Assessment of the Macquarie Harbour Broadscale Environmental Monitoring Program (BEMP) data from 2011-2020

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Acronyms

CTD/Sonde	Oceanography instrument used to measure dissolved oxygen, conductivity, temperature and depth
DO	Dissolved oxygen
DPIPWE	Department of Primary Industries, Parks, Water and the Environment
EPA	Environment Protection Authority
GAM	Generalised additive model
MHBEMP	Macquarie Harbour Broadscale Environmental Monitoring Program
MHEIS	Macquarie Harbour Environmental Impact Statement
N	Nitrogen
Total N	Total Kjeldahl nitrogen
WHA	World Heritage Area

Introduction

Salmonid aquaculture production in Macquarie Harbour (both Atlantic Salmon, *Salmo salar*, and rainbow trout, *Onchorynchus mykiss*) has steadily increased from <1,000 tonnes in the early 2000's to >20,000 tonnes in 2015/16. In 2012, an expansion of the lease area for salmonid aquaculture from 564 ha to 926 ha was approved that would enable production to increase to ~29,000 tonnes. The full level of production would be permissible provided several performance measures were met under an adaptive management framework. However, there have been notable declines in dissolved oxygen in Macquarie Harbour over recent years, which have resulted in a deterioration in sediment condition, including increased *Beggiatoa* bacteria and a decline in benthic infauna (Ross and Macleod, 2017). To reduce the pressure on the harbour and allow for environmental recovery, the maximum permissible biomass has progressively been lowered by the EPA since early 2017 (see http://epa.tas.gov.au/regulation/salmon-aquaculture/macquarie-harbour/management-determinations). In May 2020 biomass limit for salmonids (salmon and trout) in Macquarie Harbour was set at 9,500 tonnes for the next two years.

Potential environmental impacts of salmonid aquaculture, on both the water column and sediments, are well documented (Buschmann, 2006, Read and Fernandes, 2003, Cubitt et al., 2008, Soto and Norambuena, 2004). In the water column impacts stem predominantly from the introduction of nitrogen (N) and phosphorus (P) with only ~30% of the nutrients added through feed being removed as fish at harvest (Volkman et al., 2009). The availability of N has the potential to limit the primary productivity of coastal marine systems (Howarth and Marino, 2006) and consequently the extra N inputs from aquaculture have the potential to influence ecosystem dynamics. N from aquaculture enters the marine environment as overfeed, faeces and urine (Knoph and Thorud, 1996). Around 80% of the nutrients released into the environment are dissolved and immediately available to primary producers (e.g. phytoplankton, macro-algae and plants) (Volkman et al., 2009). The major nutrient released is ammonia derived from urine (Bergheim et al., 1991, Brett and Zala, 1975), which is readily available to phytoplankton.

The particulate organic N entering the environment as feed and faeces also contributes to the dissolved N pool. The particulate organic matter that settles to the seafloor is mineralised though a series of microbially mediated aerobic and anaerobic processes, and dissolved nutrients are released back into the water column. Under aerobic conditions, organic matter is mineralized and ammonia is released back into the water column, or following the conversion of ammonia to nitrate via nitrification, nitrate is returned to the water column (Ross et al., 2015).

Under anoxic conditions, nitrates are converted to gaseous N via denitrification or can be converted to ammonia via dissimilatory nitrate reduction (Ross et al., 2015).

The above processes dictate the availability of various forms of N and can therefore increase primary productivity with the potential to contribute to eutrophication and algal blooms, including harmful algal blooms (HAB), which affect salmonid aquaculture, other aquaculture operations (i.e. oysters, mussels) and the broader environment. Further, adjusted nutrient ratios can result in changes in composition of key phytoplankton species (i.e. from diatoms to dinoflagellates), which can lead to longer term shifts in ecosystem structure and function.

Much of the published research on the effects of aquaculture in Macquarie Harbour has focussed on dissolved oxygen concentrations and sediment faunal communities (MHDOWG, 2014, Ross and Macleod, 2017, Ross et al., 2016b) or the endangered Maugean skate (Bell et al., 2016, Treloar et al., 2017). The present study is the first to comprehensively assess data gathered within the Macquarie Harbour Broadscale Environmental Monitoring Program (MHBEMP), which is required to be undertaken as a license condition of the aquaculture companies operating in Macquarie Harbour. The MHBEMP gathers a suite of nutrient, physico-chemical and phytoplankton data throughout Macquarie Harbour and the major tributaries, with the goal being to determine whether aquaculture operations within the harbour are influencing the dynamics of the broader system. Here, the MHBEMP data is analysed in the context of other available information (e.g. catchment inputs, historical water quality data; see overleaf) with a focus on nitrogen[1] (N) given that coastal systems are typically N limited. The marine farming licence conditions require compliance with a set of environmental standards, this includes water column indicator limits for ammonia, nitrate, and oxygen. As such, the MHBEMP data will also be used herein to assess performance against these indicator limits. We also review the macrofaunal community data collected in Macquarie Harbour during FRDC projects 2014/038, 2015/024 and 2016/067 to provide information on harbour wide changes and recommendations for future monitoring.

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[1] This is also because phosphorus (P) is not measured in the MHBEMP program but see discussion and recommendations on the potential role of P and inclusion in subsequent monitoring.

Data used in the present study

MHBEMP data. As a requirement of Schedule 3 BEMP Macquarie Harbour - Marine Farming licence schedule relation to water quality monitoring, aquaculture companies are required to undertake monthly sampling of a suite of physio-chemical, nutrient and phytoplankton variables throughout Macquarie Harbour (Figure 1). This data, updated and maintained in the 'MacMaster' excel database, is used throughout this report, and a more detailed description of the sampling regime is detailed below.

Sense-T data. As part of a Sense-T project commissioned in 2016, permanent dissolved oxygen strings were placed at three locations in Macquarie Harbour. These comprised of a series of acoustic tags containing dissolved oxygen, salinity, temperature, and depth sensors being placed at a variety of depths, which transmitted unique signals at ~15-minute intervals to an acoustic receiver also attached to the string. Following completion of the Sense-T project, the strings were upgraded and maintained as part of FRDC 2016/067 from 2017-2020; they are currently managed and maintained by the TSGA and IMAS.

River flow and catchment inflow and nutrient input data. Data for riverine and catchment inflow were obtained from DPIPWE and Hydro Tasmania. Several imputations have been necessary throughout the time period investigated. Most notably since January 2013 the King River gauging station has been closed so a scaled relationship with the Mt Fincham gauge (1999–2013) was used to add the estimated pickup from Lake Burbury to the actual discharge from the John Butters power station. These estimations were undertaken by the Ecohydrology section of the Water Assessment Branch of DPIPWE (see Bryce Graham for further details).

Wastewater treatment plant nutrient data. One wastewater treatment plant has an outflow into Macquarie Harbour. Data pertaining to discharge volume and nutrient concentrations were supplied by the Environment Protection Authority, typically in the form of a monthly outflow and one nutrient concentration value. When monthly outflow was unavailable, the annual outflow was used to calculate the unaccounted outflow, and this was divided amongst the missing months in proportion to the contribution of that month to annual outflow during years when it was available.

Wind data. Three hour mean wind speed, wind direction and barometric pressure data were obtained for the Cape Sorell station from the Bureau of Meteorology.

Water elevation. Water elevation at Strahan wharf was obtained from Tassal. These data were initially gathered by the Ports authority but in recent years Tassal has taken over management of the gauge. Data were available from May 2002 – January 2018, however, the gauge was not operational during winters (and sometimes a portion of Autumn/Spring) of 2014, 2015 or 2016. Further, from the beginning of

October 2017 to the end of 2017 water elevation was permanently negative suggesting it was unlikely to be accurate. As such, data from this time period were removed from analysis.

Waverider buoy. Data from a Waverider buoy located at 42.12 S, 145.03 E ~10 km west of Cape Sorell were obtained from the Bureau of Meteorology. These data include information on wave height, wave period and wave energy at ten-minute intervals.

Feed and biomass data. The DPIPWE Marine Farming Branch provided a monthly breakdown of feed and nitrogen inputs for each lease in Macquarie Harbour.

Historically, N inputs were calculated based on an agreed N content of the feed (7.2%), however the exact N content of the feed is now known, and this has been used to calculate N inputs in recent years to ensure a more accurate assessment. Other factors used to convert feed quantities into N input are an agreed food conversion ratio (FCR) for the Tasmanian industry of 1.35 (i.e. 1.35 kg of dry feed produces 1 kg of fish), a digestibility coefficient of 90%, and a final estimate of the N content of the fish produced (3%) (Wild-Allen et al., 2005). When all these measures are combined ~5% of the feed enters the environment as N (with 15% of this being particulate and the remaining 85% being dissolved).

EPA water quality monitoring. The EPA has routinely undertaken quarterly water quality monitoring in Macquarie Harbour since 1993, which represents the longest time series available for the system (Figure 2). Three sites are sampled in the central harbour (Sites 12, 27 and 34; see EPA (2017) for site locations) using Sonde/CTD that measures standard physico-chemical properties of the water column such as oxygen concentration (absolute and saturation), salinity and temperature. Additionally, in the mid-late 1990's the EPA undertook a very detailed water chemistry and nutrient study of Macquarie Harbour and in recent years a number of these historical sites have been resampled. To enable direct comparison, only sites 11, 12 and 14 will be reported for nutrients as these are the only sites for which data are available for the two time periods. Sites 12, 27 and 34 are used for water chemistry as these sites provide the best longitudinal representation of the harbour of the sites for which data have been monitored continually.

