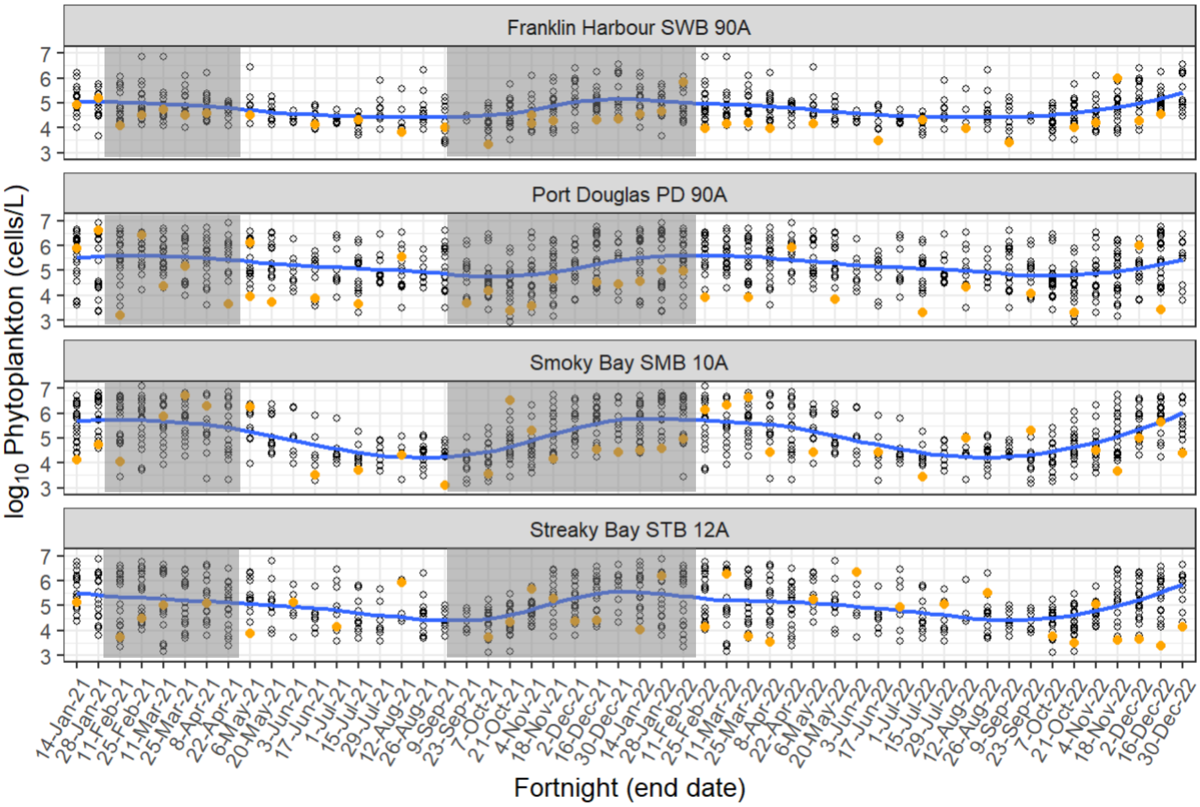


Figure 12: Phytoplankton levels from specific SASQAP algal monitoring sites in Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay. Solid line is a 19-year average total phytoplankton concentration (data from 2002 to 2020), hollow circles are individual data points from 2002 to 2020, coloured yellow circles are discrete total phytoplankton levels in 2021 and 2022. Water samples collected 1-5m below surface depending on location and date. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

The SASQAP monitoring data shows no significant anomalies in 2021 or 2022 (outbreak years). The recorded SST and total phytoplankton levels at these sites in 2021 and 2022 were generally below the 19-year historical average, whilst salinity (after removing the erroneous data points) was at or above the historical average. However, similar to the datasets above, the in-situ measurements for SST and salinity at these localised reference points were within a range that would be conducive to *Vibrio* growth during both outbreak periods.

Site specific remote sensing/satellite derived data

Satellites provide a remote sensing platform and can be used for long-term spatial and temporal monitoring of a range of environmental parameters, including SST, turbidity (via suspended particulate matter concentration, reflectance), chlorophyll-*a* (via ocean colour). Satellite-based measures provide one of the most extended available time series of marine environmental information covering South Australia's marine waters.

Australian Ocean Data Network

To assess how environmental changes may influence the presence of *Vibrio* species across South Australia's oyster-growing regions, SST and SSChl-*a* data provided by the Integrated Marine Observing System (IMOS) was obtained from the Australian Ocean Data Network (https://portal.aodn.org.au/).

The SST product from the Australian Ocean Data Network is a multi-sensor, quality-controlled, regional daily analysis of day-night infrared and microwave measurements provided in a 0.02° (~2.3km) resolution grid. The period was from July 1992 to December 2022, and the data represents the upper 10m of the water column, i.e., it is the *foundation or "bulk"* SST (see Beggs *et al.* (2011) GovekarGriffin and Beggs (2022) for a detailed description). The nearest SST pixel to each of SASQAP algal monitoring sites (see Table 10) within Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay was selected. Discrete daily data points (at weekly intervals) for 2021 and 2022 were overlayed on longer-term monthly climatology values. The localised SST from these point locations in Franklin Harbour, Port Douglas (Coffin

Bay), Smoky Bay and Streaky Bay are shown in Figure 13. The SST data points provided by IMOS were comparable to the in-situ SST data collected by the SASQAP monitoring program and have helped corroborate the lack of significant SST anomalies during the two *Vibrio* outbreaks.

The SSChl-*a* product used was based on a single instrument (MODIS – moderate resolution imaging spectroradiometer) onboard the Aqua satellite, estimated daily through three different empirical algorithms, namely:

- Ocean Color Index (OCI) (Australian Ocean Data Network, HuLee and Franz, 2012)
- Ocean Color 3 (OC3) (Australian Ocean Data Network, O'Reilly and Werdell, 2019), and
- Garver-Siegel-Maritorena (GSM) (Australian Ocean Data Network, MaritorenaSiegel and Peterson, 2002).

These empirical algorithms use surface reflectance colour differences and ratios to estimate the chlorophyll-*a* content near the ocean surface. Regionally, the OC3 provided the most reliable estimates without local adjustments in deeper water with reduced performance within the Gulfs, particularly in shallow water (Rodriguez *et al.*, 2018). Given that most algorithms are usually suited for deeper regions (i.e. beyond 30m) and oyster sites are in shallow water (less than 10m), attempts were made to reduce uncertainties in algorithm outputs by extracting and processing the SSChl-*a* related data using different estimates. For each site, three kinds of estimates (‘*point*’, ‘*box’* and ‘*deep_box*’) were considered:

- *point*: the pixel nearest the SASQAP algal monitoring reference sites (Table 10)
- *box*: all pixels within a 40km bounding box surrounding the point.
- *box_deep*: within the respective *box* only pixels within the water and beyond the 15m isobath were used. The bathymetry and land mask was estimated from WilsonSpinoccia and Buchanan (2012).

A log transformation to the data was applied and computed the daily medians for area estimates (*box/box_deep*) and the remainder estimates (monthly, seasonal cycle, anomalies). Given the highly irregular nature of SSChl-*a* in the time domain (e.g. cloud cover), the seasonal cycle was estimated via the Lomb-Scargle periodogram (Lomb, 1976, VanderPlas, 2018), fitting a daily series to a mean signal with a period close to a year. Hence, for each region, three kinds of site estimates (*point, box, and box_deep*) for SSChl-*a* through three kinds of algorithms at four levels (daily, monthly, seasonal cycle, and anomalies) were evaluated by comparing seasonal trends to the total phytoplankton concentrations from the SASQAP datasets. Whilst no single algorithm’s output followed the seasonal total phytoplankton concentrations for all four regions (data not shown), the OC3_*box_deep* estimates was the closest match overall[4]. The *box_deep* based on the OC3 algorithm was subsequently used and referred to therein simply as SSChl-*a*. The SSChl-*a* values in Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay are shown in Figure 14.

Other satellite services

There are several commercial data service providers, one such provider is Umitron Pulse (https://www.pulse.umitron.com/). Umitron Pulse is a web-based service designed for aquaculture producers to monitor changing water conditions and can provide up to 30 years of historical data depending on payment plan. Umitron Pulse sources raw dataset from Copernicus[5]. Localised climatological SST and salinity from Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay regions are shown in Figure 15. Like the other data sources (reported above), the SST during both outbreak periods showed no significant anomalies.

Satellite-based data considerations

Satellite-based measurements including SST, salinity and chlorophyll are subject to several limitations which constrain their application to environmental studies (GovekarGriffin and Beggs, 2022, O'Reilly and Werdell, 2019, Rodriguez *et al.*, 2018, Soja-Woźniak *et al.*, 2017, Werdell *et al.*, 2018), with gaps due to

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[4] It is noted that chlorophyll-*a* content provides a different metrics to total phytoplankton but was used in the absence of other in-situ data.

[5] Copernicus is the Earth Observing component of the European Union’s space programme.

cloud cover and uncertainty due to atmospheric corrections the most common limiting factors. Hence, measurements over multiple grid points and extended periods (several days to months) are usually required to reduce uncertainties at the expense of time resolution and dynamical range. Nonetheless, bias detection and reduction in the output uncertainties and errors are improving, particularly with new sensors (Ibrahim *et al.*, 2019). For shallow coastal areas, such as the regions analysed in this study, high-frequency variations in temperature and chlorophyll-*a* due to tides, diurnal changes, and light availability are likely not wholly captured. In particular, SSChl-*a* measures in shallow waters (i.e. less than 30m deep) are likely overestimated by bottom reflectance and other local inherent optical properties, such as induced changes by sediments and dissolved organic matter. Any empirical relationship should be validated against systematic in-situ observations.

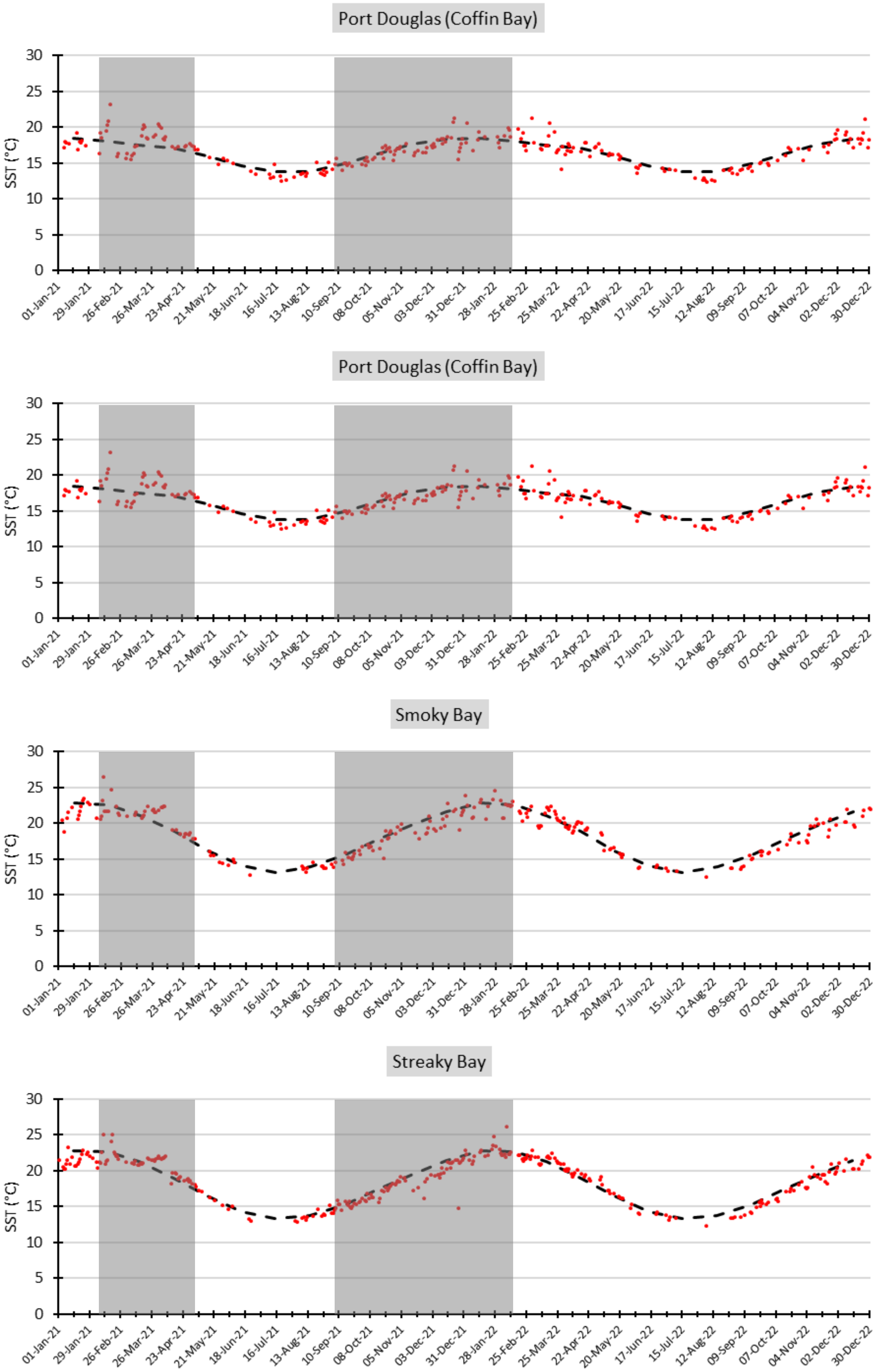

Figure 13: Monthly average sea surface temperature from Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay algal monitoring sites from IMOS data. Dashed black line is the climatological average temperature (data range from 1992 to 2020), coloured red circles are discrete daily SST data points from 2021 and 2022. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

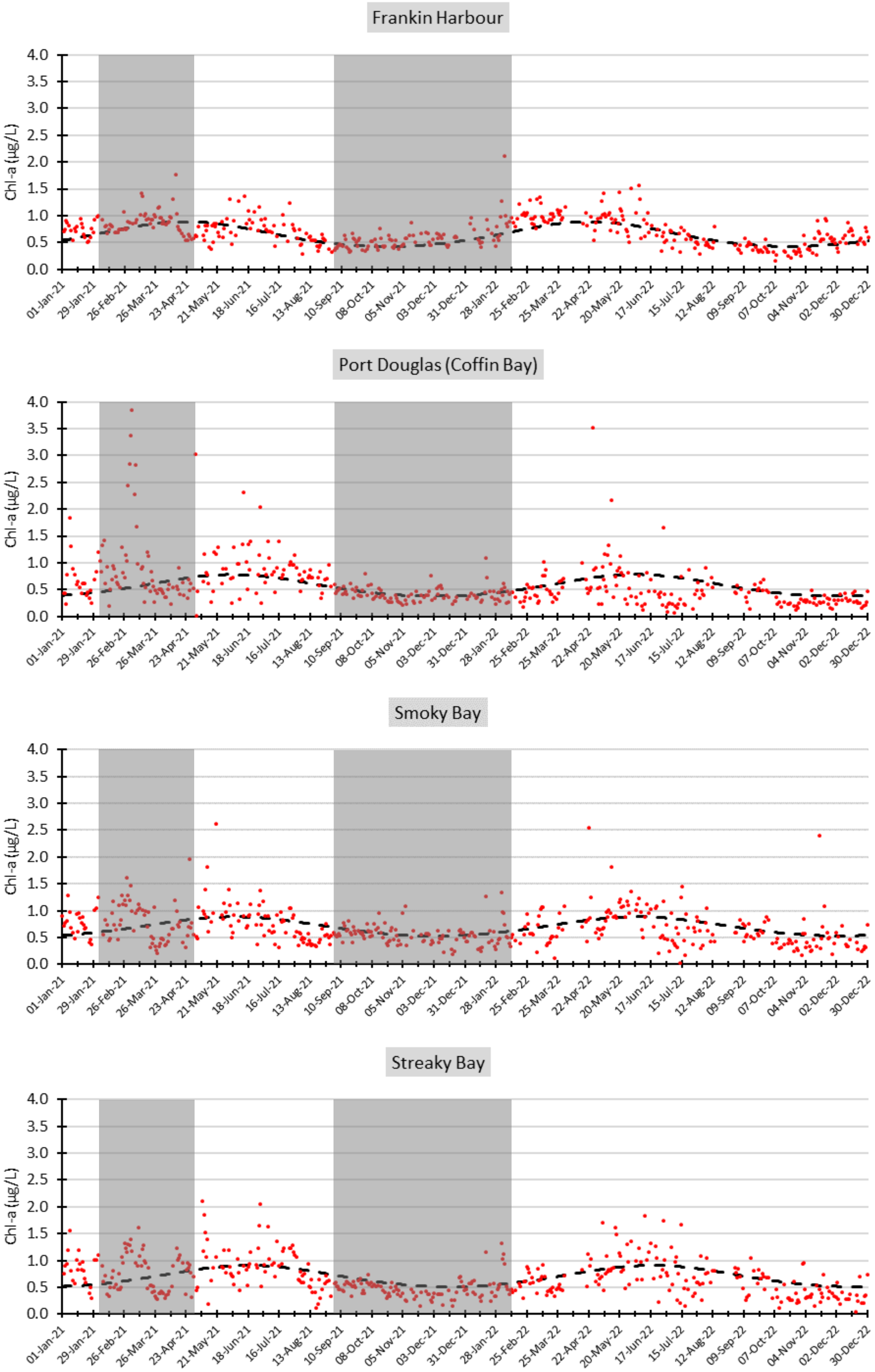

Figure 14: Sea surface chlorophyll-a values from Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay. Dashed black line is the seasonal SSChl-a values (data range from 1992 to 2020), red circles are discrete daily SSChl-a data points from 2021 and 2022. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