Benthic surveys. Fourteen benthic surveys of Macquarie Harbour have been conducted during consecutive FRDC projects (FRDC 2014-038, FRDC 2015-024, FRDC 2016-067) beginning in early 2015 with the last being January 2020 (Table 1). The work was initiated when video footage from compliance monitoring identified an increase in abundance of Dorvilleid polychaetes. FRDC 2014-038 identified four sites (leases) for assessment. FRDC 2015-024 was commissioned to review the effectiveness of current monitoring protocols in new farming areas (i.e. Macquarie Harbour and Storm Bay in Southern Tasmania) and undertook a broader suite of sampling at the same Macquarie Harbour sites (leases) that were employed in project FRDC 2014-038. A major decline in the abundance and number of benthic faunal species was observed in the final survey of FRDC 2015-024 in October 2016 and it was felt that it was important to extend the research to assess benthic recovery and the effectiveness of fallowing, and as such FRDC 2016-067 was initiated. FRDC 2016-067 extended the benthic sampling to include an additional lease (lease 5) and more external sites. In this report we consider the status of external sites as lease sites were reported on in depth in the quarterly/biannual survey reports of FRDC 2015-024 and FRDC 2016-067.

Unless otherwise specified, all the above data was obtained up to and including June 2020 with one exception: data on macrofauna abundance was last collected in January 2020. Macrofauna community data is incorporated into analyses as any recovery in macrofauna communities is seen as an important step toward improving environmental conditions in Macquarie Harbour following fallowing and a general reduction in aquaculture biomass.

Sampling details

MHBEMP

The sampling locations for the MHBEMP are shown in Figure 1. Ammonia (comprising both ammonia and ammonium) and nitrate are sampled at the surface (2 m depth) and bottom (2 m above bottom) at all sites and at 10 m intervals throughout the water column at deeper sites (KR1, CHE, CH1/CH5, PET3, WHN, WH2). Total Nitrogen, measured as Total Kjeldahl nitrogen (TKN) is only required to be measured at five MHBEMP sites (CHN, CH1, WHN, WH2 and WH1) at the same depths as other nutrients. However, the TKN samples have been filtered, and as such, represent dissolved organic N and ammonia/ammonium (i.e. particulate organic nitrogen is not included). From July 2014, unfiltered TKN samples, hereafter referred to as Total Nitrogen, have been collected at sites C8, C10, WH2, GR2, HG3 and KR4. For the purposes of this report we examine patterns for both TKN (filtered) and TN given they represent different aspects of the nutrient conditions. Measurements are made by Analytical Services Tasmania and are reported in terms of mg of the main analyte in question (e.g. mg of N as NH_3). Detailed sampling methodology can be found in [Schedule 3 BEMP Macquarie Harbour](#).

Physico-chemical variables measured include oxygen concentration (mg/l), oxygen saturation (%), temperature, salinity (all using a Sonde/CTD) and Secchi depth. Phytoplankton are required to be sampled using two methods: 1) a sample from 2 m using a Niskin bottle, and 2) a 12 m integrated sample (i.e. from the surface to 12 m). In both the 2 m and 12 m integrated samples, chlorophyll-*a* concentration (mg/l) is measured, and phytoplankton counts are made at the lowest possible taxonomic level. Nutrient and water chemistry data are available since the commencement of the MHBEMP in October 2011, whereas phytoplankton data are available from December 2012 onward. Both data sets are analysed herein from when they began until June 2020.

Analyses showed that there was minimal spatial variation in Macquarie Harbour (detailed in results). Thus, temporal analysis was undertaken at a subset of MHBEMP sites (HG1, KR1, CHN, CHE, PET3, WH1 and WH2) spread throughout the harbour, which was enough to encapsulate both spatial and water column variation.

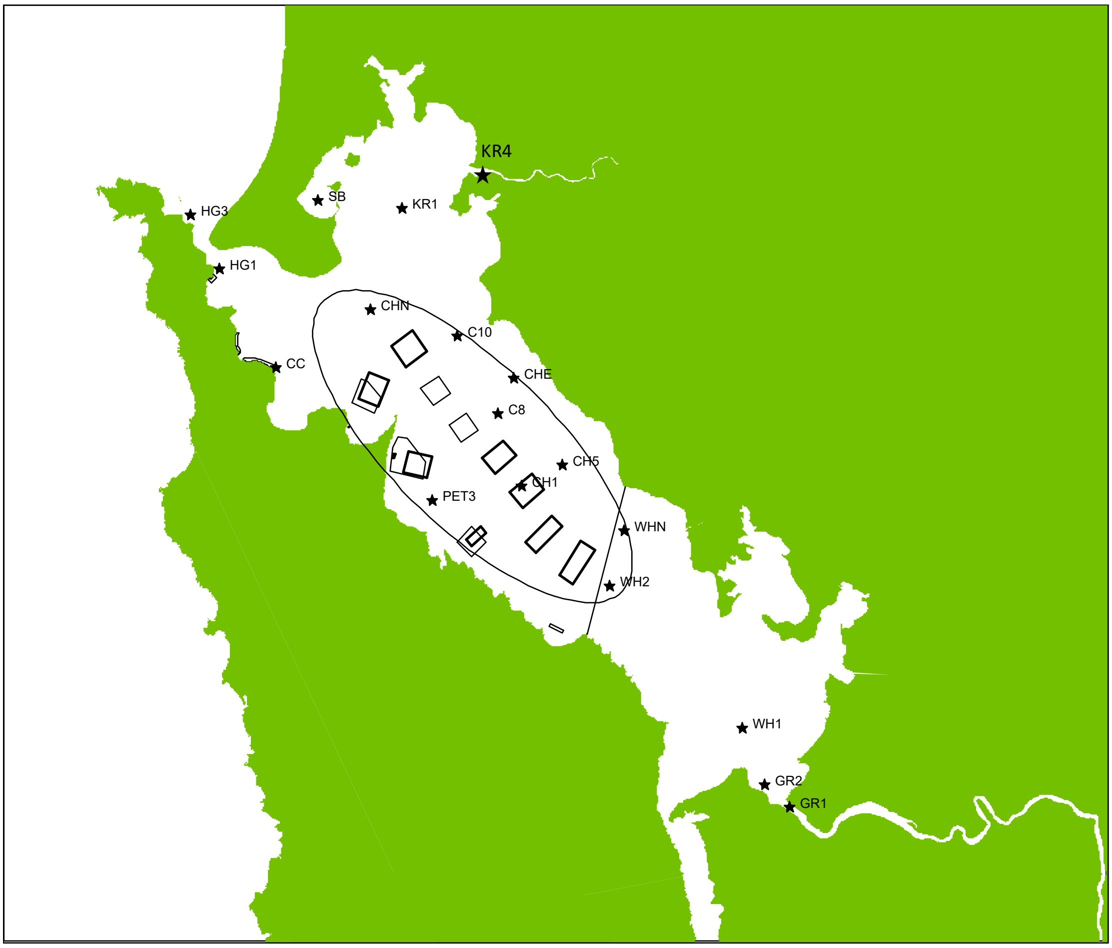


Figure 1 MHBEMP monitoring sites (stars) with lease boundaries (black squares) and the compliance region (circled). Source: MacMaster spreadsheet.

Benthic Surveys

During surveys 1, 4, and 7-14, 24 external sites in the Harbour were surveyed for benthic macrofauna and a suite of environmental sediment variables to assess the relationship between benthic communities and organic enrichment. A subset of these sites was surveyed during remaining surveys (i.e. surveys 2,3, 5 and 6). Five leases were also visited during this period; however, these will not be described here as they have been reported on extensively in Ross et al. (2020) (**Error! Reference source not found.**).

Benthic macrofauna were sampled in triplicate at each site using a Van Veen Grab (surface area 0.0675 m2). All grab samples were wet sieved to 1mm and preserved in 10% formalin: seawater (4% formaldehyde) in the field. They were then washed and stored in ethanol before being sorted, and the fauna were counted and identified to the lowest possible taxonomic level.

Redox potential and sulphide concentrations were measured from triplicate cores, using a quad-corer consisting of Perspex tubes (250mm long, 45mm internal diameter). In the laboratory, redox was measured at 3cm depth using a Hach HQ30d oxidation-reduction potential (ORP or redox potential) probe, calibrated with ZoBell's solution prior to analysis using the method described in Macleod et al. (2004). Redox potential was recorded once the probe had stabilised (i.e., when the meter displayed constant values for approximately 10 seconds). The probe was re-calibrated after every three measurements.

Sulphide concentrations in sediments were measured using a TPS WP-90 meter. Sub-samples of sediment (2 mL) were extracted from a port in the side of each core tube 3cm below the sediment surface using a 5 mL syringe. The samples were then placed in a glass vial containing 2 ml SAOB (refer to Macleod and Forbes, 2004). Sulphide levels were measured (mV) by placing the probe into the vial, and slowly stirring the sediment / buffer mix until the reading stabilised. The mV readings were converted to sulphide concentration using a calibration curve as outlined in Macleod and Forbes (2004).

A profile of the physio-chemical properties of the overlying water column (dissolved oxygen, salinity, pH and temperature) was obtained at each sampling location using a YSI EXO2 Sonde, with measurements recorded every 5m.