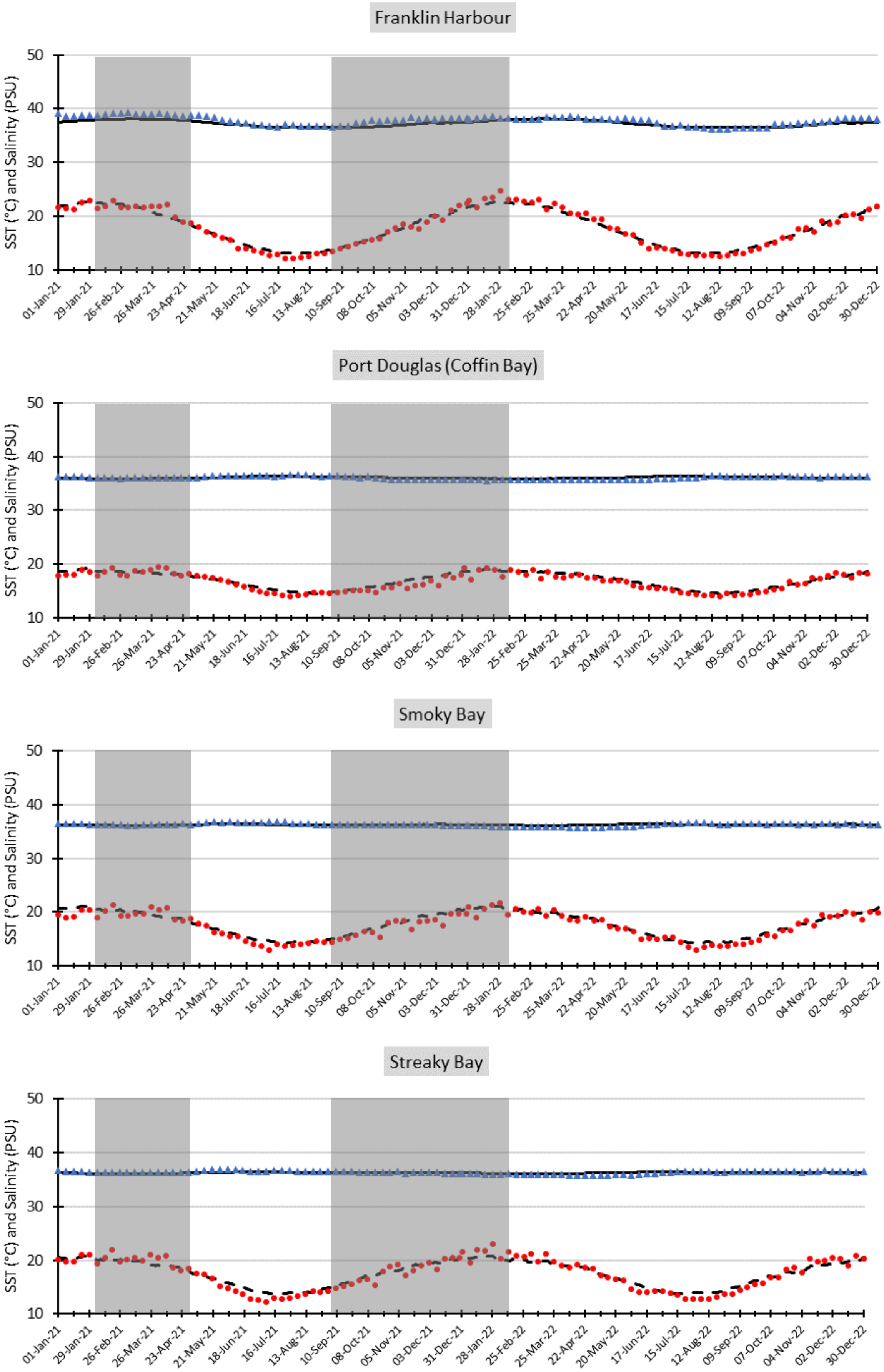

Figure 15: Umitron Pulse’s satellite based sea surface temperature and salinity from specific SASQAP algal monitoring sites in Franklin Harbour, Port Douglas (Coffin Bay), Smoky Bay and Streaky Bay. Solid line is a 20-year salinity average (data from 2001 to 2020), dashed line is the 20-year SST average (data from 2001 to 2020), coloured blue triangles are discrete salinity readings in 2021 and 2022, coloured red circles are discrete SST readings in 2021 and 2022. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

Site specific oyster basket temperatures

Temperature data from commercial oyster baskets were provided by Australian Seafood Industries and Cameron of Tasmania. Both companies had loggers attached inside baskets that recorded the surrounding temperature (seawater when the basket/oyster is submerged or air temperatures when the basket/oyster is out of the water, i.e. at low tides) at regular intervals. The temperature traces from these loggers are shown in Figure 16. Whilst average SST were between 15-25°C during the outbreak periods, there were frequent short-duration high (35-40°C) and low (8-10°C) temperature '*spikes*' which would likely correspond to when the baskets are positioned out of the water due to physical handling or tidal movements. The high/low temperature spikes are well above/below the SST data that was sourced from SASQAP and IMOS monitoring programmes but would be more representative of what the oysters experience. The basket temperature data helps to demonstrate the importance of harvesting oysters before, or as soon as possible after, oysters are exposed by receding tides. Once baskets/oysters are out of water and exposed to ambient air conditions the temperature of the oysters can rapidly increase which would facilitate faster *Vibrio* growth.

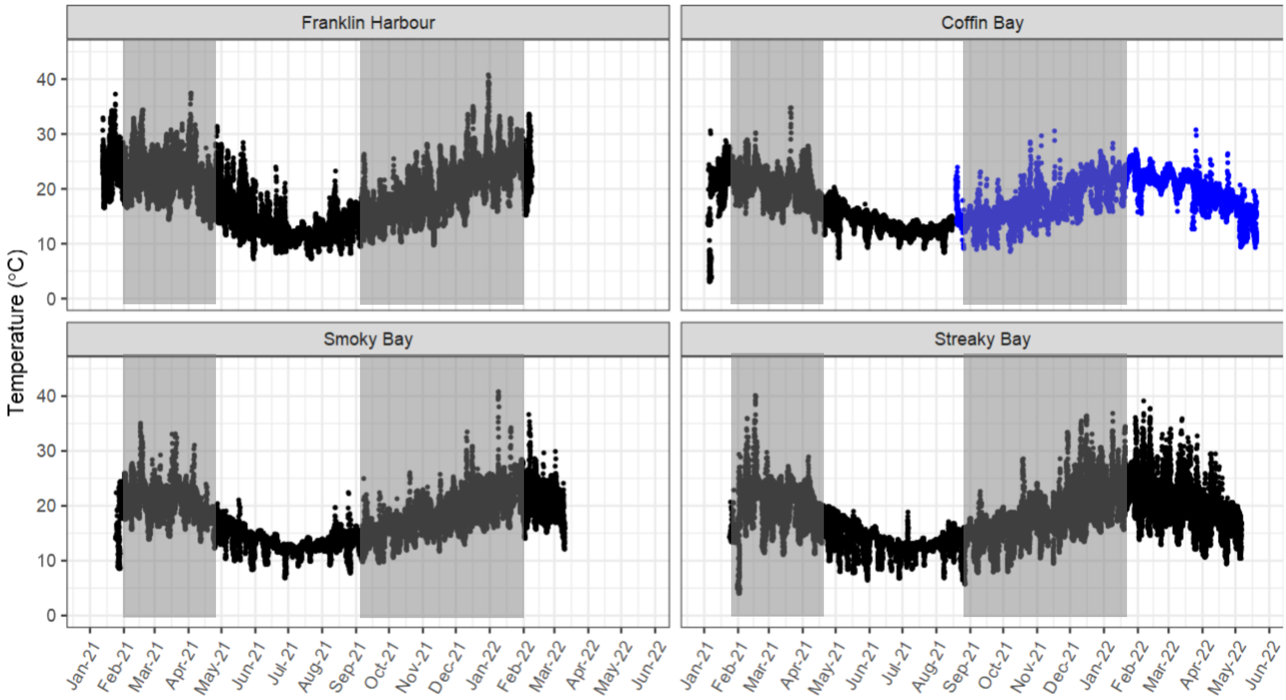

Figure 16: Temperature logs from oyster baskets from leases within Franklin Harbour, Coffin Bay, Smoky Bay and Streaky Bay harvest areas. Raw data provided from Australian Seafood Industries (black circles) and Cameron of Tasmania Pty Ltd (blue circles). For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

Bureau of Meteorology daily weather observations

The Bureau of Meteorology (BOM) monitors and records weather observations for a number of locations on the Eyre Peninsula. Not all weather stations monitor and record the same information. Weather stations of closest proximity to the implicated harvest locations include the following:

- Smoky Bay (Station No. 18077); Lat 32.38°S; Long 133.94 °E
- Streaky Bay (Station No. 18079)[6]; Lat 32.81°S; Long 134.20°E
- Cleve Aerodrome (Station No. 18116)[6]; Lat 33.71°S; Long 136.50°E
- Coffin Bay (Point Avoid) (Station No. 18230)[6]; Lat 34.68°S; Long 135.34°E
- Cowell (Station No. 18022; Lat 33.68°S; Long 136.91°E.
- Ceduna Amo (Station No. 18012)[6]; Lat 32.13°S; Long 133.70°E

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[6] Weather station includes monitoring wind speed and wind direction.

Minimum and maximum air temperatures, daily rainfall and daily wind speed and direction from 1 January 2021 through to 31 December 2022 at Cleve Aerodrome (proximity to Cowell/Franklin Harbour), Coffin Bay, Streaky Bay and Ceduna (proximity to Smoky Bay) are shown in Figures 17 and 18, respectively. Whilst there were no significant rainfall events prior to the outbreak, maximum ambient air temperatures were generally above 15°C. The only significant rain event in the region during the outbreak periods occurred towards the end of the second outbreak (21-24 January 2022) where a total of between 20-85mm was recorded.

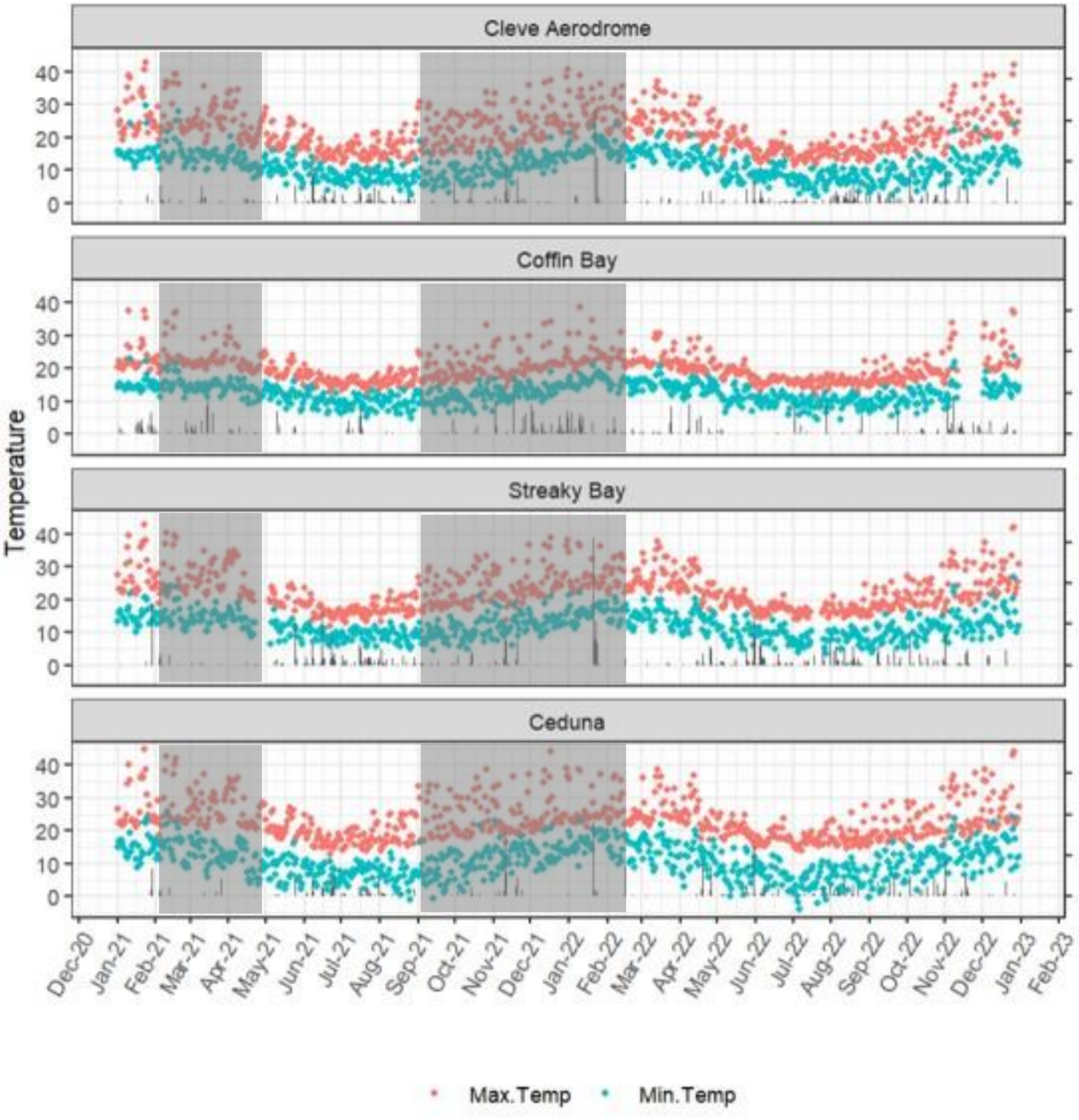


Figure 17: Daily minimum and maximum air temperature and rainfall from Cleve Aerodrome (Station No. 18116), Coffin Bay (Station No. 18230), Streaky Bay (Station No. 18079) and Ceduna Amo (Station No. 18012) weather stations. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

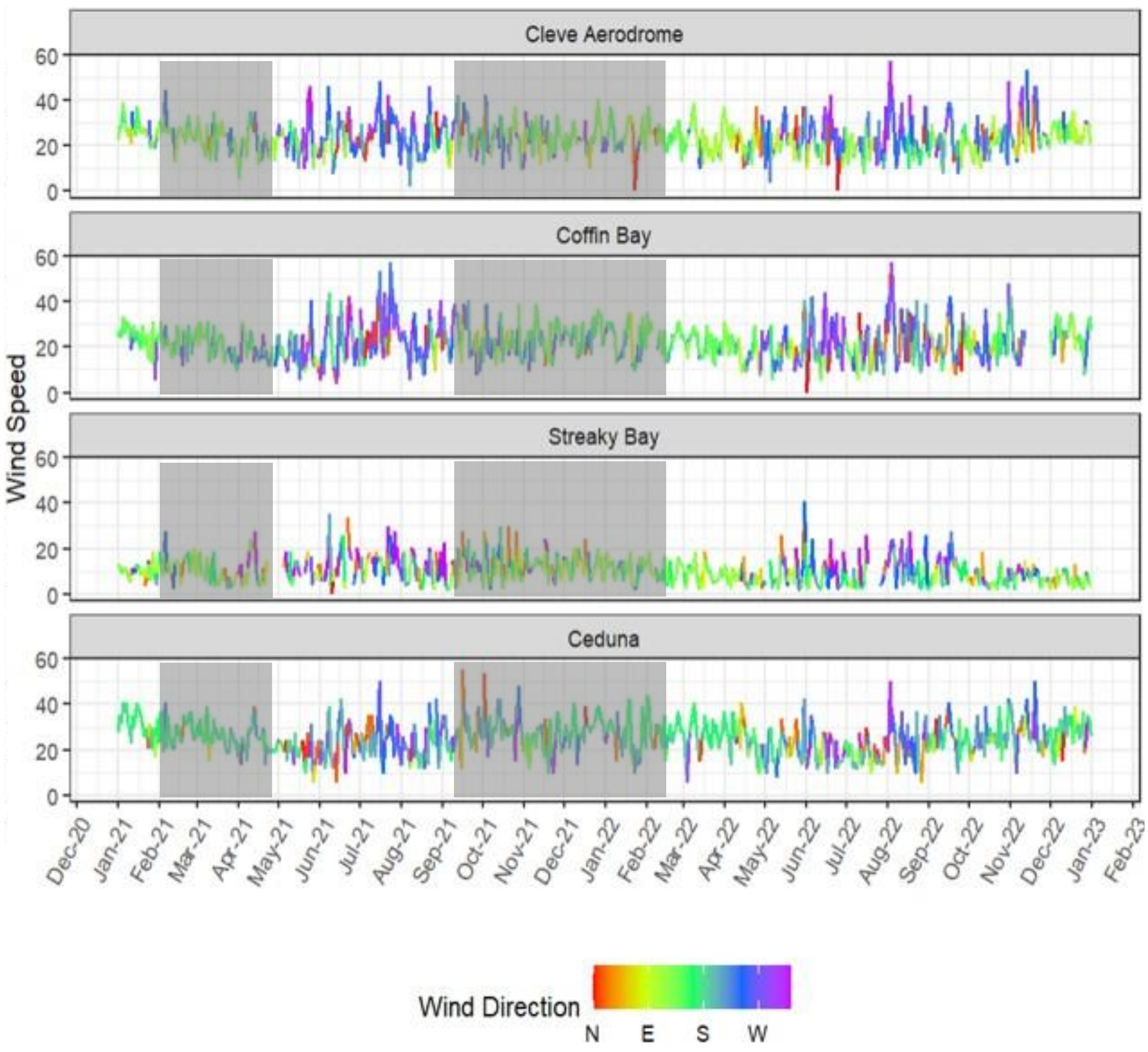


Figure 18: Daily wind speed/direction from Cleve Aerodrome (Station No. 18116), Coffin Bay (Station No. 18230), Streaky Bay (Station No. 18079) and Ceduna Amo (Station No. 18012) weather stations. Wind speed recorded at 3pm for all locations except for Streaky Bay where only 9am was available. For comparative purposes the period of illness notifications have been shaded grey for all sites and do not infer *Vibrio* cases at these sites.