Table 1 Benthic survey details

Survey	Survey period	Reference in report	Study
1	6/1/2015 - 30/01/2015	January 2015	FRDC 2014-038
2	25/5/2016 - 4/06/2016	May 2015	FRDC 2015-024
3	8/9/15 - 18/9/2015	September 2015	FRDC 2015-024
4	9/2/2016 - 18-2-2016	February 2016	FRDC 2015-024
5	31/5/2016 - 21/06/2016	June 2016	FRDC 2015-024
6	11/10/2016 - 3/11/2016	October 2016	FRDC 2015-024
7	17/1/2017 - 16/2/2017	January 2017	FRDC 2016-067
8	16/5/2017 - 7/6/2017	May 2017	FRDC 2016-067
9	10/10/2017-25/10/2017	October 2017	FRDC 2016-067
10	16/01/2018-25/01/2018	January 2018	FRDC 2016-067
11	5/06/2018 - 20/06/2018	June 2018	FRDC 2016-067
12	15/01/2019 – 30/01/2019	January 2019	FRDC 2016-067
13	12/06/2019 – 26/06/2019	June 2019	FRDC 2016-067
14	21/01/2020 – 6/2/2020	January 2020	FRDC 2016-067

Data analysis

Nutrient conditions of Macquarie Harbour

To describe spatial and temporal patterns in nutrient concentrations at each depth for which data were consistently measured, generalised additive models (GAMs) were fitted to the time series at each site using the default settings of the stat_smooth function of the ggplot R package (version 3.2.2; http://www.r-project.org), which uses the 'mgcv' package (Wood, 2011) for GAM fitting. Analyses were limited to the following sites that have been consistently sampled throughout the time series: HG1, KR1, CHN, CHE, PET3, WH1, WH2, WHN and depth classes with fewer than 10 observations were also removed from analysis.

At each site, a nutrient measurement is taken at a depth of 2 m from the bottom. To incorporate this into analysis, 2 m was subtracted from the maximum depth at each site.

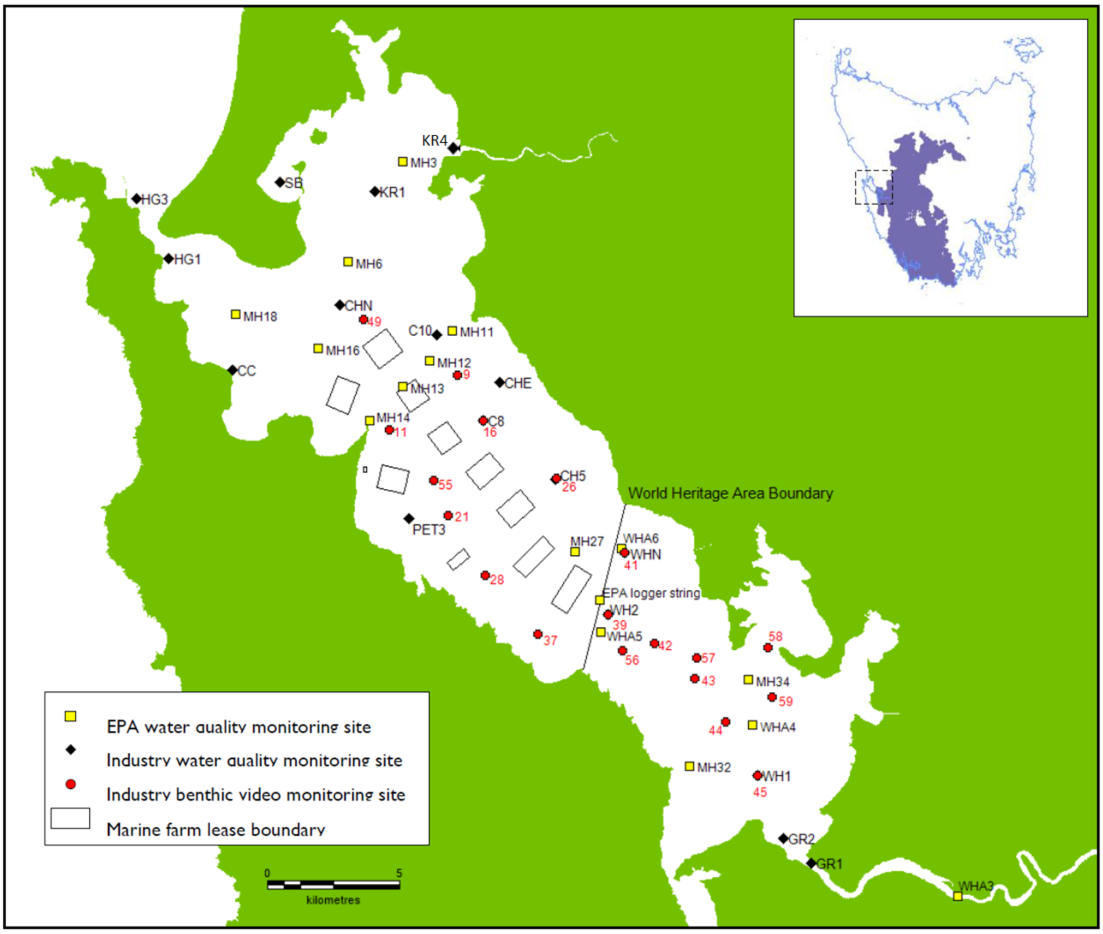


Figure 2 EPA monitoring sites. Source: EPA (2017).

To determine potential sources of nutrients (i.e. marine, freshwater or within Harbour), linear models were fitted to nutrient concentration and salinity data under the hypothesis that nutrients sourced from freshwater inputs would be negatively correlated with salinity and nutrients sourced from marine sources would be positively correlated with salinity.

It is important to note that it was not possible to analyse the importance of phosphorus, or in particular, dissolved forms such as phosphate in detail in the present study. These are not measured in the MHBEMP and the available data from the EPA sampling is limited and does not align with the more detailed MHBEMP sampling.

Riverine nutrient inputs

To estimate the nutrient concentration of riverine and catchment inflows into Macquarie Harbour, data from the two MHBEMP sites located in the King (KR4) and Gordon (GR1) Rivers were used. As there was no data available to estimate the nutrient concentration of inputs from the minor tributaries that discharge into Macquarie Harbour, it was assumed that concentrations were the same as from the Gordon River. The basis for this decision was that the Gordon River is fed from largely pristine environs, as are most of the minor tributaries discharging directly into Macquarie Harbour, whereas the King River is influenced by mining tailings which are less likely to be representative.

Dissolved oxygen analysis

To investigate spatial and temporal trends in dissolved oxygen concentration, GAMs were applied to the time series data at each site for each available 5 m depth category. This was undertaken identically to nutrient data, and depth categories with <10 samples were removed from analysis.

Phytoplankton analysis

Macquarie Harbour is highly stratified with tannin stained freshwaters on the surface resulting in low light penetration that limits primary productivity in deeper marine waters (Edgar et al., 1999a). As such, spatial variation in Secchi depth was compared using a Kruskal-Wallis test and pairwise comparisons made using Mann-Whitney tests to determine whether variation in light penetration is likely to influence phytoplankton communities. Additionally, analyses of spatial and temporal surface nutrient concentrations were undertaken using GAMs.

Chlorophyll-*a* data are in a similar format to nutrient data (i.e. both are received from AST within the same spreadsheet) so identical processing was used. As phytoplankton communities are not quantified at the MHBEMP river sites, chlorophyll-*a* concentration at these sites was investigated as a proxy for phytoplankton inputs into Macquarie Harbour. Chlorophyll-*a* is measured at 2 m at most sites, but in the King River it has been measured at 1 m at times. To facilitate temporal analyses, the chlorophyll-*a* measured at 1 m depth at this site was treated as 2 m, to ensure temporal compatibility.

Phytoplankton species were grouped into their major lineages (termed Phyla groups hereafter) to simplify analysis and reporting of the results: Bacillariophyta (diatoms), Chlorophyta (green algae), Cryptophyta (Chryptophytes), Chrysophyta (golden-brown algae), Dinophyta (Dinoflagellates), Prasinophyta (Prasinophytes), Prymnesiophyta (Haptophytes), Raphidophyta (Raphidophytes), Euglenophyta (Euglenoids), Cyanobacteria (blue-green algae) and "Unidentified nanoflagellates". In addition to simplifying the results, this grouping reduced the frequency of zero observations, which can compromise some analyses.

To determine the salinity preference of each Phyla group present in Macquarie Harbour, their abundance in the 2 m Niskin and 12 m integrated sample was compared using Welch t-tests. The assumption being that groups that were more abundant in the 2 m sample prefer low salinity, whereas groups that were more abundant in the integrated sample prefer high salinity. It must be noted that due to dilution in the 12 m integrated sample, the results are indicative only and may contain unrepresentatively high abundances of groups that aggregate at the pycnocline, which may prefer fresh water.

The influence of nutrients (ammonia, nitrate, total nitrogen, ammonia:nitrate ratio) and physico-chemical properties (temperature, salinity, Secchi depth and photoperiod) in dictating phytoplankton abundance and composition (Phyla group level) was investigated using the "bioenv" function of the R Vegan package. This method calculates the Spearman rank correlation between phytoplankton composition and the available environmental variables, which is used to determine which variable(s) best determine community structure and abundance. Detailed description of this technique can be found in Clarke and Ainsworth (1993). The lowest level of replication (i.e. each site/survey combination) was used for the analysis.

To further investigate the effects that the different environmental variables had on the abundance of the different phyla, a multivariate species distribution model was fitted to the data using a negative binomial distribution to account for overdispersion (due to the presence of multiple zeroes). The standardized model coefficients (forth coefficients) were optimized by incorporating a LASSO (Least Absolute Shrinkage and Selection Operator) penalty to improve interpretability. Given that coefficients are standardized, the resulting species-specific interaction terms indicate the relative importance of each variable for abundance. The model was fitted using the mvabund library in R.