Post-harvest supply chains

The supply network of Pacific Oysters produced in Australia (see Figure 19) is quite complex, often with multiple entities involved throughout the processing and wholesale segments. There is limited published information on temperature profiling of Pacific Oyster supply chains from South Australia. However, in a study by Madigan (2008), 50% (11 out of 22) of Pacific Oyster supply chains investigated from South Australia were non-compliant against the state Shellfish Quality Assurance Program, typically due to slow cooling rates following harvest. Whilst more recent/current compliance rates are unknown, all major commercial freight companies that collect oysters from South Australia growers utilise some form of temperature monitoring within their refrigerated freight compartments. Some oyster growers also utilise temperature loggers on individual consignments. These temperature loggers used by growers are typically at the pallet level. Freight companies have an ‘oyster run’ collection consignments from growers across the bays, and those oysters collected at the start of the ‘run’ typically had a shorter time-period to get down to temperature. Refrigerated truck compartments are often set at around 4°C and are designed to maintain product temperature and not to cool product temperature down to its carriage temperature. However, some refrigeration units are capable of pulling down the temperature. Oysters are typically collected from growers and transported to Adelaide-based depots overnight. Some consignments are then reloaded onto interstate routes, where it can take in the order of an additional 24 hours for product to reach Melbourne or Sydney and 36 hours to Brisbane or Perth depots. Other destinations require further transfers with unloading and storage in chillers, prior to being reloaded and transported to their final destinations.

Following the second *Vibrio* outbreak, all accredited oyster bivalve mollusc producers in South Australia that are harvesting for human consumption have been required to validate the initial cooling phase of oyster muscle temperature and demonstrate compliance with the time and temperature requirements. It is also recommended that operators determine the chiller's capacity and the refrigeration unit's capacity to reduce the oyster temperature to the specified value within the specified time (Department of Primary Industries and Regions (PIRSA), 2023a). Freight companies are now also required to check the temperature of consignments and ensure it is less than 10°C prior to loading. They often initially use an infrared thermometer and if in doubt then use a temperature probe to measure the muscle temperature. If the muscle temperature is not below 10°C they will not accept the consignment.

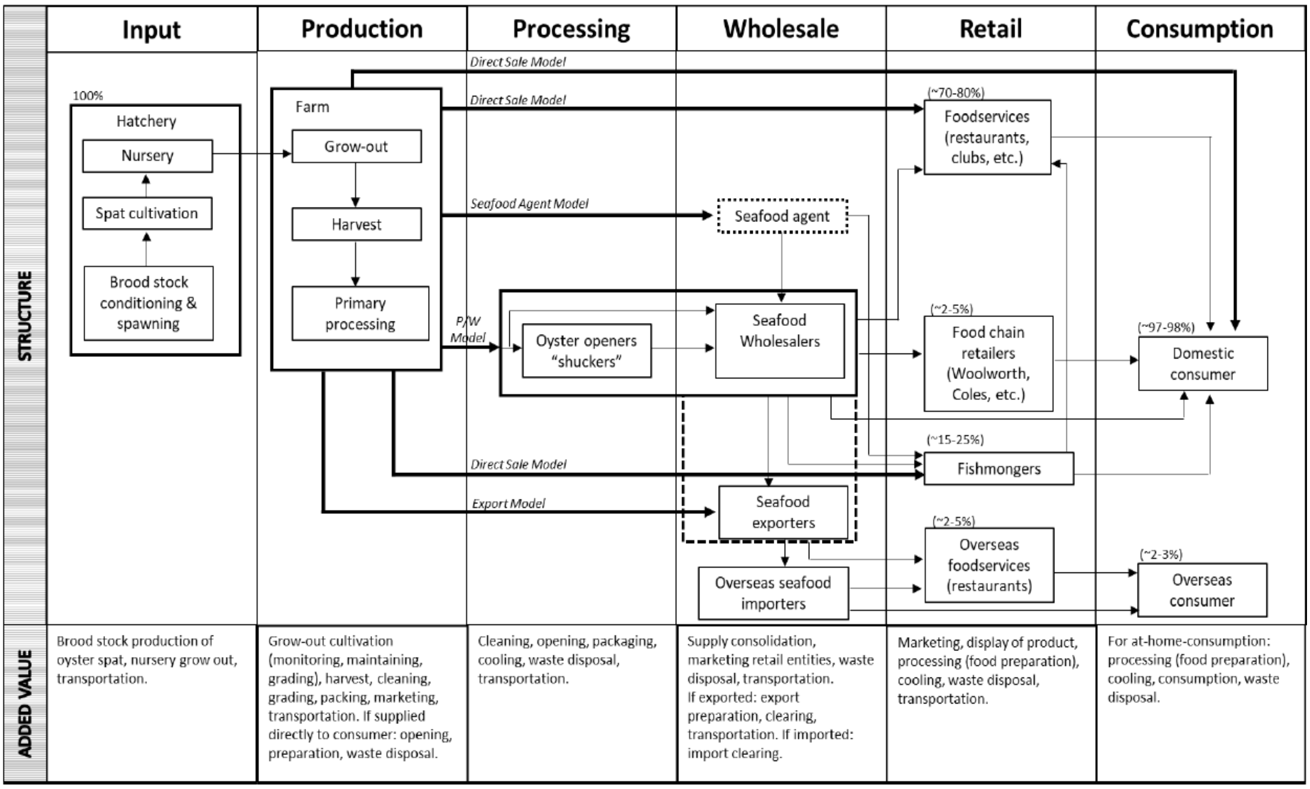


Figure 19: Generic Pacific Oyster supply chain network. Black boxes around multiple entities within the supply chain indicate integration of processes or entities. Black dotted box indicates a broker, no physical flow through this entity. 'P/W' for processor/wholesale model. Reproduced from Schrobback et al. (2020).

Comparison of collated environmental and climatological data and risk factors

Table 11 compares potential risk factors against the collated environmental and climatological data surrounding the February 2021 and September 2021 *Vibrio* outbreaks. During the two outbreaks there was either an active La Niña episode or a La Niña Watch/Alert phase. The collated data was from a combination of in-situ monitoring and remote surveillance with various spatial and temporal dimensions. The growing areas cover a large geographical range and in-situ data did not exist or was not readily available from impacted leases.

Table 11: Comparison of *V. parahaemolyticus* risk factors and the collated environmental data.

Risk factors	Collated data summary
Sea-surface temperature (SST)	SST during the outbreak periods were identified from remote monitoring outputs and the SASQAP monitoring program. SSTs were between 15-25°C and in the range conducive to growth of *V. parahaemolyticus*. SST anomalies during the two outbreaks were at or below average. However, there is potential for more localised variations of SST in shallow coastal leases due to effects of the local geographical features, tides, currents and solar radiation.
Oyster basket temperature	The collation of oyster basket temperature data was limited, but highlights that the actual temperatures experienced by oysters on lease are highly variable. Temperature spikes (35-40°C) frequently occur as the baskets (loggers and oysters) were exposed to ambient conditions. These temperature spikes would facilitate faster *Vibrio* growth and are likely to be a strong contributing factor in the outbreak.
Salinity and rainfall	In South Australia, oysters are grown in high salinity environments (>35PSU) with no significant freshwater in-flows. There were no significant salinity anomalies based on SASQAP algal monitoring sites, with salinity at or above the historical average. There were also no significant rain events in the days/weeks leading up to either outbreak. The only significant rain event during either outbreak occurred towards the end of the second outbreak.
Chlorophyll/phytoplankton	Total phytoplankton levels were obtained from the SASQAP monitoring program at reference sites and were generally at or below the seasonal average. Chlorophyll-*a* levels were estimated through various empirical algorithms, with estimated values lower than internationally published studies.
Turbidity	Turbidity on leases was unknown – no data sources could be identified. Wind speed, wind direction and farming practices can influence turbidity by resuspending sediments and sediment-residing bacteria into the water column. Turbidity can also be used as an indicator of water quality, nutrient loadings and plankton concentrations.
pH	pH values unknown – no data sources could be identified. Vibrios tolerate a wide range of pH levels. pH levels are more stable in open oceanic waters, but there can be more pronounced shifts in estuarine, near-shore and in coastal upwelling locations.
Dissolved oxygen	Dissolved oxygen levels unknown – no data sources could be identified. However, vibrios are facultative anaerobes and oxygen levels are unlikely to play a direct role in the abundance of *V. parahaemolyticus*.
Production and harvest practices	Oysters produced in South Australia are grown in intertidal and subtidal waters, on various production systems that are suited to the environmental conditions within a particular growing area and growers' operational preference. Bivalve molluscs licence holders must implement and maintain an approved Food Safety Arrangement with PIRSA. There were no known changes in production or harvest practices in the leadup to the outbreaks.
Post-harvest supply chains	No known changes in post-harvest supply chain practices in the leadup to the outbreaks. The ability to trace (rapidly trace) implicated oysters back to harvest leases was hampered due to complexity of the supply chains, multiple exposure to oysters during case onset periods, suspected co-mingling of product, and inaccurate or incomplete labelling of product.

Vibrio control measures in South Australia

The Australian Shellfish Quality Assurance Program Operations Manual (ASQAAC, 2022) is a national guideline for managing risks in the harvesting, relaying, depuration and wet storage of shellfish. It stipulates that when a suspected shellfish-borne illness outbreak is reported, appropriate authorities investigate the proposed epidemiological link. If an aetiologically confirmed outbreak is demonstrated to implicate a shellfish harvest area, then the harvest area should be promptly closed. At the time of the outbreaks, some control measures to minimise the proliferation of human pathogens (including *Vibrio*) in bivalve molluscs produced in South Australia were already required under existing Food Safety Arrangements. Since the two *Vibrio* outbreaks the control measures in South Australia have been strengthened to minimise the risk of future outbreaks (see Table 12). It is the responsibility of accredited bivalve molluscs producers in South Australia to implement and maintain a HACCP plan for the production and sale of live bivalve molluscs as part of their approved Food Safety Arrangement with PIRSA. Each accredited producer must demonstrate the requirements of the approved Food Safety Arrangement have been achieved through verification and validation activities.

In addition, PIRSA in collaboration with SA Health have developed a guideline protocol on proposed actions to be taken when notifications of *V. parahaemolyticus* cases are received and linked to oysters harvested from South Australian, or when *V. parahaemolyticus* is detected in shellfish (>3 MPN/g) within a classified harvest area through a surveillance program. Industry and individual growers also have the option to voluntarily cease their harvesting operations (prior to any formal closures) but would need knowledge of an early warning *Vibrio* forecast and/or near-real time reporting of any illnesses.

The South Australian Oyster Growers Association (SAOGA) with support from PIRSA have also developed a guide for South Australian oyster growers that aims to provide some best practice options to mitigate the risk of V*. parahaemolyticus*.

Table 12: Mandatory and/or recommended control measures in South Australia to reduce the risk of *Vibrio in oysters*

Stage of supply chain	Description
Pre-harvest	Good farming practice – Maintain oysters off the sea floor as *V. parahaemolyticus* can live in sediments on the sea floor and contaminate oysters.
	Good farming practice – Minimise increases in seawater turbidity during harvest by reducing disturbances of the sea floor. *V. parahaemolyticus* can live in sediments on the sea floor and if disturbed may contaminate oysters.
	Harvest as soon as possible once oysters are exposed by the tide. **The time into active refrigeration starts from when oyster is out of the water.** If oysters are harvested from intertidal areas during a low tide event, the time of harvest commences when the shellfish is first out of the water as the water is receding during a tide event. This time may vary from site to site. (Mandatory)
	Localised weather conditions should be considered. Ideally harvest during periods in the day when the air temperature is cooler.
	Good farming practice – Lease Management, infrastructure and practices. Maintain leases in good conditions and plan maintenance activities during non-harvest periods. Be aware of maintenance activities on adjacent or nearby leases and sites.
Post-harvest	Oysters need to be under shade within 4 hours of harvest and under active refrigeration (recommend less than or equal to 5°C) within 7 hours. If product not within temperature control with required time (7 hours), return product to harvest area. (Mandatory)

Stage of supply chain	Description
	Remove any external sediment from the oysters as soon as possible after harvest. If using water, it is mandatory that the water is potable or approved. Sediment often contains bacteria, including *V. parahaemolyticus*, that can contaminate the oysters.
	Harvested product intended for sale must achieve an internal product temperature of less than or equal to 10°C within 24 hours from harvest. If product internal muscle temperature is greater than 10°C after 24 hours, do not despatch product, retain under active refrigeration. Return product to harvest area. Returned product cannot be harvested for 48 hours. (Mandatory)
	Product stored under air temperature control to maintain internal product temperature of less than or equal to 10°C. If air temperature is above 10°C, confirm internal muscle temperature remains less than or equal to 10°C. If internal muscle temperature is greater than 10°C, return product to harvest area. Returned product cannot be harvested for 48 hours. (Mandatory)
Labelling	Labelling, invoices and tags to contain (Mandatory): • Species of Oyster (Pacific Oysters) • Food Safety Accreditation Number • Aquaculture Licence number • Name and address (optional) of this business • Date of oyster harvest • Name of the Area as specified by SASQAP from where oysters were harvested • Quantity of oysters in the shipment e.g. dozen or bag, (optional, as may appear on sales invoice) • Storage/transit temperature control requirements for storage (no more than 10°C).
Distribution	Approved supplier to maintain active refrigeration during supply chain. Where product is packed and transferred to third party logistics company (3PL) for storage and transport within the processing times listed, an arrangement must be in place between the producer and the 3PL to maintain product under active refrigeration, internal muscle temperature is 10°C within 24 hours of harvest and not despatch products until the internal muscle temperature of the oyster is less than or equal to 10°C at time of despatch. (Mandatory)

Tools for improved surveillance

Globally, most *Vibrio* surveillance systems commence with an understanding and monitoring of trends and patterns of vibriosis. Identifying the true extent of vibriosis can be challenging as individuals with mild, self-limiting infections may not seek medical care, or if they do seek medical care, medical practitioners might treat them symptomatically without a formal diagnosis. Whilst the under reporting of illness is acknowledged worldwide, illness reports remain the best source of on-going information to inform human health risk levels. In the United States of America, where vibriosis has been a nationally notifiable disease since 2007, it has been estimated that only 1 in 20 cases are reported (US Food and Drug Administration, 2012). In Australia, this could be further compounded as *V. parahaemolyticus* is not currently a notifiable disease in all Australian states and territories. It is only notifiable in the Northern Territory, South Australia (since 19 February 2016), Tasmania and Western Australia, and is not notifiable in other jurisdictions unless cases are identified as part of a foodborne outbreak. Greater understanding of the epidemiology of *Vibrio* illnesses at a national level may assist in surveillance, response to potential outbreaks and development of public health policies and resource allocation. In 2022, HarlockQuinn and Turnbull (2022) recommended a national discussion is required to consider if *V. parahaemolyticus* infection should become a nationally notifiable disease.

The serotyping and genotyping of *Vibrio* isolates are important epidemiologic tools used during outbreak investigations but provide little value for routine monitoring (FAO and WHO, 2016). Multi-locus sequence typing (MLST) and whole genome sequencing (WGS) in particular, provide detailed genome information that can assist the investigation of sources, and geographical and temporal spread of *Vibrio* strains (Baker-Austin *et al.*, 2018, Jesser *et al.*, 2019). The routine monitoring for *V. parahaemolyticus* in harvest areas has generally not been undertaken as part of a control programme, as controlling temperature between harvest and consumption is seen as the most significant element in controlling risk (FAO and WHO, 2016). However, it is only through continuous monitoring of the environmental conditions that the specific conditions driving the onset of *Vibrio* infections can be determined (Trinanes and Martinez-Urtaza, 2021). In Tasmania, the Oysters Tasmania's Sensor Network provides stakeholders with real-time remote monitoring capabilities for salinity, water temperature and depth in growing areas (Oysters Tasmania, 2023). This is seen as a useful tool to raise awareness of increased temperatures and to provide high quality information to relate growing areas conditions to *Vibrio* levels/infections. Remote monitoring can also be used, but empirical relationships should be validated against systematic in-situ observations.

A range of commercial temperature loggers, time and temperature indicators and other sensors can be used to monitor the temperature within consignments. In Tasmania, at least one grower has used a Tive Tag temperature logger (https://www.tive.com/tag) on individual consignments. Some oyster growers also use Hobo® waterproof loggers for monitoring water temperature, chiller temperature and through transit. Tamplin and Howieson (2014) provides several other examples of commercial temperature loggers and related sensors. However, most loggers will measure the exposed temperature of the shipment and not the oyster meat/muscle temperature. Nevertheless, the use of supply chain monitoring systems can increase the trust between supply chain partners and improve product quality and food safety outcomes (if any temperature abuse is detected) (Skawińska and Zalewski, 2022). The utilisation of consignment level temperature loggers generally requires support from supply chain actors (particularly in terms of return of any re-usable loggers, infrastructure requirements or remote scanning actions) as well as considerations on cost and utility.

Conclusion

Vibrios are naturally occurring bacteria and are widely distributed in fresh, estuarine and marine environments throughout the world. Pathogenic vibrios are a food safety concern in raw, undercooked or cross-contaminated seafood and *V. parahaemolyticus* is the leading cause of bacterial gastroenteritis associated with the consumption of seafood products globally. Oysters are a high-risk food category because they are filter feeders and are often eaten raw or minimally cooked with the viscera and other organs intact. Oysters naturally accumulate and depurate vibrios through filter feeding activities; however, once oysters are no longer underwater, depuration can no longer occur. Consequently, *Vibrio* levels increase quickly unless the oysters are held at less than 10°C. Environmental and biological factors and impact of harvest practices are being studied internationally to find relationships between risk factors and the prevalence and concentration of *V. parahaemolyticus* in bivalve shellfish, as well as determining *Vibrio* growth rates in oysters. It is the responsibility of accredited oyster producers in South Australia to implement and maintain an approved Food Safety Arrangement with PIRSA. Approved Food Safety Arrangements are designed to produce safe and suitable bivalve molluscs safely and to comply with regulations, legislations and standards.

This project collated and recorded several readily available environmental conditions associated with the February 2021 and September 2021 *Vibrio* outbreaks and compared the data to known risk factors. The environmental conditions, notably sea surface and oyster basket temperatures, during the onset periods of the two outbreaks were conducive to the growth of *V. parahaemolyticus*. There were no clear climatological anomalies in the available data sets that were reviewed which would help to substantiate why these outbreaks occurred in South Australia at these times given that there had not been any significant changes in oyster production, harvest and post-harvest practices. MLST typing detected one *Vibrio* sequence type (ST36) from clinical isolates during the February 2021 outbreak whilst two *Vibrio* sequence types (ST417 and ST50) were recovered from clinical isolates during the September 2021 outbreak. Only one *Vibrio* sequence type (ST417) was detected from some oyster samples during the second outbreak. Whilst these were the first *Vibrio* outbreaks attributed to oysters produced in South Australia, several sporadic and locally acquired cases have occurred since *V. parahaemolyticus* infections became a notifiable condition in 2016.