Macrofauna community analysis

To determine if there has been harbour wide benthic changes in response to farming, the temporal change in the key response parameters (i.e. redox, sulphide and macrofauna) was investigated. To visualise temporal change in total abundance, the number of species and environmental variables at each external site, data were graphed using bar or stacked dot charts produced in R (R Core Team 2014). Plots of response variables by time were fitted with a smoother using loess (± SE).

To examine the relationships between sites and time in macrofaunal communities and their relationship with environmental variables, community data was analysed using PCO and MDS ordinations in PRIMER (PRIMER 7). Macrofaunal species and environmental variables that were associated with the observed patterns of macrofaunal assemblage distribution in the MDS were visualised using vector overlays of Pearson correlations ($R>0.5$). Macrofauna and environmental variable data from surveys 1, 4, 7-14 was used in this analysis as these surveys had the most complete number of sites and a full suite of environmental variables were sampled. Sulphides were removed from the analysis as they were only measured from surveys 7-14 and preliminary analyses (not shown here) revealed that there were no temporal patterns detected and that they contributed little to the patterns of macrofaunal distribution ($R < 0.1$).

Results and discussion

Nutrient inputs into Macquarie Harbour

Riverine nutrient inputs

To ensure that the two downstream sites (KR4 & GR1) were representative of river nutrient concentrations and not influenced by harbour sources, the salinity at the time and depth (0 m and 1 m for the King River and 2 m for the Gordon River) that nutrient samples were taken was investigated. Salinity was frequently elevated in the Gordon River but rarely in the King River (

Figure 3). Thus, to prevent harbour sources from influencing the calculation of riverine nutrient inputs, nutrient concentrations were eliminated from analysis when salinity was >1 ppt.

Linear models were used to investigate the influence of river flow on nutrient concentration (Figure 4). There was a significant relationship with river flow for nitrate in the Gordon and ammonia in the King River; total nitrogen increased with flow in the King River, but the relationship was marginally not significant (**Error! Reference source not found.**). However, given that the significance of the relationship between nutrients and flow was not consistent between the two rivers, and that model fit was relatively poor across all variables, end member contributions were calculated based on median concentrations.

To calculate riverine nutrient inputs at the sites located near the river mouths, the median concentration of each analyte was multiplied by flow (Figure 5, Figure 6). Monthly inputs are provided for the years of the MHEMP monitoring period only (2011 – 2020) to highlight intra-annual variation (Figure 7). Not surprisingly, there is a strong seasonal trend with the greatest inputs typically occurring during the winter months, when catchment rainfall is high. This seasonal trend was less apparent for the Gordon River from 2012 – 2014, when unseasonably high nutrient inputs entered the harbour during the summer and autumn of 2013/14 presumably following increased river flows due to hydro-electricity generation from the Gordon power plant (MHDOWG 2015). This trend was not observed in the King River or from the rest of the catchment; thus, increased flows in the Gordon River was likely responsible for the high nutrient inputs in 2013.

Annual riverine and catchment nutrient inputs are dominated by the Gordon River with the King River having secondary inputs and the remaining catchment having only minor inputs (Figure 8). Inputs of nitrate are approximately double that of ammonia with total nitrogen being approximately an order of magnitude greater than nitrate inputs.

The riverine and catchment nutrient loads calculated herein are 20–30% lower than those obtained by previous studies (MHEIS, 2011, MHDOWG, 2015). These studies either purely (MHEIS, 2011) or heavily (MHDOWG, 2015) relied on riverine nutrient concentrations gathered between late 2010 and early 2012 for the Environmental Impact Statement (EIS) when nutrient concentrations were higher than most of the subsequent sampling undertaken during the MHBEMP. Additionally, the 80th percentile was used during the latter assessment (MHDOWG, 2015) to estimate loadings from the King River to minimise the risk of underestimating river loadings, however, in the present study we used the median throughout as there is more data available now and there was no reason to believe this was not appropriate.

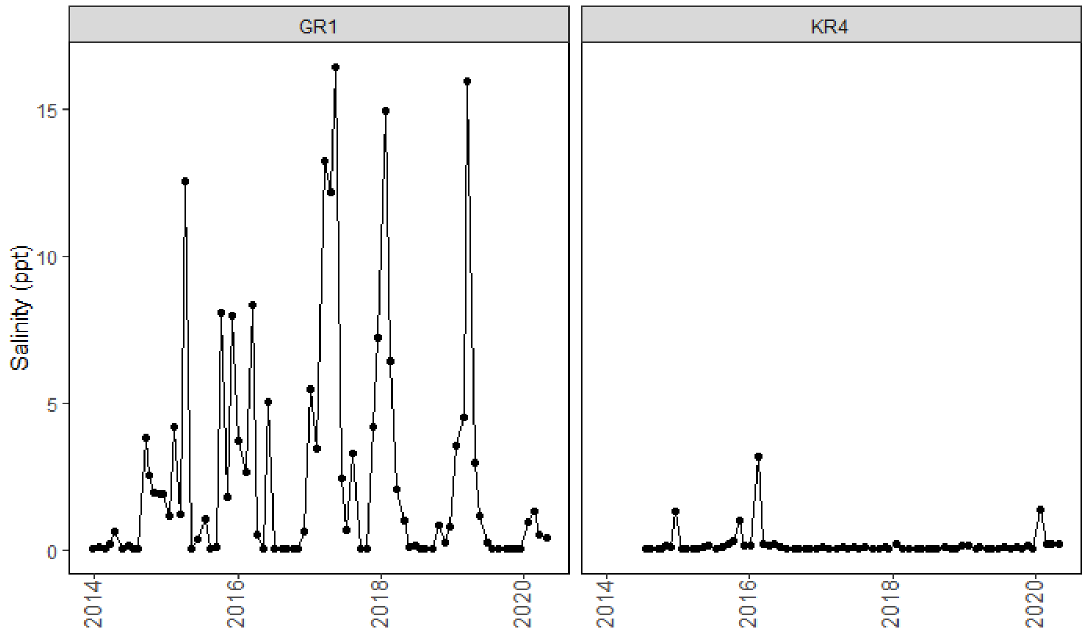


Figure 3 Salinity at the time of Macquarie Harbour BEMP sampling in the Gordon (GR1 2m) and King (KR4 <2m) River mouths

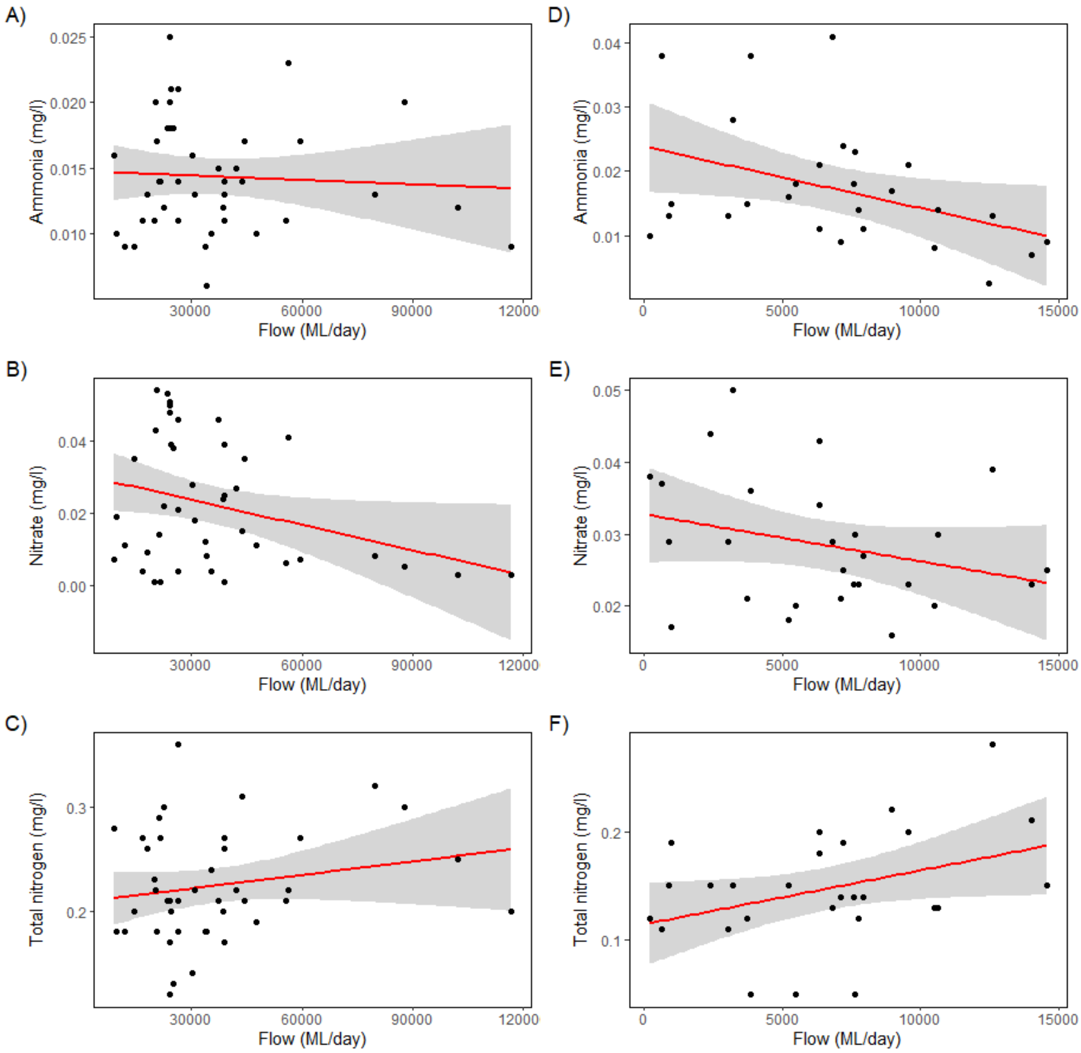


Figure 4 Linear models of Gordon (A-C) and King (D-F) River flow and nutrient concentration. The shaded area represents 95% confidence intervals of the model.

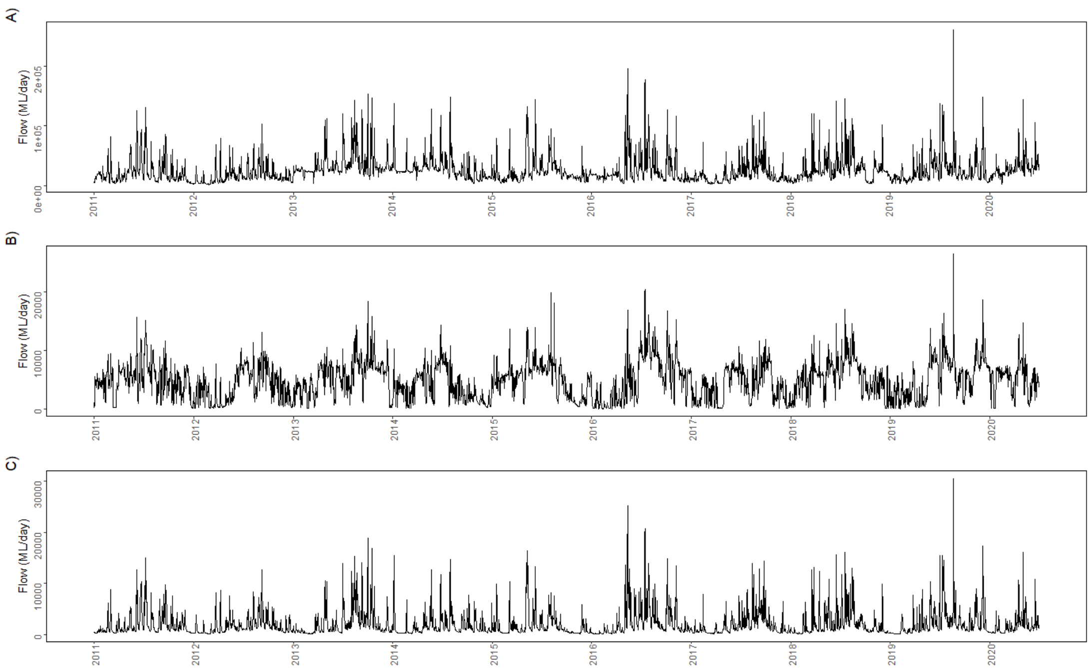


Figure 5 Daily inflows (ML/day) from the Gordon (A) and King Rivers (B) and the smaller tributaries (C) (termed 'Catchment') between 2011 and 2020.

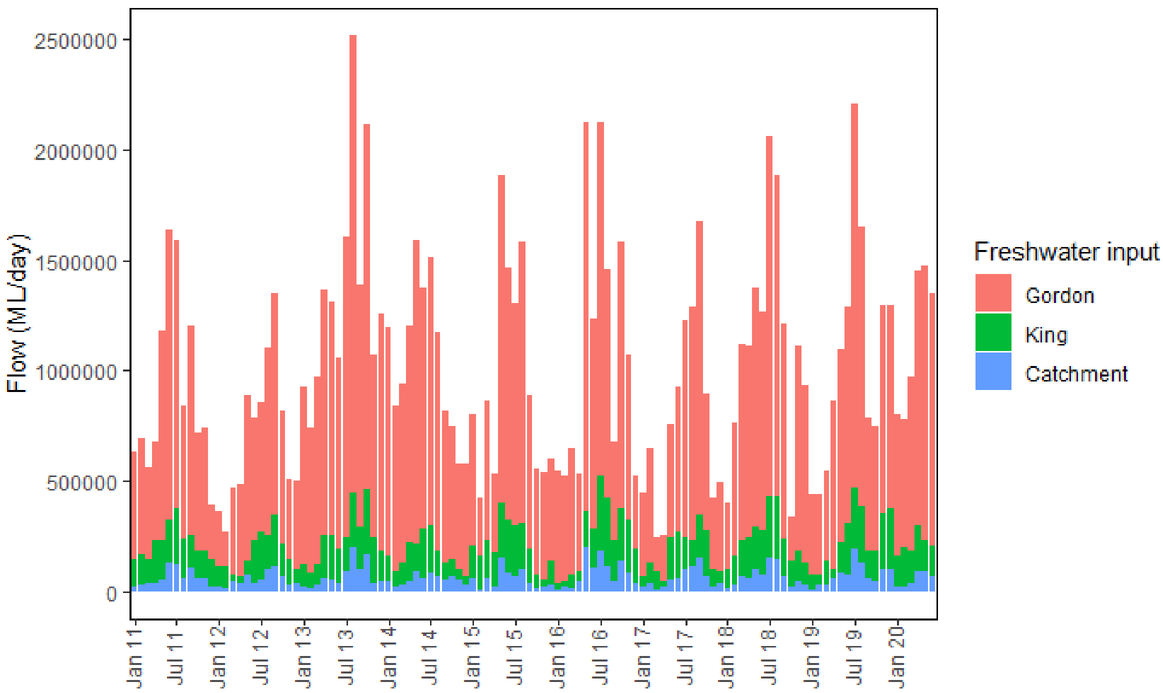


Figure 6 Monthly inflows from the Gordon and King Rivers and the smaller tributaries (termed 'Catchment').

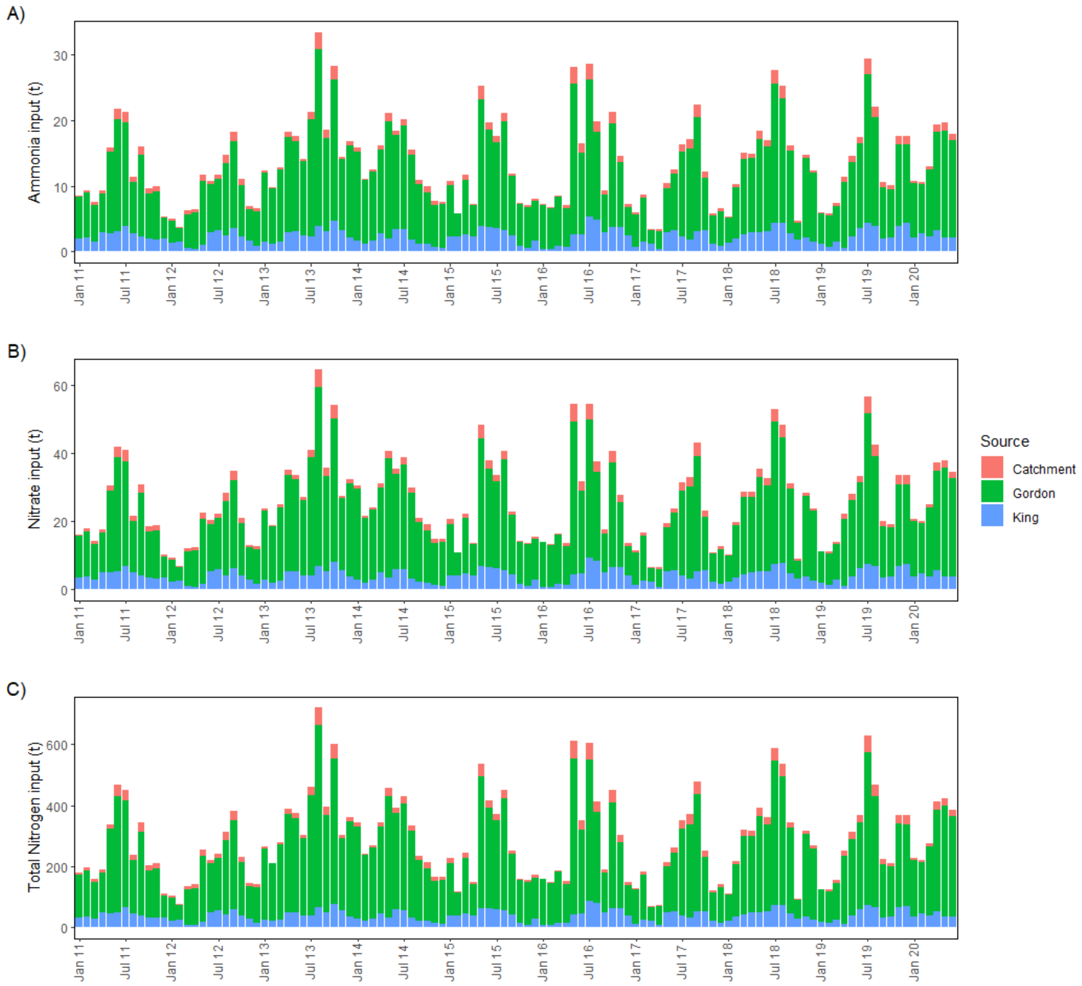


Figure 7 Monthly ammonia (A), nitrate (B) and total N (C) inputs from the Gordon and King Rivers and the smaller tributaries (termed 'Catchment')