A number of tools and approaches are available that could be used to identify and assess potential risk factors and improved surveillance. These tools include in-situ data collection, remote sensing of the environment, microbiological sampling and molecular diagnostics. Large-scale oceanographic data is appropriate for investigating interannual/seasonal variations and making predictions, but has limited value in determining daily environmental conditions or the temperature oysters experience on lease. Similarly, the fortnightly/monthly SASQAP monitoring data has limited value for the purpose of determining daily temperatures at the growing leases scale. The best source of information on temperature experienced on the leases came from data loggers attached to oyster baskets. These loggers showed that a) temperatures were highly variable on an hourly basis and in particular in relation to the tidal cycle; and b) temperatures reached much higher levels than expected in early spring at the start of the outbreak and are likely to be a strongly contributing factor to the growth of *Vibrio*. The size and geographically variability in the South Australian growing areas is given in Appendix 5. The growing environment between and within each harvest zone varies depending on proximity to the bay opening, water depth, tidal channels, sand banks, or enclosed embayments. Thus, multiple temperature loggers would be required to adequately reflect the temperature of the range of micro-climates within each growing area.

The 2021 outbreaks were the largest vibriosis outbreaks on record associated with Australian product and resulted in substantial costs for industry, both economically and reputationally. The magnitude and severity of the September 2021 outbreak was likely compounded by several factors, including post-harvest temperature controls, timely reporting of illnesses, and poor traceability along the supply chain, which impacted traceback and the timing of growing area closure. Traceback through the supply chain to growing area, lease and harvest date was often incomplete or confounded when cases had consumed oysters from multiple outlets during the onset period or the co-mingling of oysters could not be excluded. Further work needs to be undertaken within the supply chain to ensure that legislated responsibilities on appropriate labelling and control of co-mingling are adhered to. Similarly, a review and refresh of growers recall plans is necessary and growers should participate in simulation training of recall events to improve the practices supporting speedy recalls.

The scale of this second outbreak could have been largely avoided with timely closure of growing areas following multiple illnesses in line with ASQAP guidelines. The South Australian authorities have recognised this and have developed a guideline protocol on proposed actions to be taken when notifications of *V. parahaemolyticus* cases are received and linked to oysters harvested from South Australian, or when *V. parahaemolyticus* is detected in shellfish (>3 MPN/g) within a classified harvest area through a surveillance program. Mandatory and/or recommended control measures to reduce the risk of *Vibrio* in South Australian oysters have been identified. Industry and individual growers also have the option to voluntarily cease their harvesting operations (prior to any formal closures) but would need knowledge of an early warning *Vibrio* forecast and/or near-real time reporting of any illnesses.

Communication during public health outbreaks of any nature is complex for many reasons: privacy concerns for the outbreak cases and implicated businesses (sometime legislated); traceback difficulties particularly when a large volume of product is involved; investigations take time; multiple agencies are involved across multiple jurisdictions; and importantly the stakes are high in terms of health and economic outcomes. Open lines of communication were established during the outbreaks and stakeholders are to be commended on the volume of information they were able to share on a regular basis. However, a post-event review that included industry members did not occur and would have been a valuable tool to enable a deeper understanding of the type of data that can be shared and when, in preparation for future events.

This project has also highlighted several data gaps. Poor traceability through supply chain hampered traceback investigations and the identification the unique harvest date, harvest location, and subsequent production, harvest and post-harvest conditions was limited. There is no publicly available information on the concentration of *V. parahaemolyticus* in the implicated oysters, or the prevalence and levels of *Vibrio* from other oyster samples collected during the events.

Implications

Globally the prevalence of vibriosis is linked to the effects of climate change, aging populations, dietary changes and improved detection methods. There is also some evidence internationally that vibrios are constantly evolving, creating more resilient and virulent strains. In recent decades, incidences of vibriosis have been occurring in regions with cooler climates and as well as upward trends in case numbers in affected jurisdictions. This project has confirmed that the sea surface temperatures and salinity levels at the time of illness onsets for both outbreaks could support *Vibrio* growth. However, there were no clear climatological anomalies in the environmental data sets investigated within this project that can help to explain why these outbreaks occurred in this particular year. Predicted increases in seawater temperature as a result of global warming will only increase the risk in the future. Consequently, *Vibrio* cases and outbreaks have the potential to become more common. A greater awareness and understanding of pathogenic vibrios and the implementation of effective control mechanisms are necessary to reduce the risk.

Routine monitoring for *Vibrio* is used in some jurisdictions (e.g. Japan and Canada) to control risk but detected levels are not always indicative of risk. This is because not all *Vibrio* are pathogenic, and illnesses occasionally occur when reported *Vibrio* numbers are low. Vibrios can also enter a viable but non-culturable state during periods that are not conductive for growth or when facing stressful conditions. Establishing relationships between environmental parameters and *Vibrio* prevalence and abundance in growing areas can help to provide an early warning system. PIRSA Biosecurity is currently conducting some routine surveillance for *V. parahaemolyticus* in oysters from classified growing areas between the months of September and March, and the usefulness of this approach should be reviewed when a suitable dataset becomes available. Any detections should also be related to the environmental parameters at the time.

Pre- and post-harvest control measures are critical to manage the risk of vibrios and accredited operators should pay special attention to their approved Food Safety Arrangements. Approved Food Safety Arrangements are designed so that growers produce safe and suitable oysters and to comply with legislative requirements. A key consideration to reduce the number of illnesses and maintain confidence in consumers is to close growing areas in a timely manner following outbreak onset. This requires timely reporting of illness and detailed traceback investigations. The difficulties in traceback found during the 2021 outbreaks indicate that improvements can be made in the oyster supply chain and a concerted effort is needed to improve the adherence of the supply chain to traceability requirements. The South Australian authorities have already responded to the need for timely closures by developing a guideline protocol on proposed actions to be taken when vibriosis notifications are received or when *V. parahaemolyticus* is detected in shellfish (>3 MPN/g).

Recommendations

1. In-situ environmental monitoring is improved through use of loggers in more growing and harvest areas.
2. Further work needs to be undertaken within the supply chain to ensure that legislated responsibilities on labelling, traceability and control of co-mingling are adhered to.
3. *Vibrio parahaemolyticus* isolates should be collected during vibriosis events (clinical and oyster) and an Australian isolate collection curated and maintained.
4. A review and refresh of growers recall plans is necessary and growers should participate in simulation training of recall events to improve the practices supporting speedy recalls.
5. Open lines of communication between regulators and industry should be maintained to determine what type of data can be shared and when.
6. Authorities should implement timely closure of growing areas following multiple illnesses in line with ASQAP guidelines.
7. Food Safety Management plans should be reviewed and closely adhered to, especially if there are any future outbreaks.
8. Regulators should hold a post event review that includes industry and research representatives to strengthen working relationships and improve joint outcomes.

Further development

- ***In order to determine the effectiveness of the existing control strategies an assessment and benchmarking of domestic post-harvest handling practices and temperatures through the supply chain should be considered.***

  Both the South Australian and Tasmanian *Vibrio* guides for growers recommend that following land-based oyster activities (i.e. sorting, rumbling and grading), the oysters are returned to the growing area for at least two tidal cycles before harvesting for human consumption. However, several international studies have reported it can take longer and up to 7-14 days for *V. parahaemolyticus* levels to return to background levels.

  Rapid post-harvest cooling of oysters and maintaining the cold chain through distribution and storage are considered fundamental *Vibrio* risk management strategies. Growers are required to demonstrate compliance with time/temperature requirements and are recommended to consider worse-case scenarios. Post-harvest cooling, distribution and storage time/temperature requirements should be based on product quality and safety (including potential for *Vibrio* growth) and practicalities.

- ***Promote and support digital traceability through the supply chain.***

  Traceability is the ability to track any food through all stages of production, processing and distribution. The minimum traceability requirement in Australia is "*one step back and one step forward*". The ability to track food through all stages of production, processing and distribution makes it easier and quicker to recall product(s) if something goes wrong. The ability to (rapidly) trace oysters back to the production leases was hampered in the 2021 outbreaks due to complexity of the supply chains, multiple exposure to oysters during case onset periods, suspected co-mingling of product, and inaccurate or incomplete labelling of product. In South Australia all shellfish must be labelled as per the requirements of the *Primary Produce (Food Safety Schemes) (Seafood) Regulations 2017*. Records need to be maintained for a period of 4 years and should be made available upon request during regulatory and third party audits. The implementation of digital traceability systems can assist in capturing product movement along the supply chain and facilitate product recalls.

- ***Consider determining the prevalence, levels and strain types in of V. parahaemolyticus in South Australian oyster growing regions.***

  There remains limited information detailing the specific risk factors of *V. parahaemolyticus* in South Australian oyster growing regions. Recently, Torok *et al.* (2023) determined the prevalence, levels and strain types of *Vibrio* at harvest in various Tasmanian growing areas, included the development of harvest area specific *Vibrio* predictive models and the development of the first Australian risk profile for *Vibrio* in Tasmanian commercial oysters. A similar approach could be followed in South Australia.

- ***Consider assessing Vibrio growth in oysters by using local clinical and/or environmental isolates and review post-harvest time/temperature requirements***.

  There are numerous studies predicting *V. parahaemolyticus* growth as a function of temperature. Each study uses different approaches. The study by Fernandez-Piquer *et al.* (2011) injecting a cocktail of strains into the adductor muscle of Pacific Oysters and observing growth during storage at different temperatures. Fernandez-Piquer *et al.* (2011) reported that *V. parahaemolyticus* levels were stable at 14.9°C and increased/decreased at storage temperatures above/below this value. However, FletcherCruz and Hedderley (2024) recently reported observing slow *V. parahaemolyticus* growth at 10°C in naturally contaminated New Zealand Pacific Oysters. Growth rates were also slower at higher temperatures. In Australia, regulatory requirements for Pacific Oysters include storage at temperatures of 10°C or less within 24 hours of harvest, whereas in New Zealand, *Vibrio* management plans (when operative) are more stringent and include refrigeration at ≤7°C.

- ***Promote and support national decision to make V. parahaemolyticus nationally notifiable.***

  V. parahaemolyticus is not currently a nationally notifiable disease (currently only notifiable in Northern Territory, South Australia, Tasmania and Western Australia; other jurisdictions only need to notify if cases are identified as part of a foodborne outbreak). Due to recent non-choleragenic *Vibrio* infections HarlockQuinn and Turnbull (2022) recommended that a national discussion to consider *V. parahaemolyticus* infection as a nationally notifiable disease is warranted. Notifiable conditions help to provide early warning of potential threats to public health, identify emerging trends to guide policy responses and interventions and to aid in responding to prevent or control the spread of diseases.

Extension and Adoption

In 2021 a formal ‘Extension and Adoption’ plan was developed to clearly indicate who the target audience was, the key messages we were aiming to deliver and a method/action plan for our communication and research outputs.

As part of this project the following presentations were given:

- 13th October 2022: Stephen Pahl presented project overview at the South Australian Oyster Industry 2022 Seminar Program (Port Lincoln, SA)
- 28th April 2023: Stephen Pahl presented research findings at the SARDI/FRDC Milestone Day (West Beach, SA)
- 26th October 2023: Stephen Pahl presented project update and findings at the South Australian Oyster Industry 2023 Seminar Program (Stansbury, SA)

Project materials developed

- 1-2 page “grower friendly” summary of the project outcomes (to be developed)
- Short 2-5 minute video summary of the work and findings (to be developed)

Appendices

Appendix 1 – List of researchers and project staff

Position	Name	Organisation
Principal investigator	Dr Stephen Pahl	SARDI Food Sciences, Department of Primary Industries and Regions
Co-investigators	Ms Navreet Malhi	SARDI Food Sciences, Department of Primary Industries and Regions
	Dr Hugo Bastos de Oliveira	SARDI Aquatic and Livestock Sciences, Department of Primary Industries and Regions
	Dr Alison Turnbull	Institute of Marine and Antarctic Sciences, University of Tasmania

Appendix 2 – Intellectual Property

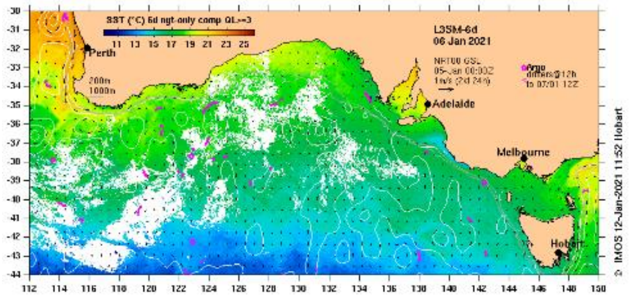
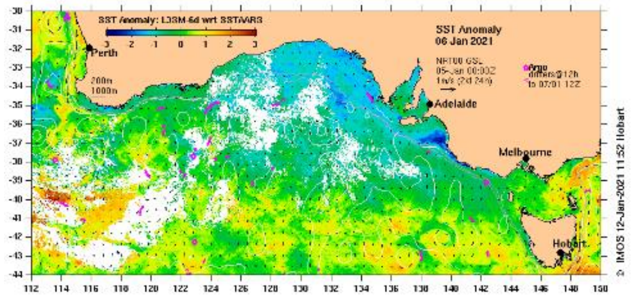
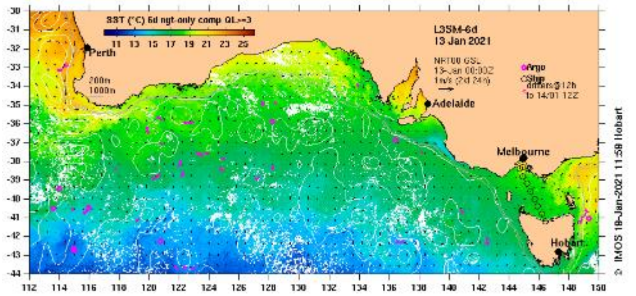
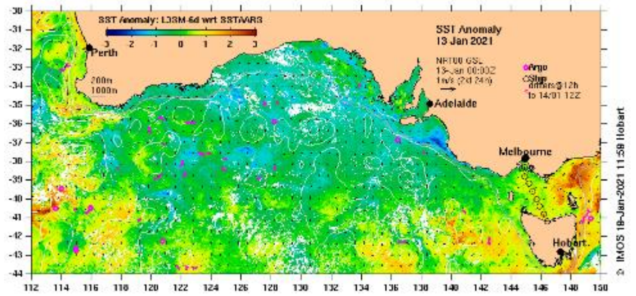
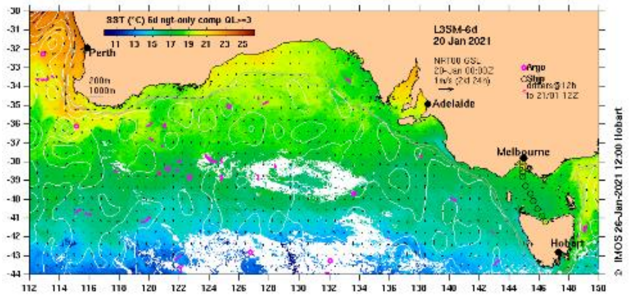
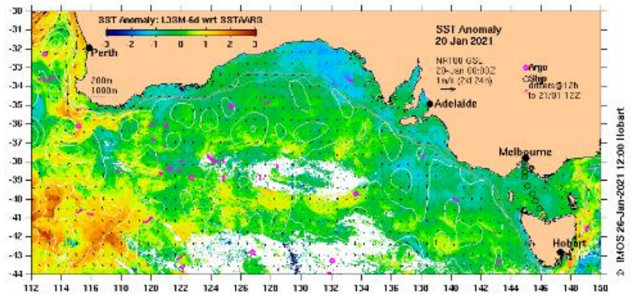
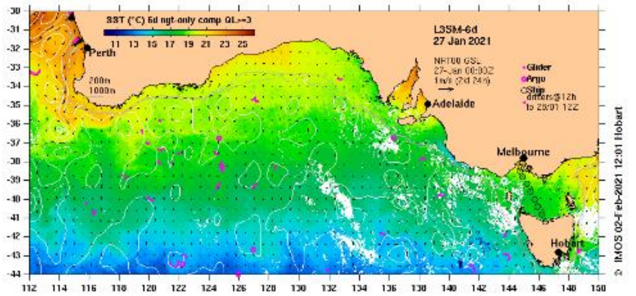
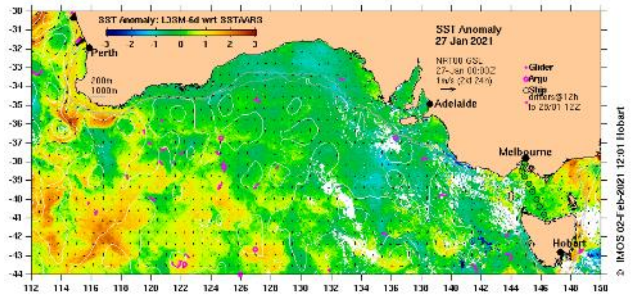
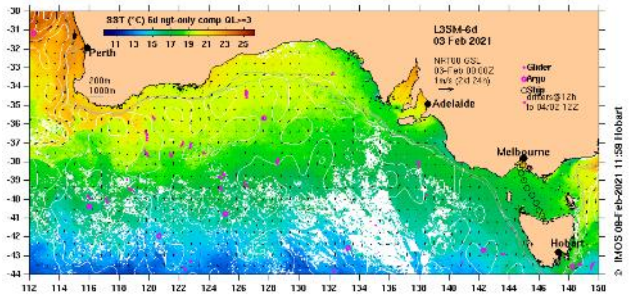
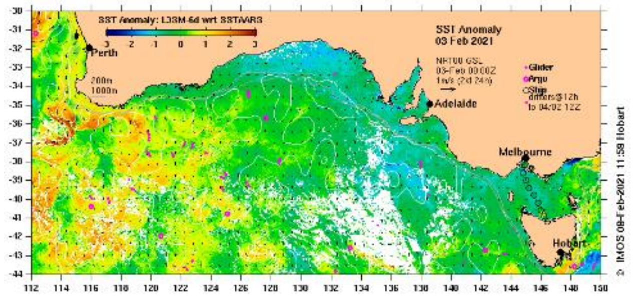
None

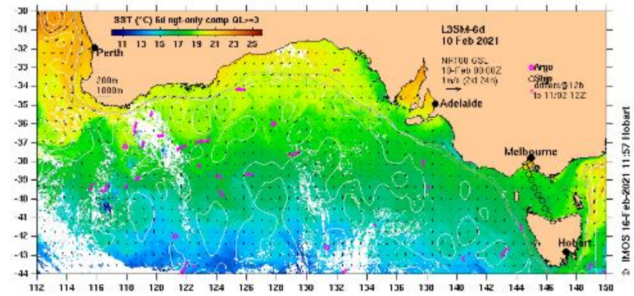
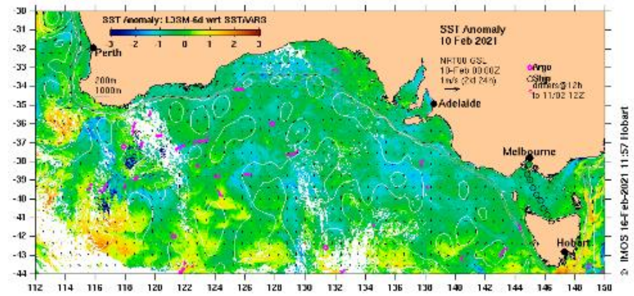
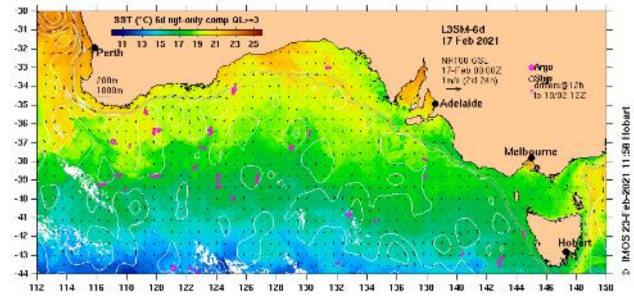
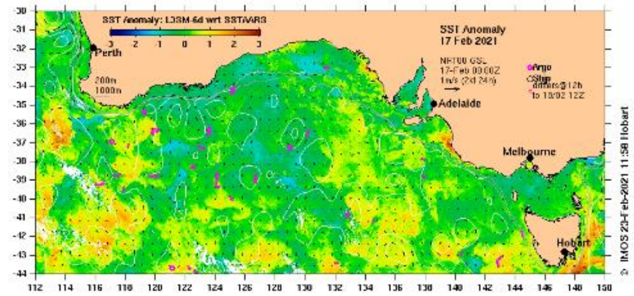
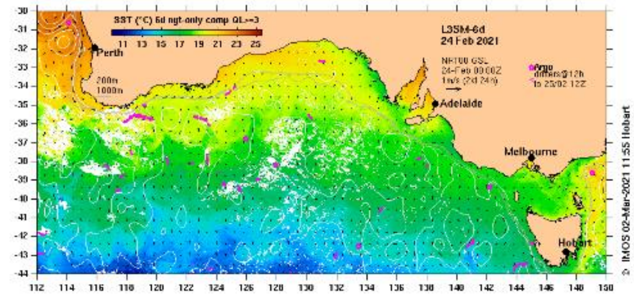
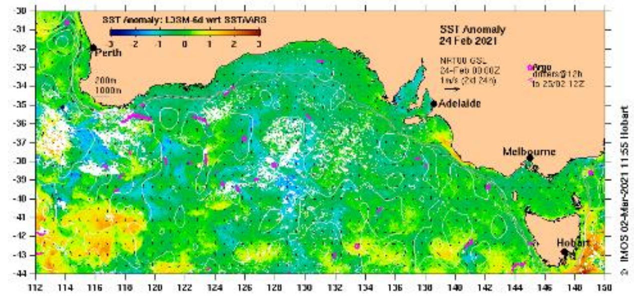
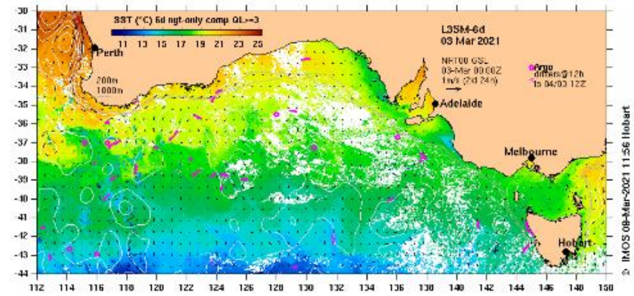
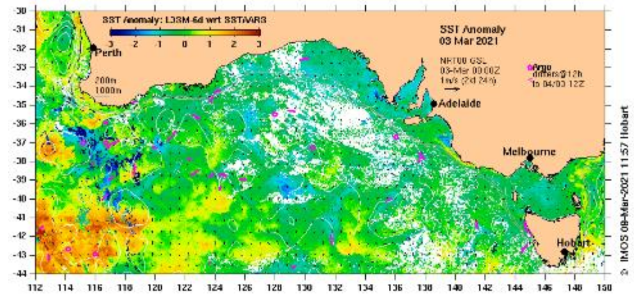
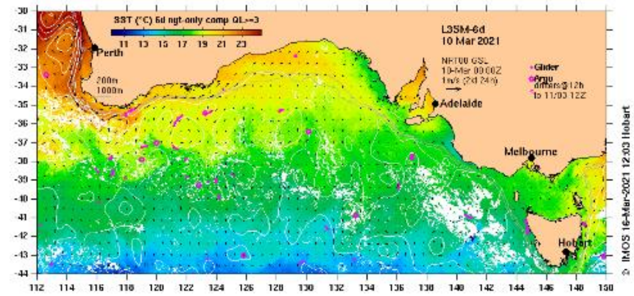
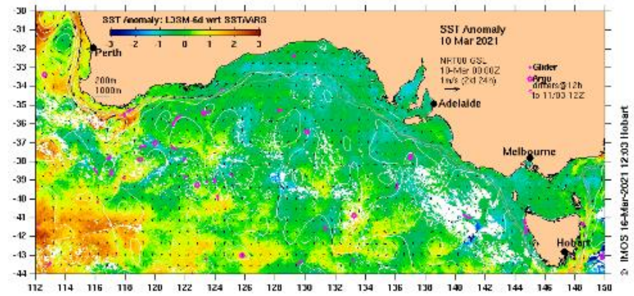
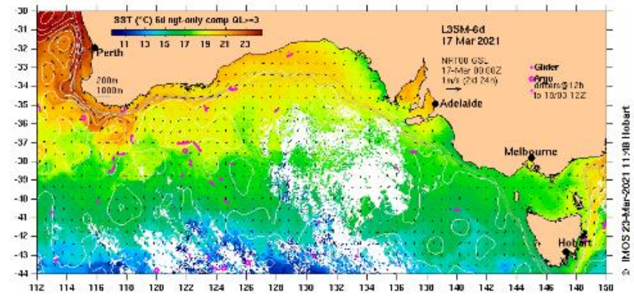
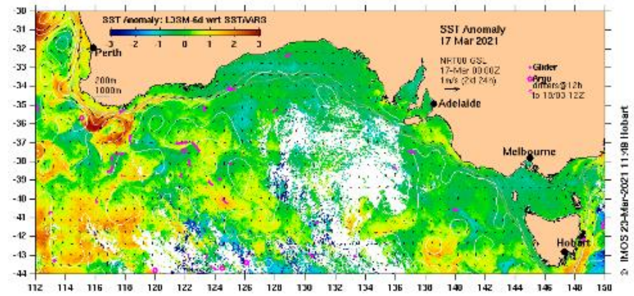
Appendix 3 – Temperature specific growth rates of *Vibrio parahaemolyticus*

Table 13: Temperature specific *V. parahaemolyticus* growth rates and doubling times. Adapted from US Food and Drug Administration (2019).

Oyster temperature		Growth rate (logs/hr)	Doubling time (hrs)
(°C)	(°F)		
10.0	50.0	0.008	35.80
10.6	51.0	0.011	28.40
11.1	52.0	0.013	23.10
11.7	53.0	0.016	19.20
12.2	54.0	0.019	16.10
12.8	55.0	0.022	13.80
13.3	56.0	0.025	11.90
13.9	57.0	0.029	10.40
14.4	58.0	0.033	9.14
15.0	59.0	0.037	8.11
15.6	60.0	0.042	7.24
16.1	61.0	0.046	6.50
16.7	62.0	0.051	5.87
17.2	63.0	0.056	5.33
17.8	64.0	0.062	4.86
18.3	65.0	0.068	4.45
18.9	66.0	0.074	4.09
19.4	67.0	0.080	3.77
20.0	68.0	0.086	3.49
20.6	69.0	0.093	3.24
21.1	70.0	0.100	3.01
21.7	71.0	0.107	2.81
22.2	72.0	0.115	2.63
22.8	73.0	0.122	2.46
23.3	74.0	0.130	2.31
23.9	75.0	0.139	2.17
24.4	76.0	0.147	2.05
25.0	77.0	0.156	1.93
25.6	78.0	0.165	1.83
26.1	79.0	0.174	1.73
26.7	80.0	0.183	1.64
27.2	81.0	0.193	1.56
27.8	82.0	0.203	1.48
28.3	83.0	0.213	1.41
28.9	84.0	0.224	1.34
29.4	85.0	0.235	1.28
30.0	86.0	0.246	1.23
30.6	87.0	0.257	1.17
31.1	88.0	0.268	1.12
31.7	89.0	0.280	1.07
32.2	90.0	0.292	1.03
32.8	91.0	0.304	0.99
33.3	92.0	0.317	0.95
33.9	93.0	0.330	0.91
34.4	94.0	0.343	0.88
35.0	95.0	0.356	0.85
35.6	96.0	0.370	0.81
36.1	97.0	0.383	0.79
36.7	98.0	0.397	0.76
37.2	99.0	0.412	0.73
37.8	100.0	0.426	0.71

Appendix 4 – 6-day composite SST and SST anomaly for the Southern Australia region