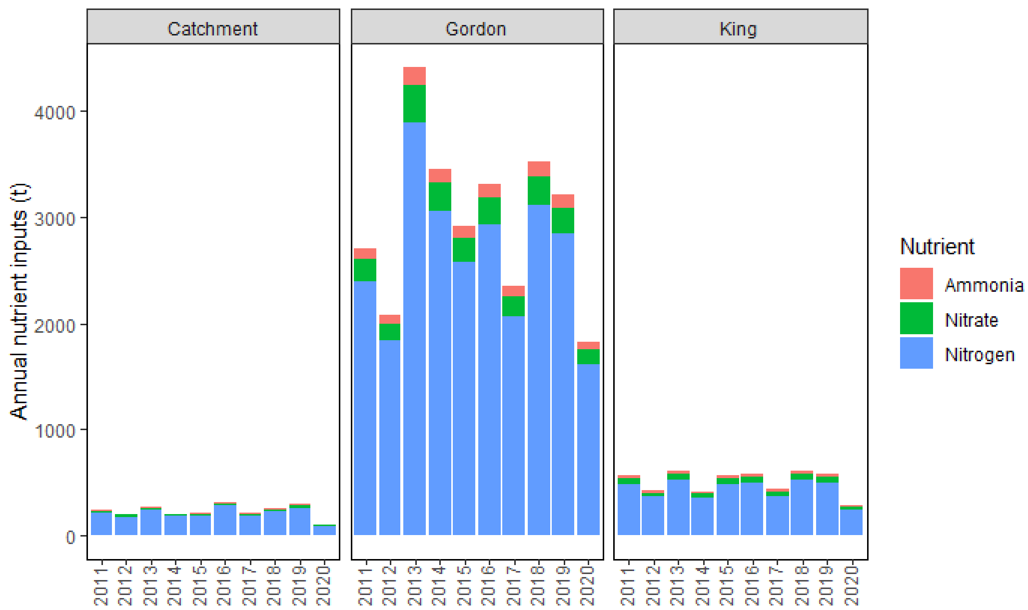


Figure 8 Annual ammonia, nitrate and total N inputs from the Gordon and King Rivers and the smaller tributaries (termed 'Catchment').

Wastewater treatment plant nutrient inputs

Annual nutrient inputs (ammonia, nitrate, nitrite, NOx, total N, and total phosphorus) from the WWTPs have been relatively consistent throughout the time period investigated (Figure 9). Although there are not clear and consistent seasonal patterns, inputs do often appear to be greater through summer and autumn (Figure 10). Within the context of nutrient cycling in Macquarie Harbour, the inputs from the WWTPs are relatively small (e.g. compared to river and aquaculture inputs), and given there has been no observable temporal trend, WWTP nutrient inputs are unlikely to have a major influence on harbour wide nutrient trends.

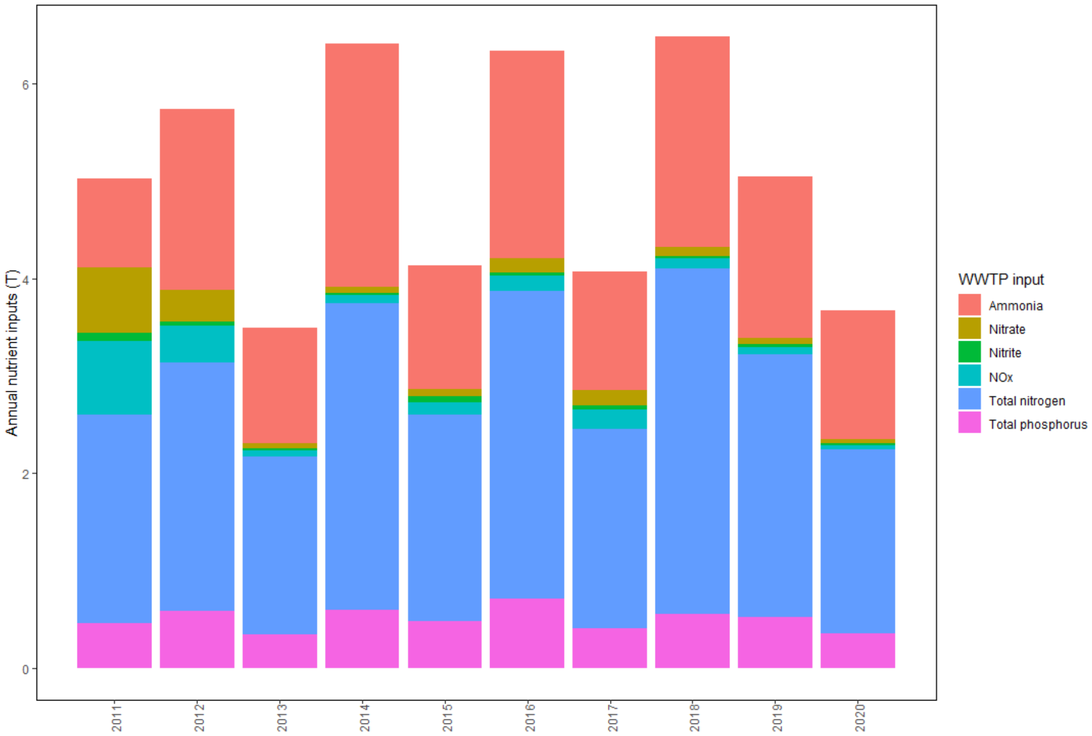


Figure 9 Annual nutrient inputs from the Strahan WWTP. Data from 2020 only represents 6 months of sampling.

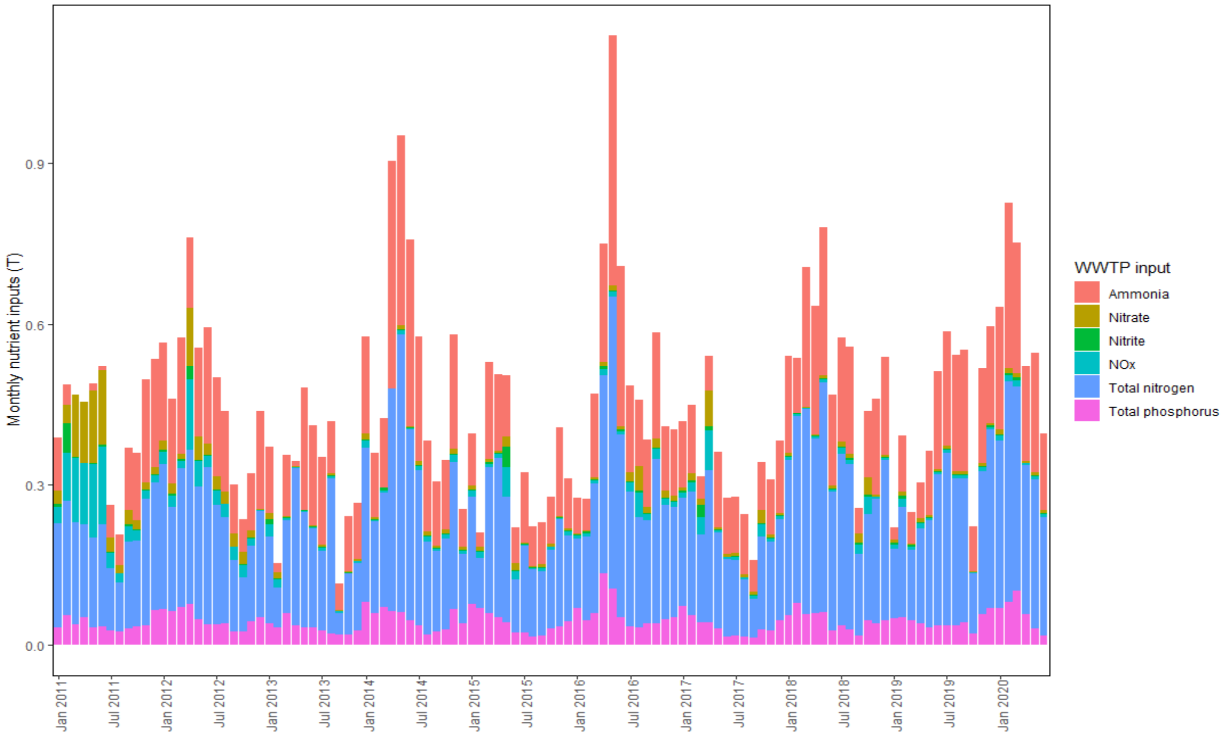
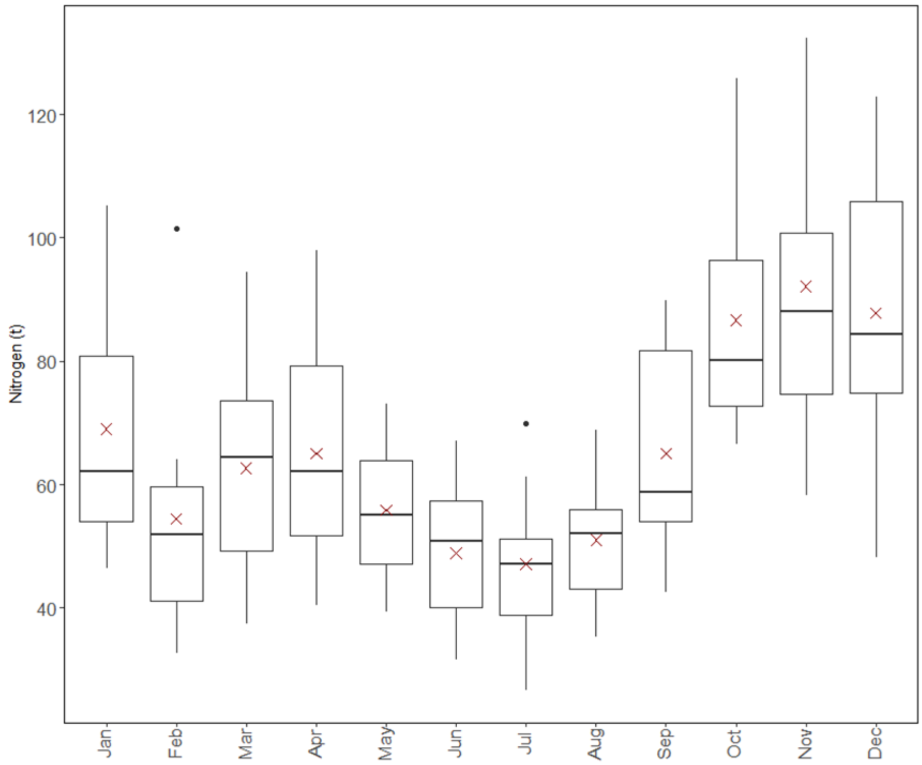


Figure 10 Monthly nutrient inputs from the Strahan WWTP.