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	
	

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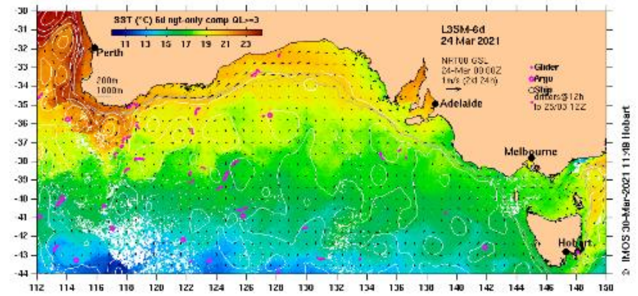
<tr><td>
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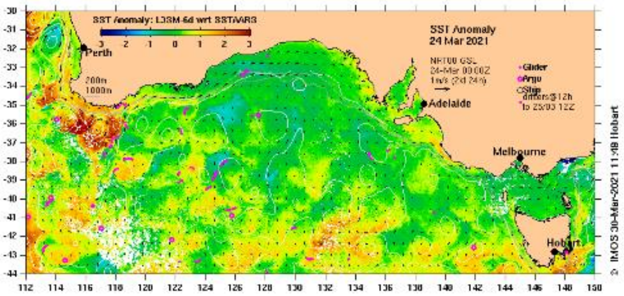
<td>
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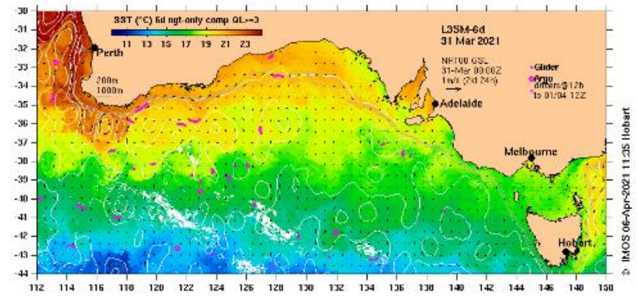
<tr><td>
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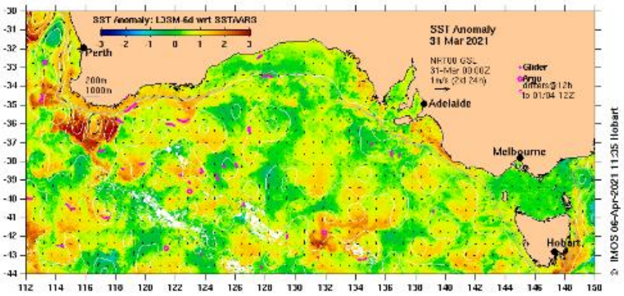
<td>
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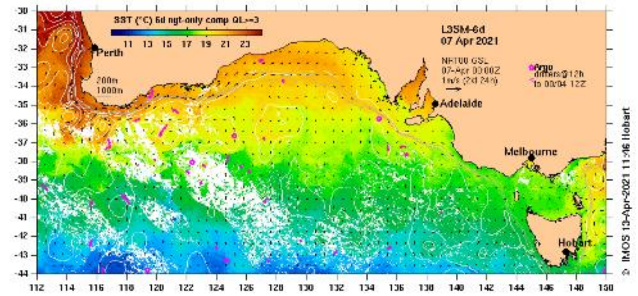
<tr><td>
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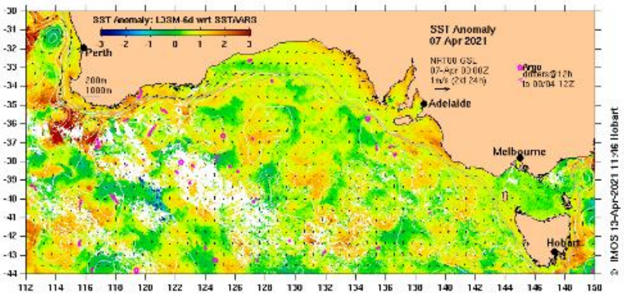
<td>
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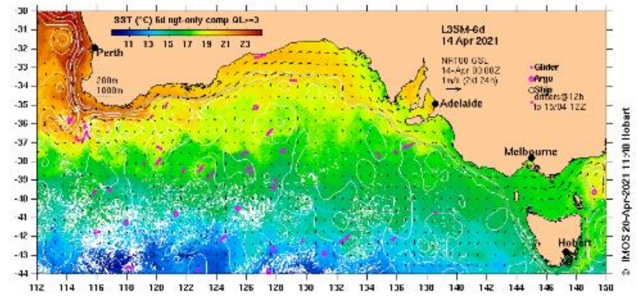
<tr><td>
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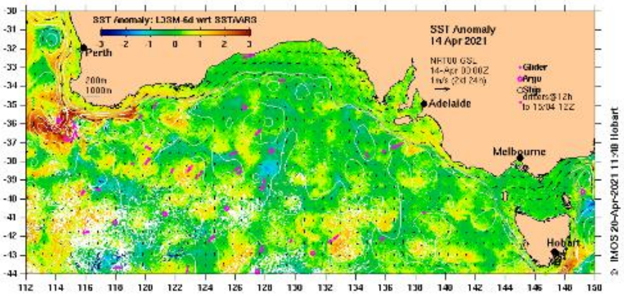
<td>
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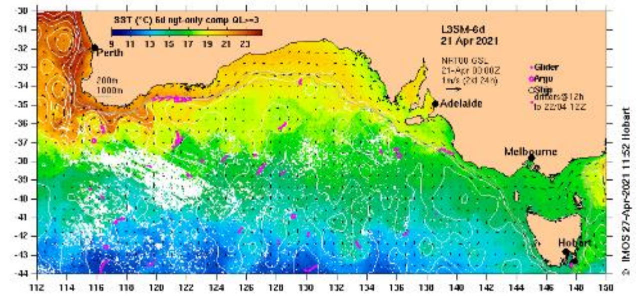
<tr><td>
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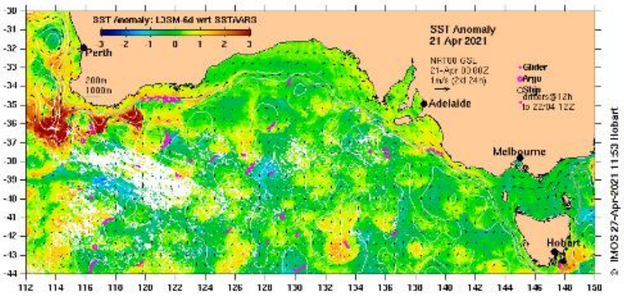
<td>
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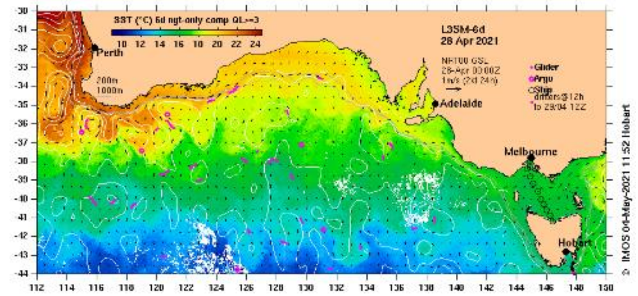
<tr><td>
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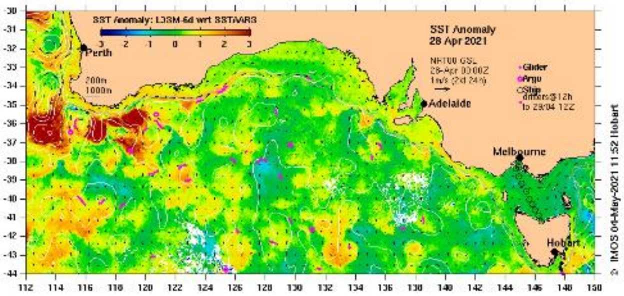
<td>
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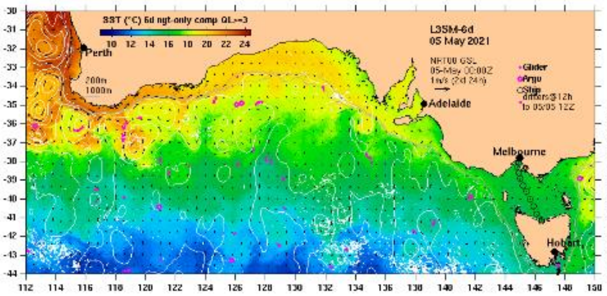
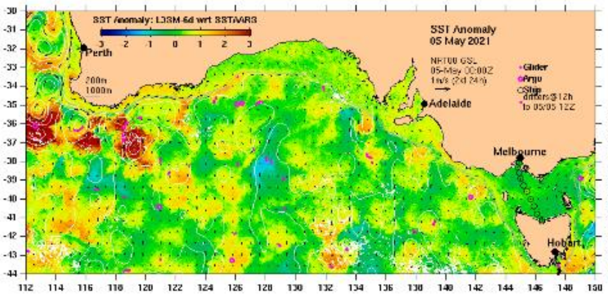
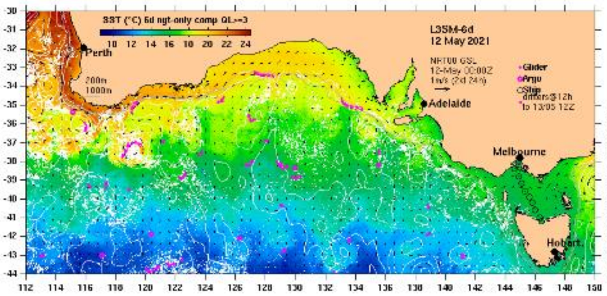
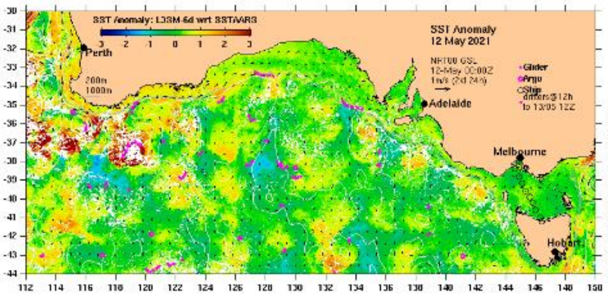
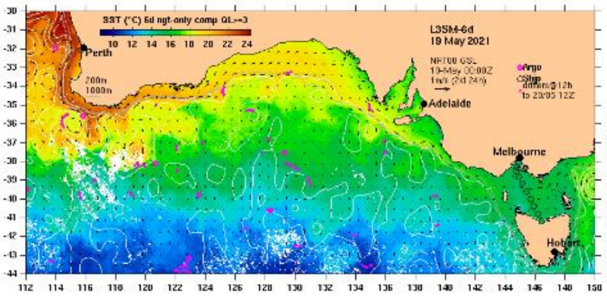
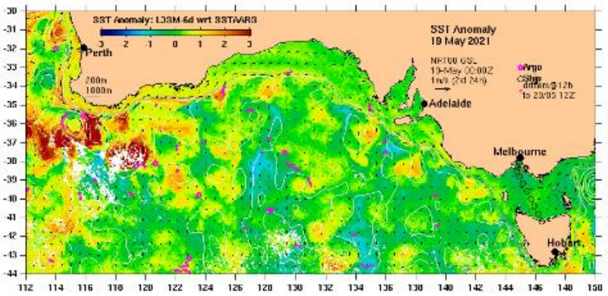
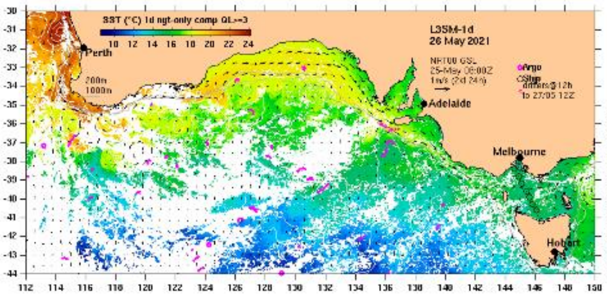
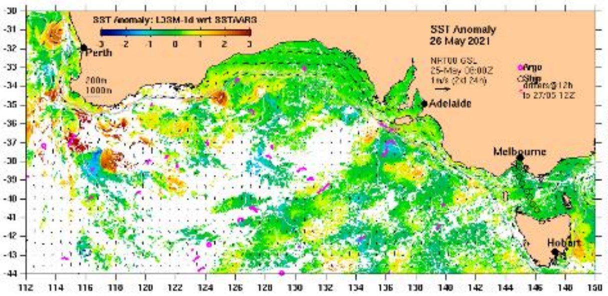
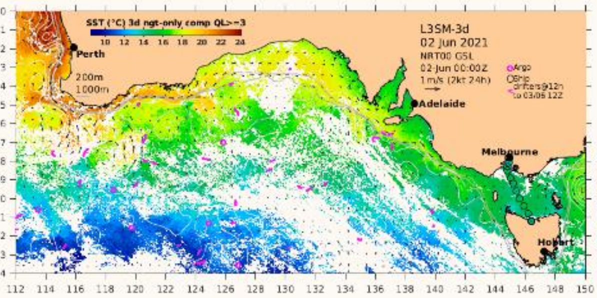
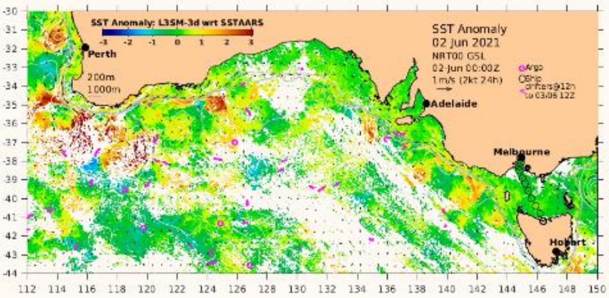
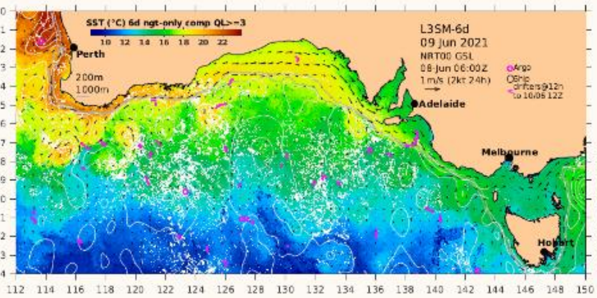
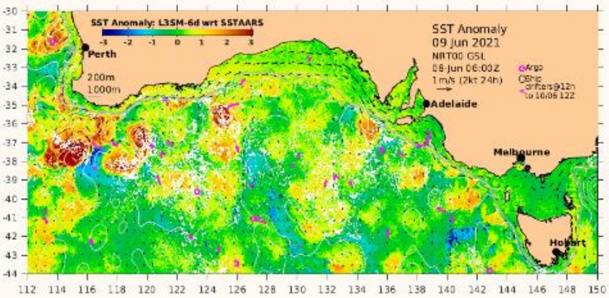
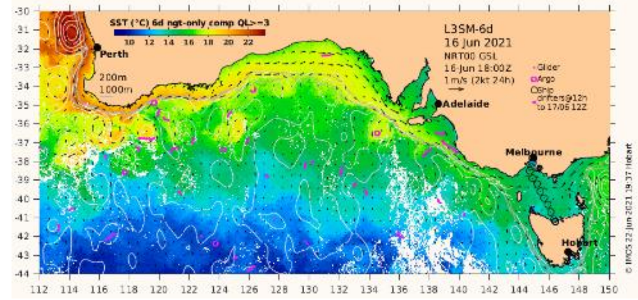
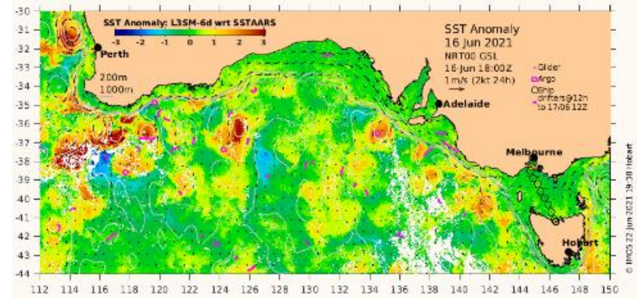
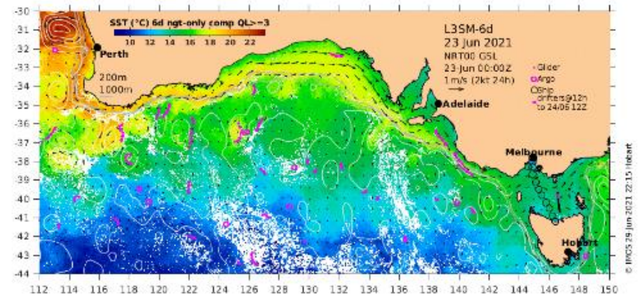