Aquaculture nutrient inputs

Nitrogen inputs from aquaculture follow a consistent seasonal pattern with peak inputs reached in late spring to early summer and the lowest inputs occurring in late autumn to winter (Figure 11). Inputs increased steadily from 2008 to reach a maximum of ~1000 tonnes in 2014 and 2015 (Figure 12). Following harbour wide declines in DO and associated declines in benthic faunal abundance (Ross et al., 2017), the permissible aquaculture biomass in Macquarie Harbour has been reduced, and this has seen nitrogen inputs decline since 2017 (except for a slight increase in 2019) .

In 2011-12, nitrogen inputs from aquaculture were restricted to the northern area of the main Macquarie Harbour basin, but from 2013 onward, inputs extend southwards associated with the expansion of the industry (Figure 13).



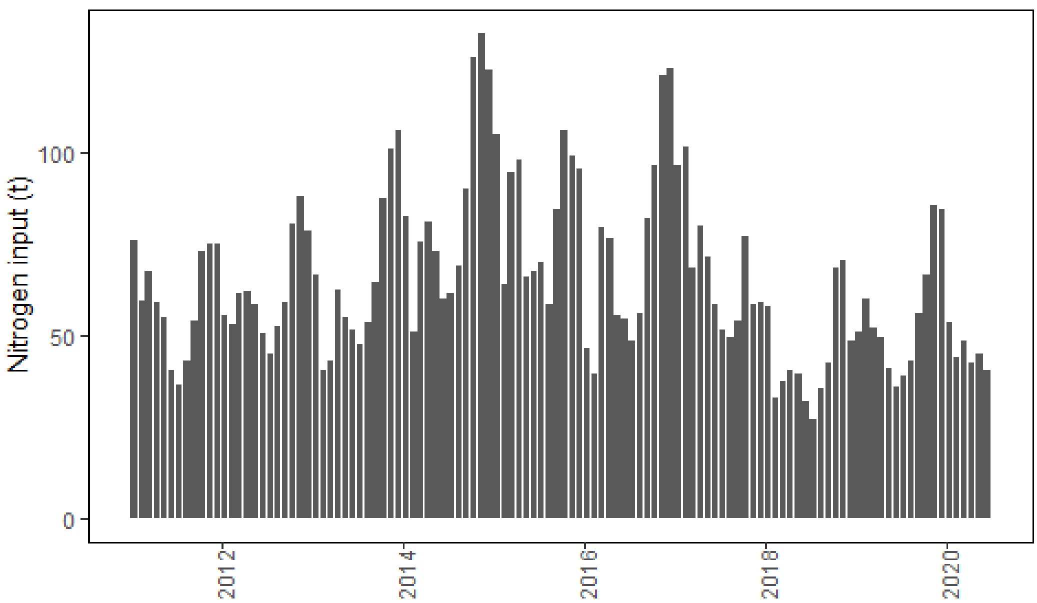


Figure 11 Seasonal (top) and monthly (bottom) nitrogen inputs from aquaculture in Macquarie Harbour.

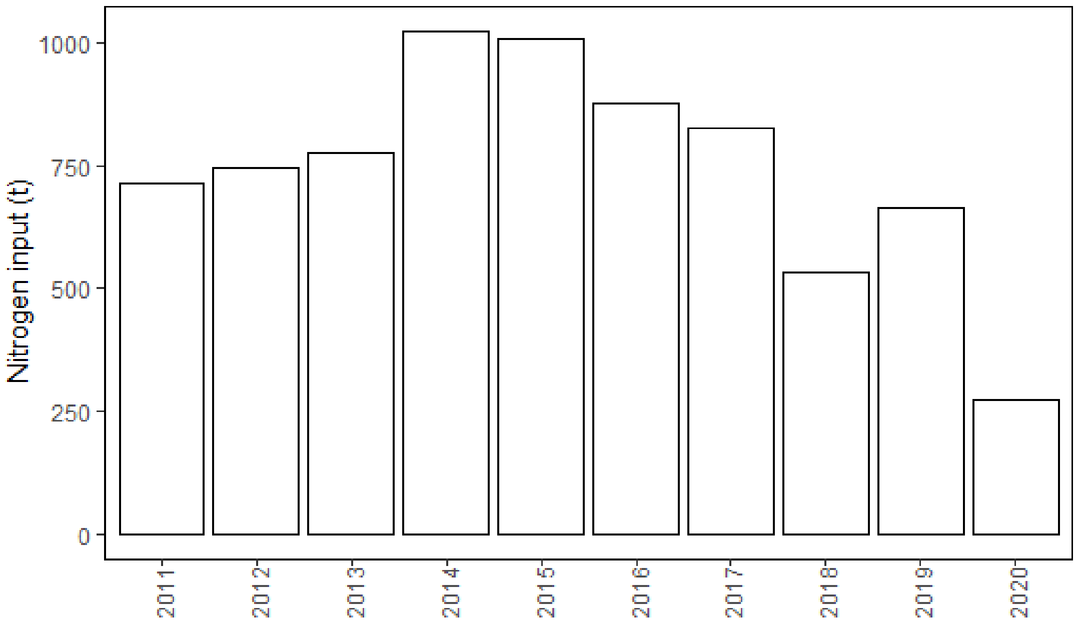


Figure 12 Annual nitrogen inputs from aquaculture in Macquarie Harbour. *2020 total nitrogen (t) only includes values from Jan to Jun.

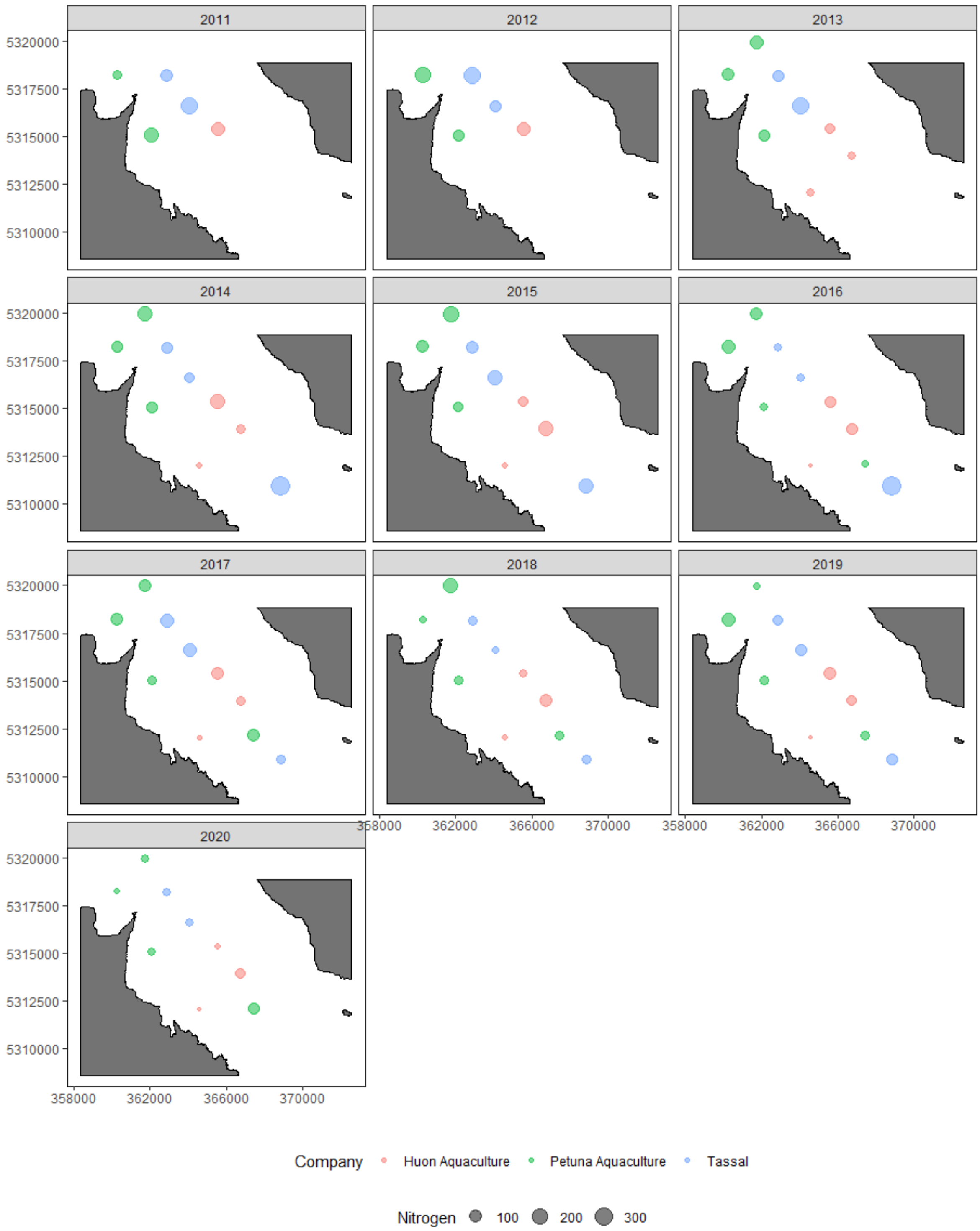


Figure 13 Annual nitrogen inputs from aquaculture in Macquarie Harbour from 2011-2020. Coordinates in m (UTM zone 55S). *2020 total Nitrogen (t) only includes values from Jan to Jun.