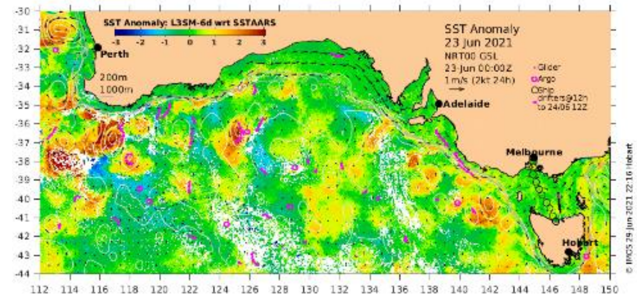
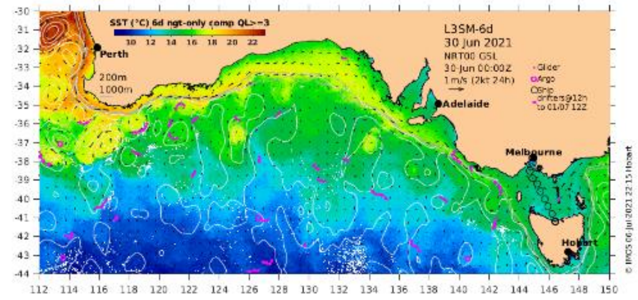
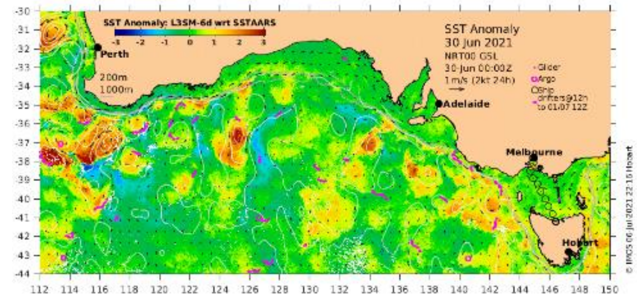
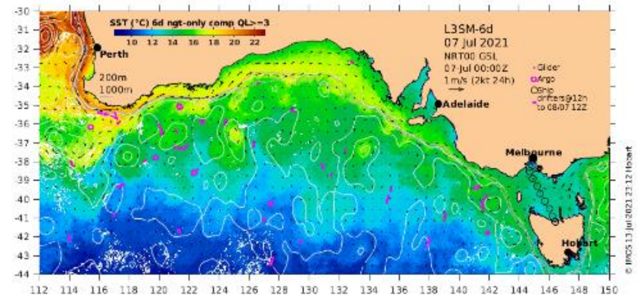
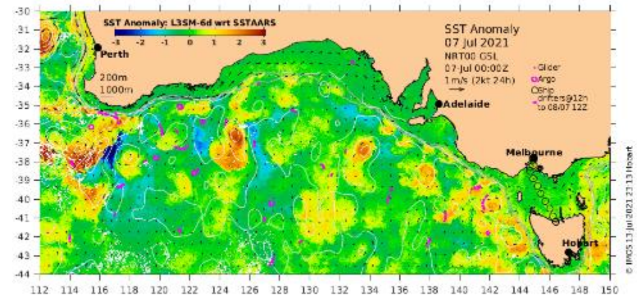
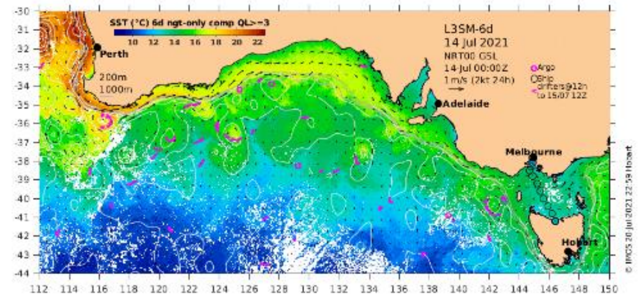
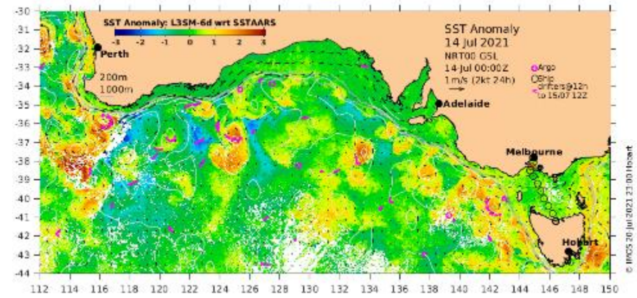
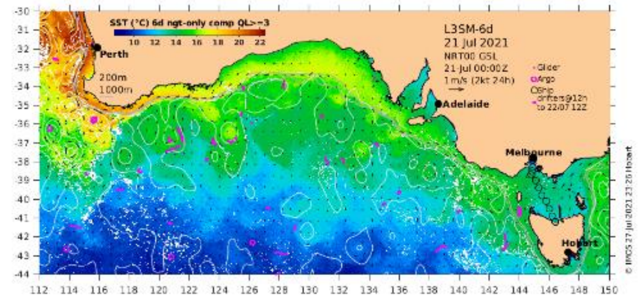
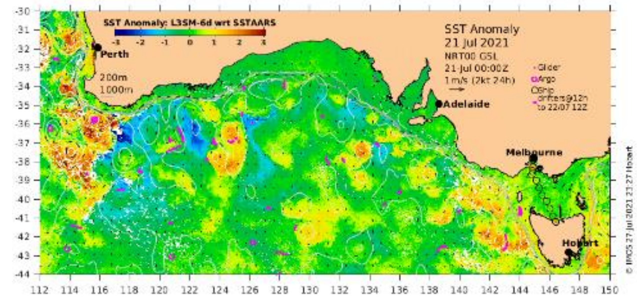
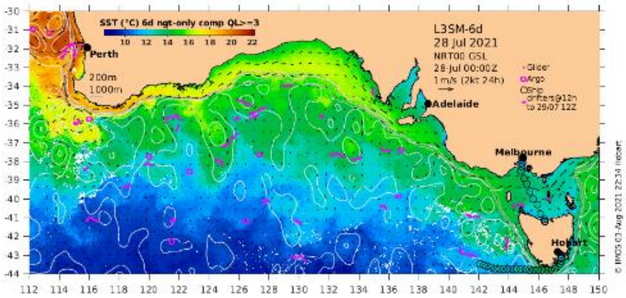
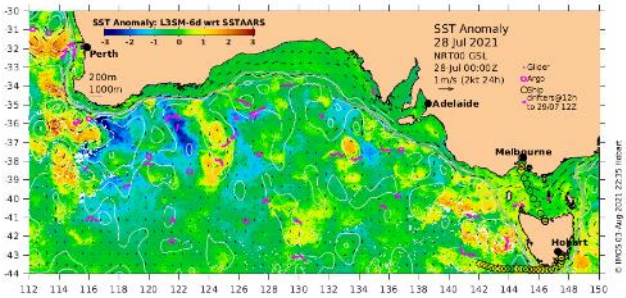
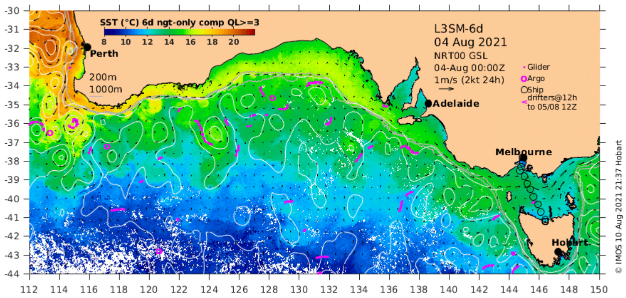
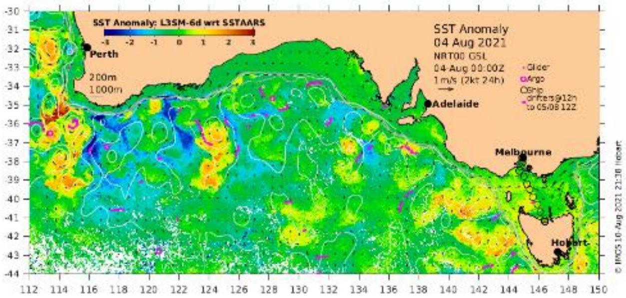
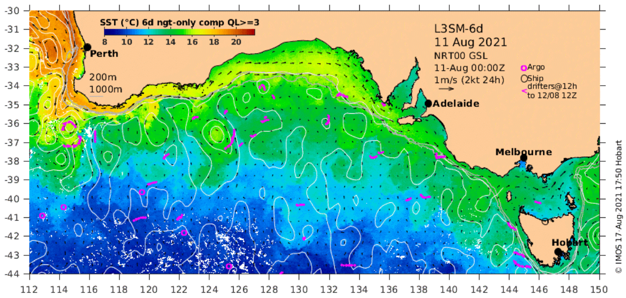
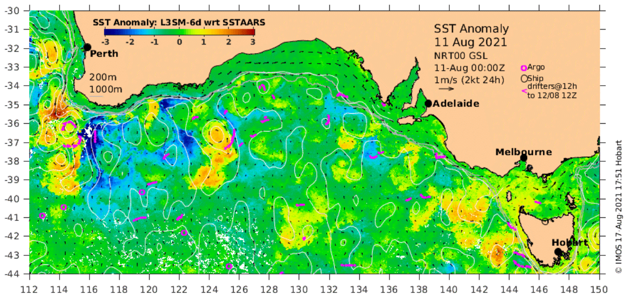
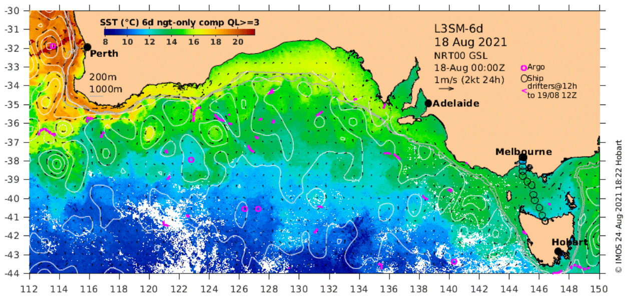
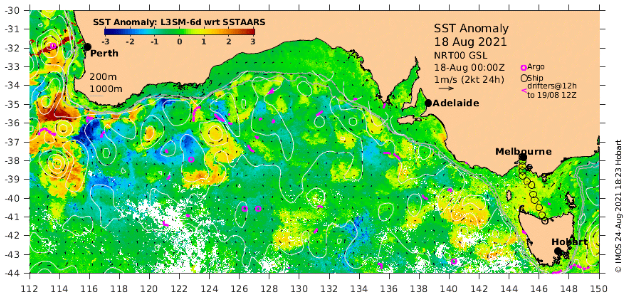
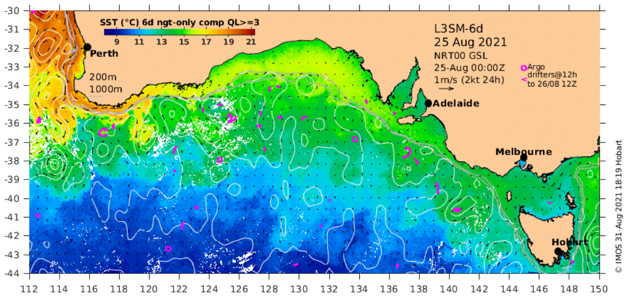
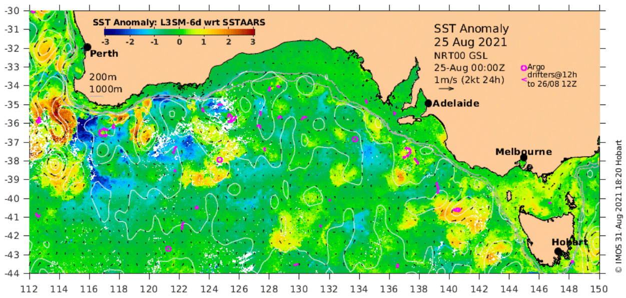
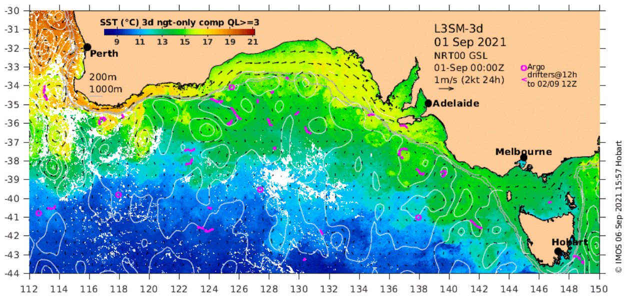
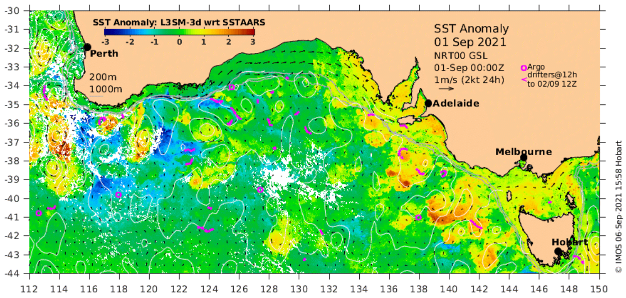
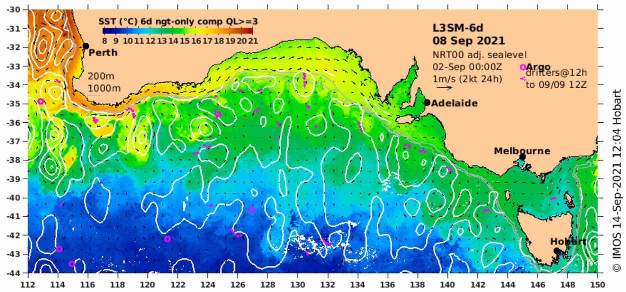
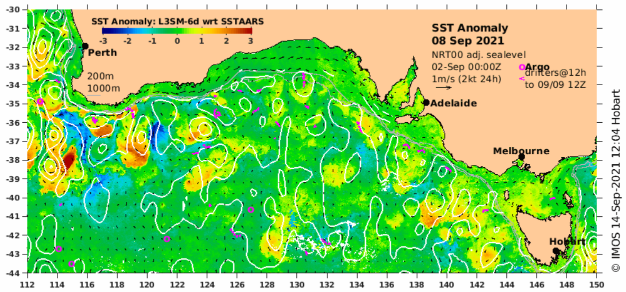
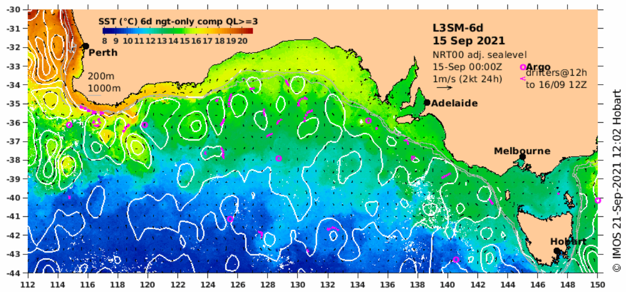
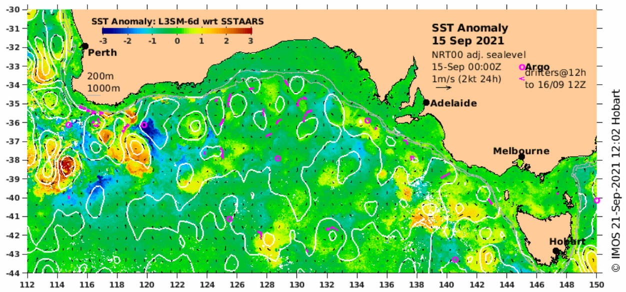
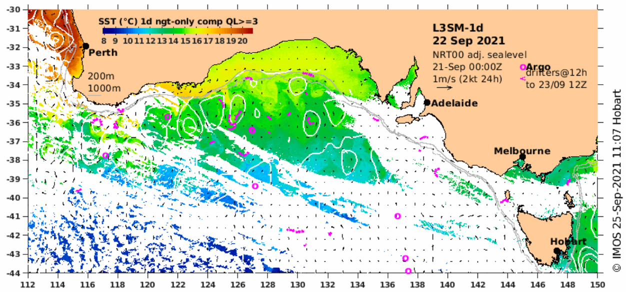
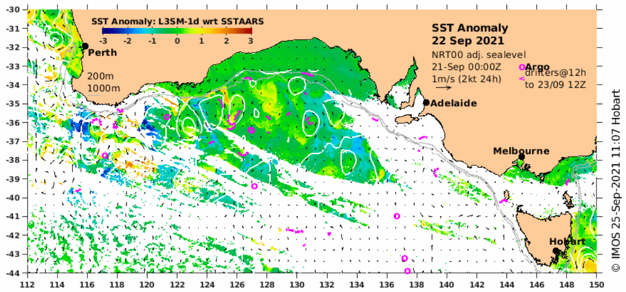
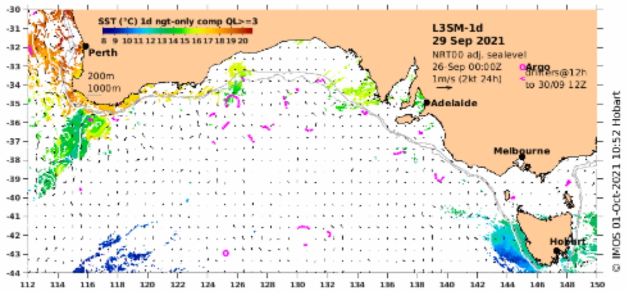
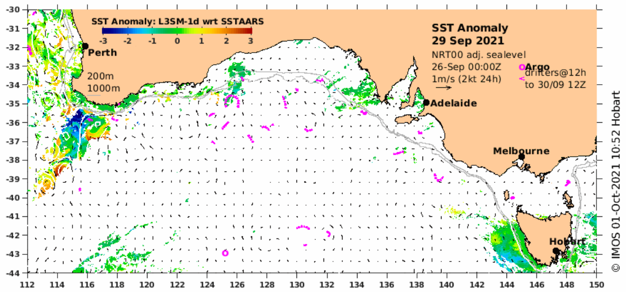
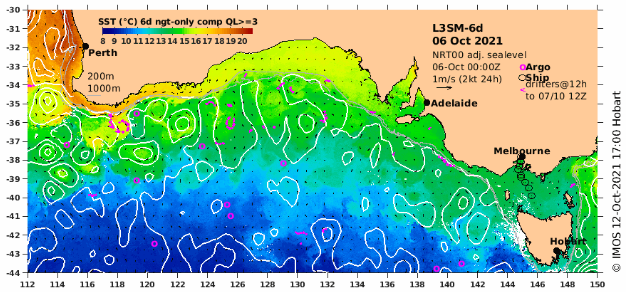
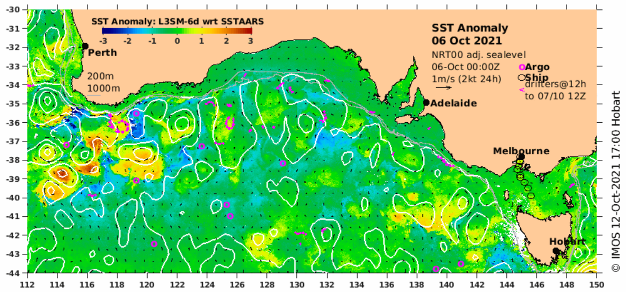
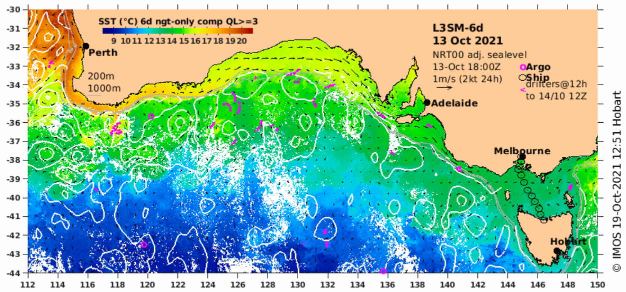
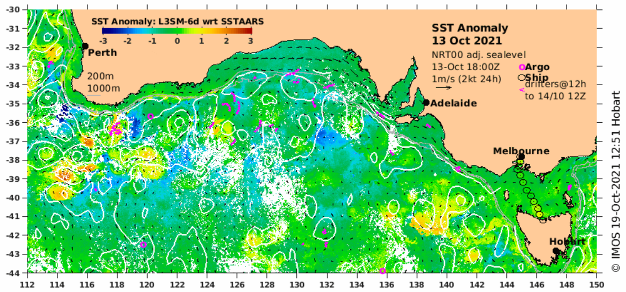
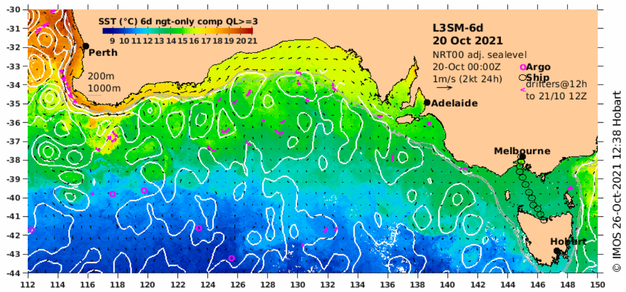
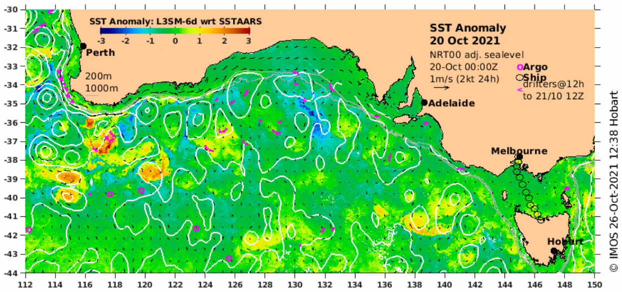
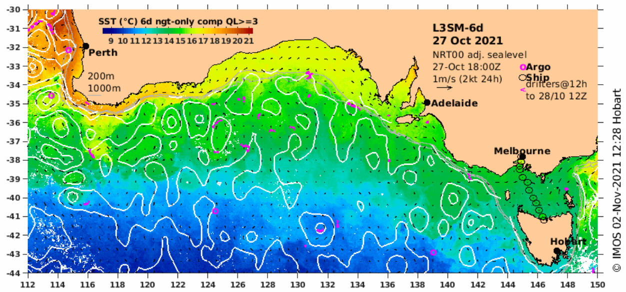
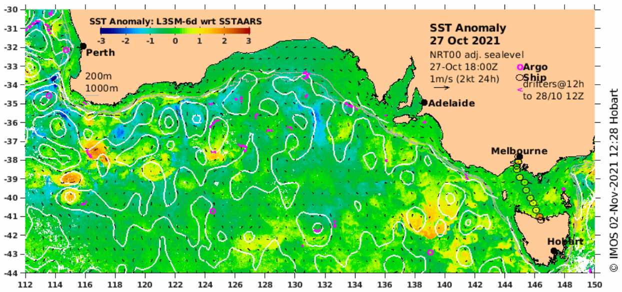
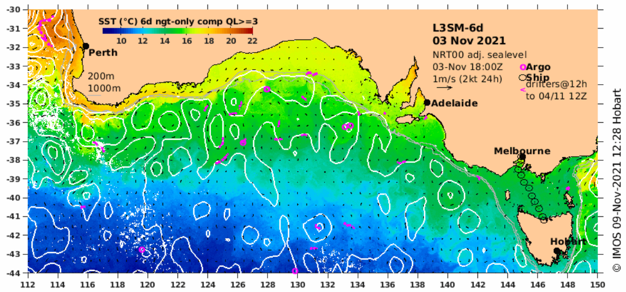
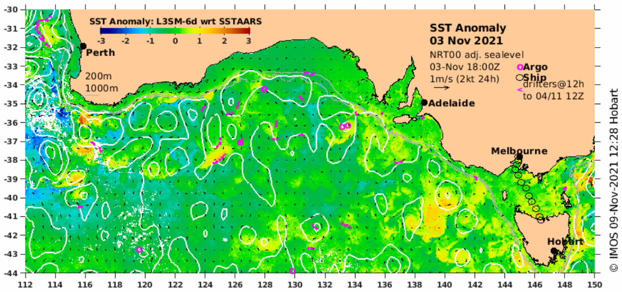
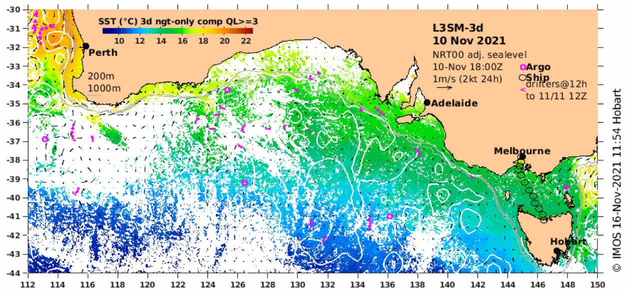
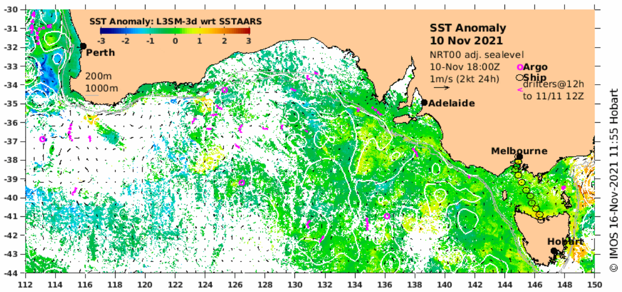
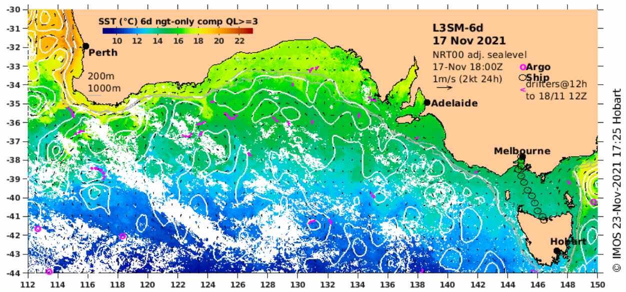
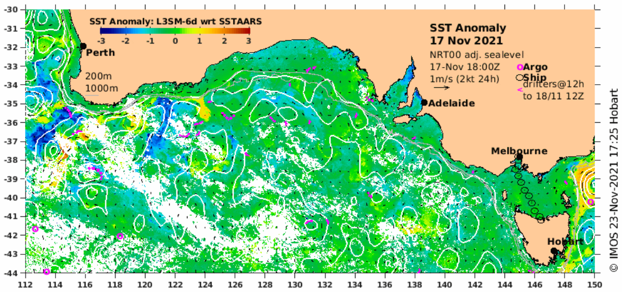
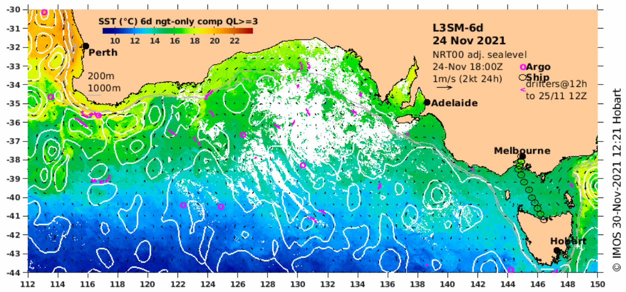
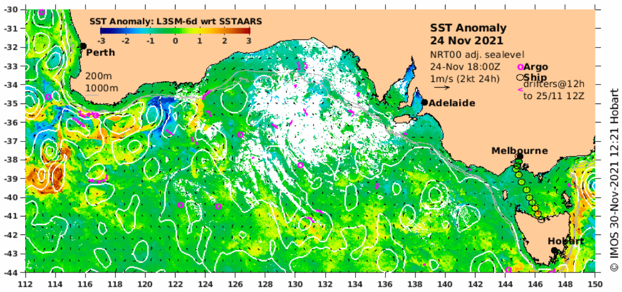
6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	
	

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	
	

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	
	

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	
	

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region
	
	
	
	
	
	

6-day composite SST for the Southern Australia region	6-day composite SST anomaly for the Southern Australia region

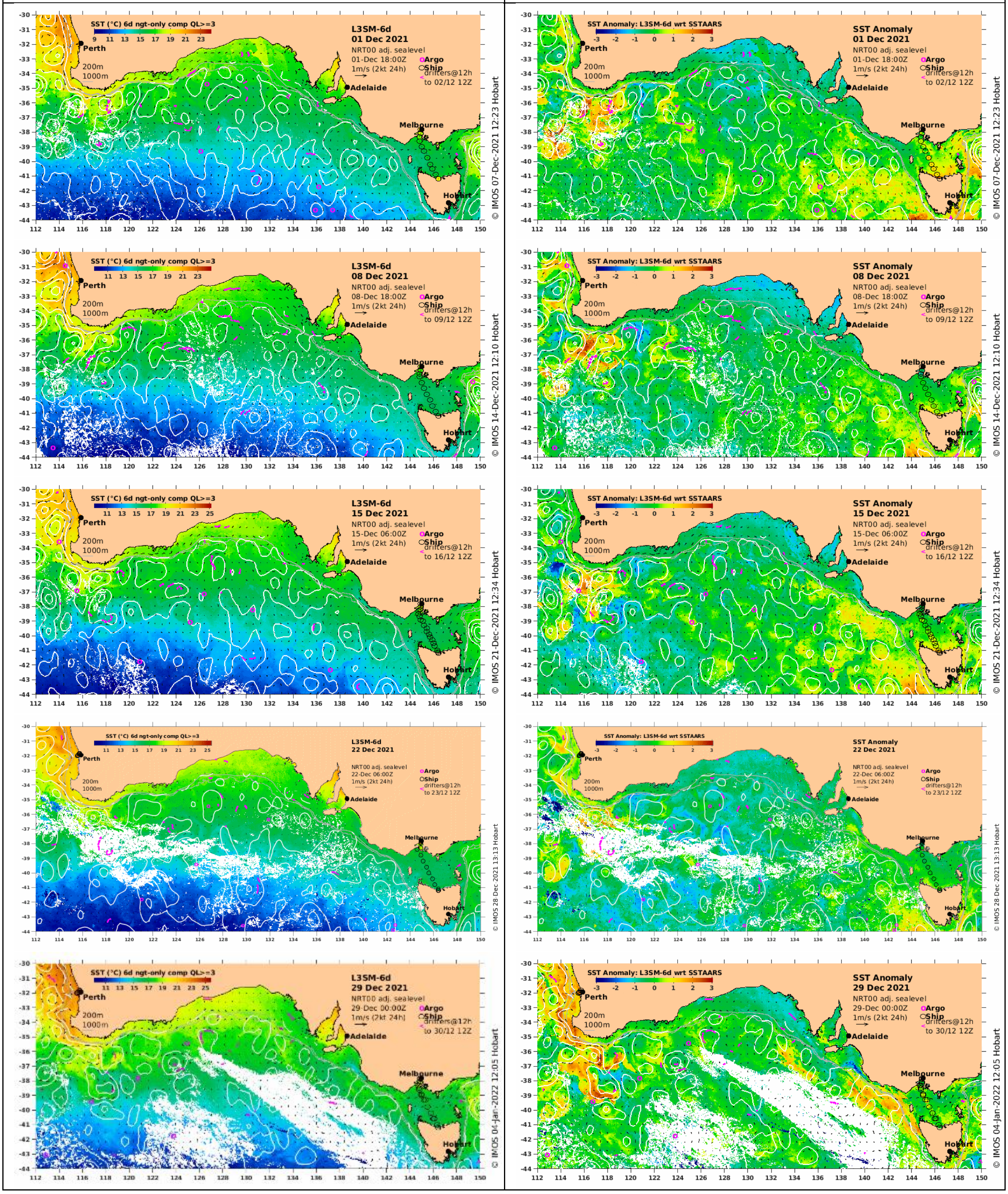
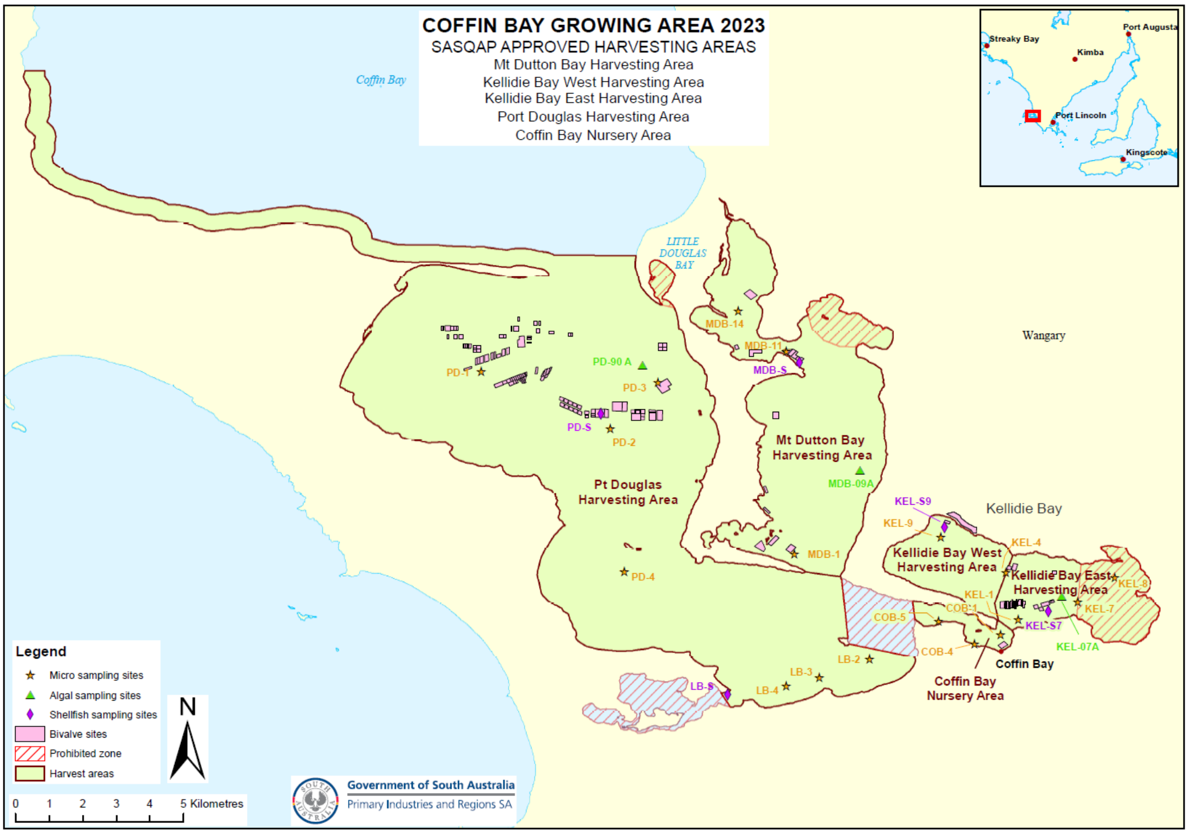
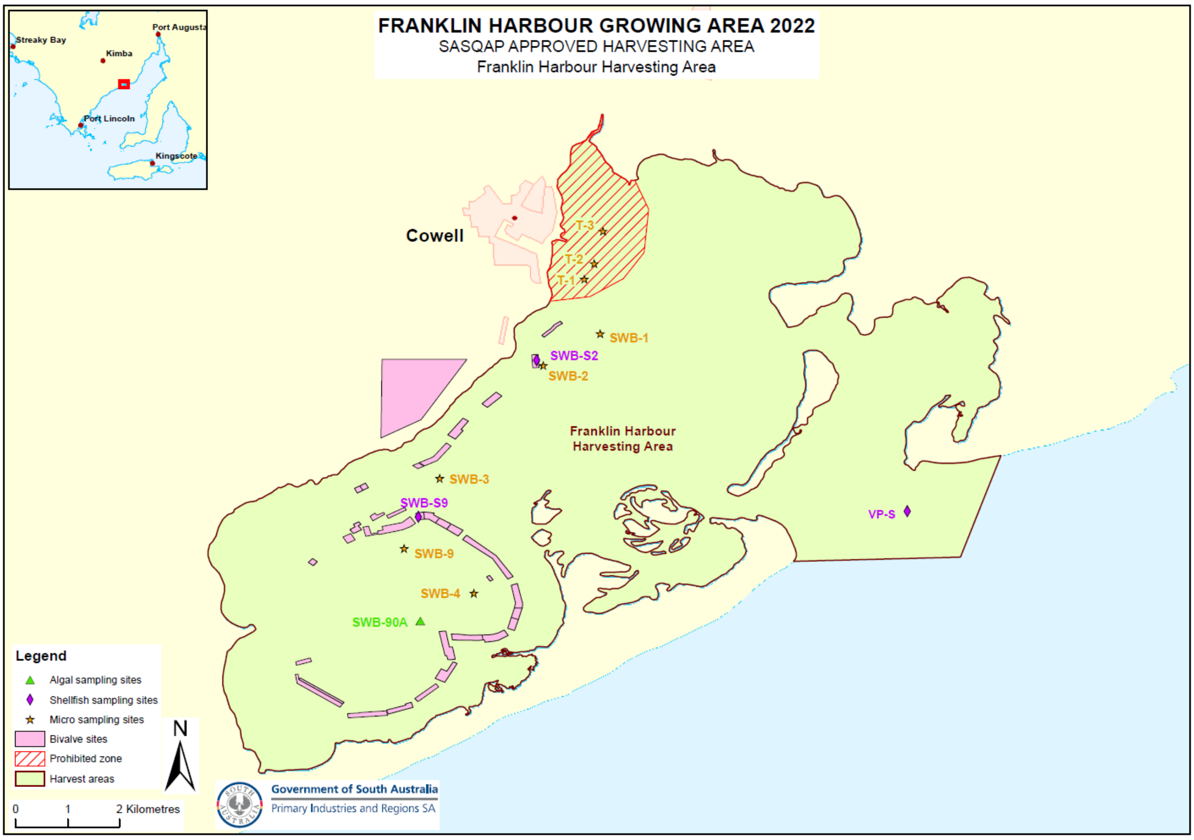
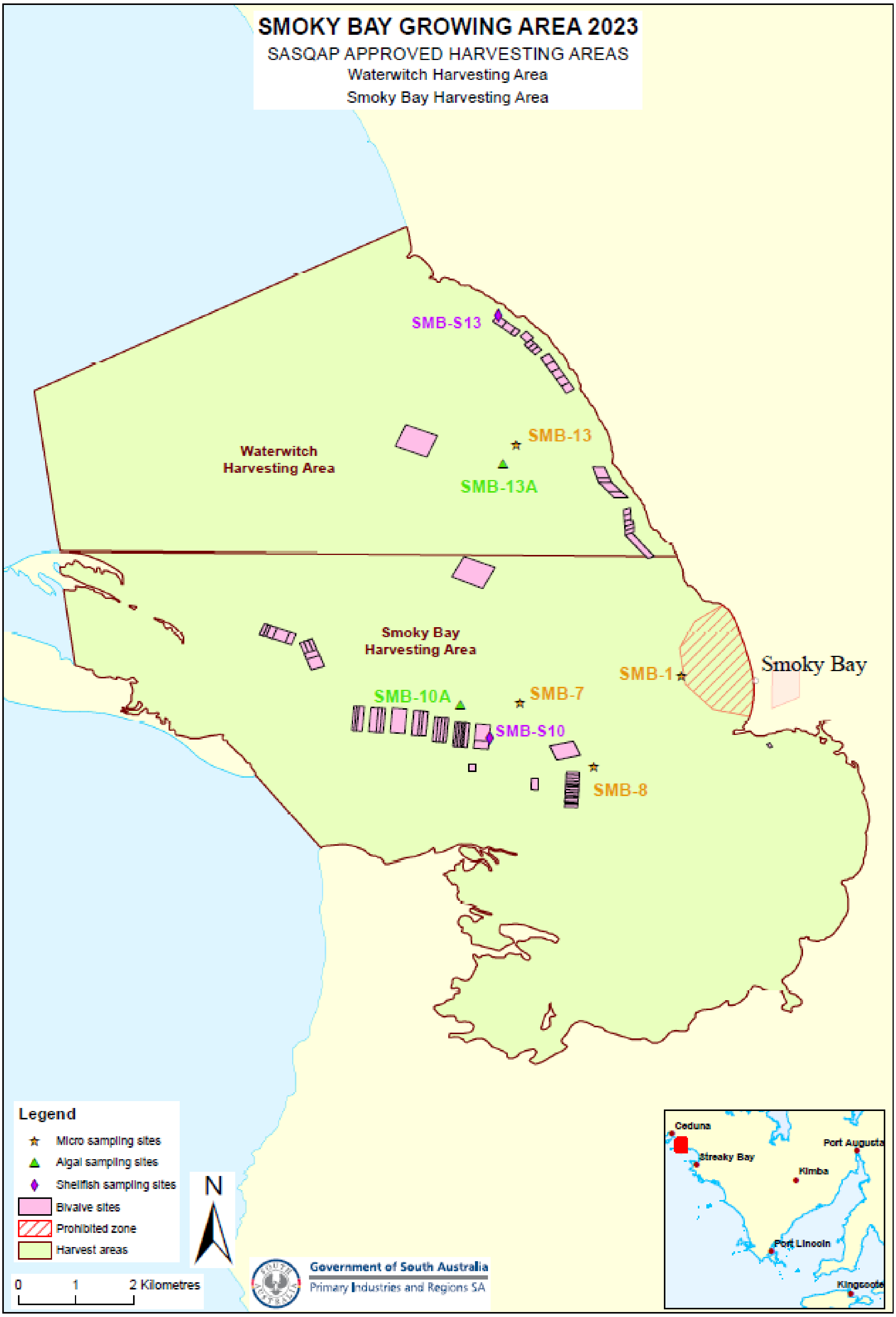


Figure 20: 6-day composite SST for the Southern Australia region from January 2021 to December 2021 (one image per week). Reproduced from IMOS (2023). Note the colour scale can vary from image to image.

Appendix 5 – SASQAP monitoring sites for Coffin Bay, Franklin Harbour, Smoky Bay and Streaky Bay growing areas





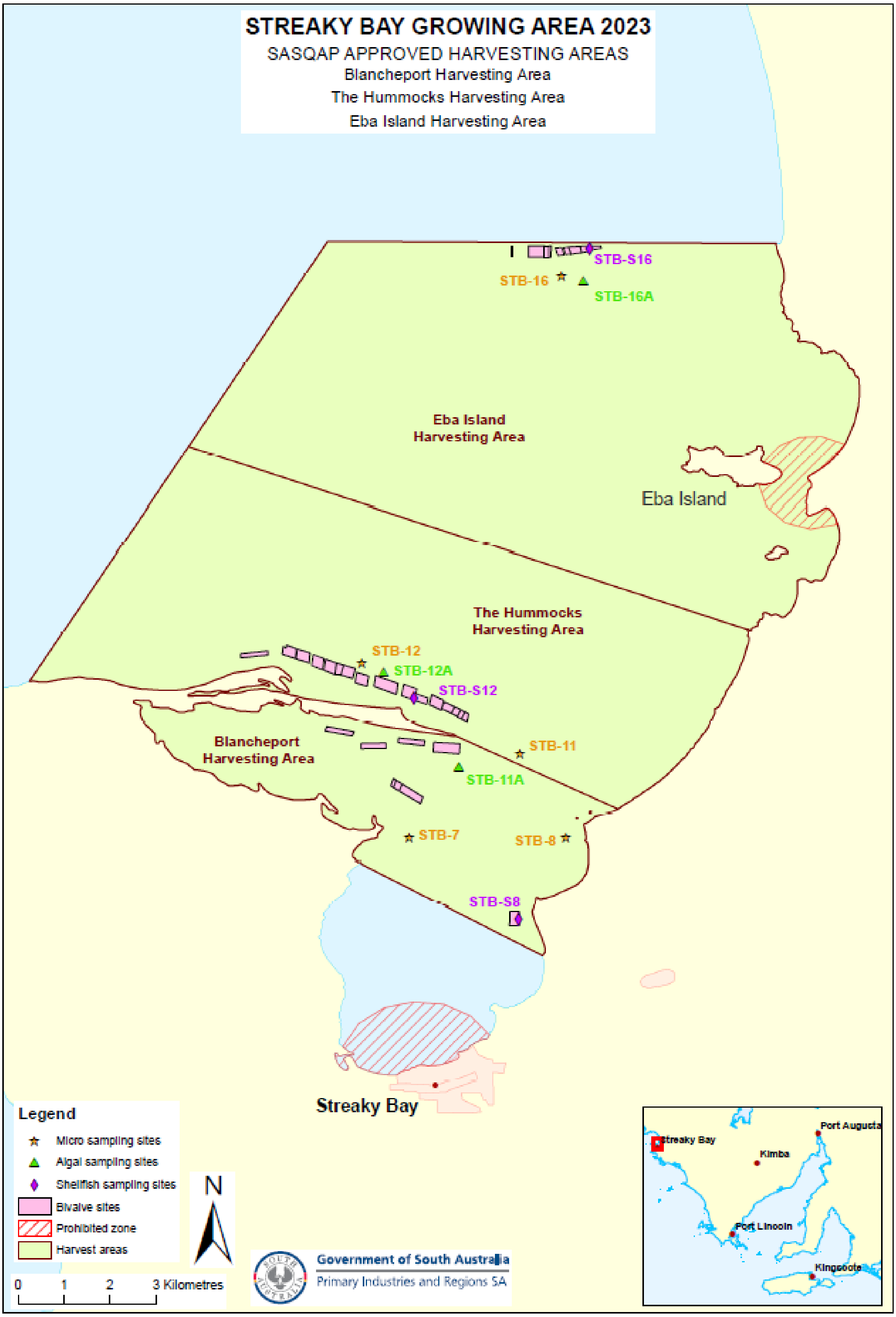


SMOKY BAY GROWING AREA 2023

SASQAP APPROVED HARVESTING AREAS

Waterwitch Harvesting Area

Smoky Bay Harvesting Area



STREAKY BAY GROWING AREA 2023

SASQAP APPROVED HARVESTING AREAS

Blancheport Harvesting Area

The Hummocks Harvesting Area

Eba Island Harvesting Area

Appendix 6 – References

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