Origin of nutrients in Macquarie Harbour

To help determine the origin of nutrients in Macquarie Harbour (i.e. oceanic, riverine or internal), the relationship between nutrient concentration and salinity (where these variables were measured concurrently) was explored at the end member MHBEMP sites (GR1, KR4and HG3) and one internal MHBEMP site (WH2). Linear regression was used to determine whether a significant relationship between these variables existed. WH2 was chosen as the internal site because it is one of the deepest and most southern of the MHBEMP sites where nutrient residence times (e.g. greater capacity for nutrients to accumulate) are likely to be longer.

Ammonia concentration has a significant negative relationship with salinity (Table 2) suggesting ammonia is sourced from riverine inputs and/or in the surface freshwaters of the harbour. Ammonia concentration was low at the Hells Gate site and in the deeper waters at the internal site (Figure 14) suggesting minimal influx from the ocean into deeper waters, or that inputs reaching deeper waters are rapidly nitrified. The latter is supported by a strong positive relationship that exists between nitrate and salinity at WH2 and to a lesser extent GR1 (Figure 15). The relationship for nitrate suggests that the end members (rivers and/or ocean) aren't the major sources of nitrate with lower concentrations in low salinity waters at the river mouths (KR4 and GR1) and high salinities at Hells Gate (HG3). Thus, the elevated nitrate concentrations at high salinities at WH2 are most likely due to a source of nitrate within the harbour and it seems likely that ammonia is being rapidly nitrified and converted to nitrate. As described above, aquaculture is the major source of ammonia in the harbour, however, ammonia is also produced following the mineralisation of particulate nitrogen inputs from the catchment, ocean, and aquaculture, and as such they may also be important indirect sources of ammonia in the harbour. Although it is unclear whether the conversion of ammonia to nitrate occurs throughout the entire water column, the increase in nitrate concentrations with salinity in deeper waters suggests that nitrification is significant below the halocline, either in the sediments or the water column. This is consistent with the findings of Ross et al. (2016a) and Maxey et al. (2016) who found that the abundance of ammonia oxidising archaea and rates of nitrification, respectively, increased markedly with depth in the harbour.

There is a weak, but significant, positive relationship between total N and salinity (Figure 16; Table 2). At all four sites, there was high variation in total N at low salinity, which may represent seasonal variation in the abundance of phytoplankton or prokaryotes. The elevated total N at WH2 in deeper waters, potentially represents a high concentration of prokaryotic nitrifiers, which would explain the pattern in elevated nitrate in the deeper waters at this site.

Table 2 Linear models of the influence of salinity on ammonia, nitrate and total nitrogen at end member (GR1, KR4 and HG3) and one internal (WH2) MHBEMP site.

Nutrient	Coefficient	df	Adjusted R^2	Estimate	Std. Error	t	Pr(>|t|)
Ammonia	Intercept	3027	0.31	0.0155	2.00E-04	78.6763	<0.001
	Salinity			-3.00E-04	0	-37.302	<0.001
Nitrate	Intercept	3027	0.4	0.016	0.001	14.477	<0.001
	Salinity			0.002	0	44.986	<0.001
Nitrogen	Intercept	3030	0.13	0.176	0.002	111.307	<0.001
	Salinity			0.002	0	21.014	<0.001

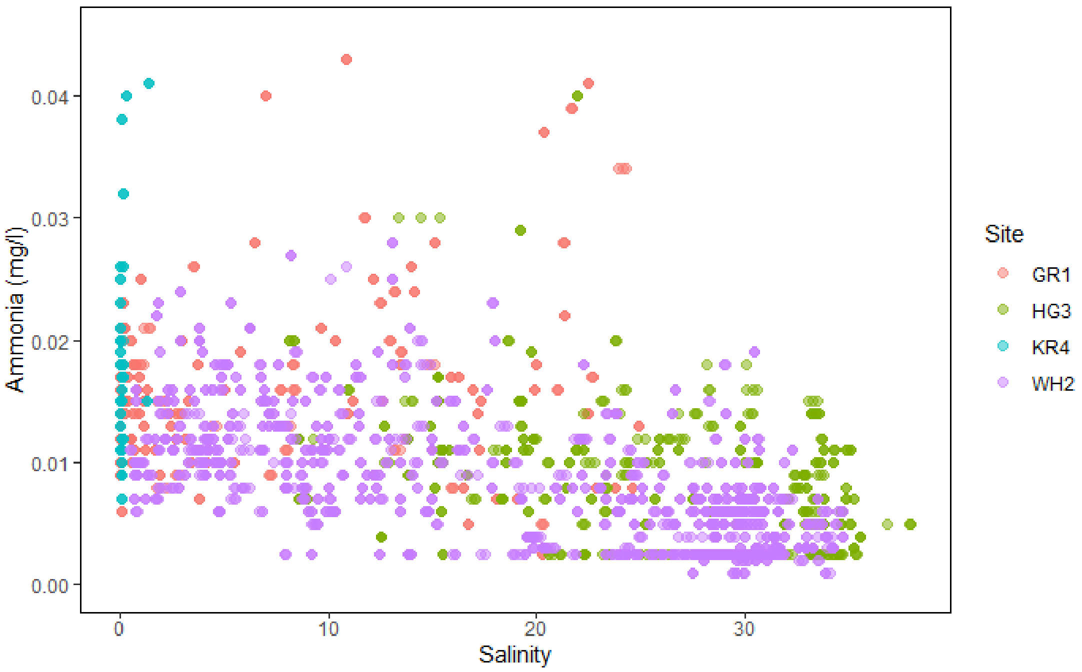


Figure 14 The relationship between salinity and ammonia at the entrance to Macquarie Harbour (HG3), the two main rivers (Gordon – GR1 and King – KR4) and one central MHBEMP site (WH2).

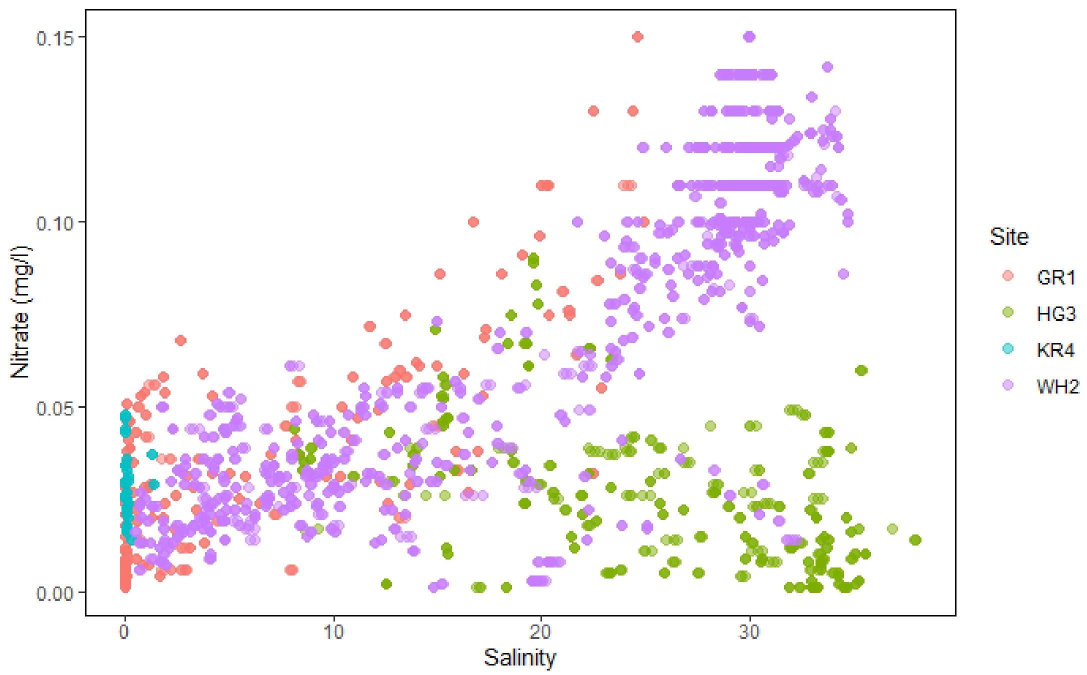


Figure 15 The relationship between salinity and nitrate at the entrance to Macquarie Harbour (HG3), the two main rivers (Gordon – GR1 and King – KR4) and one central MHBEMP site (WH2).

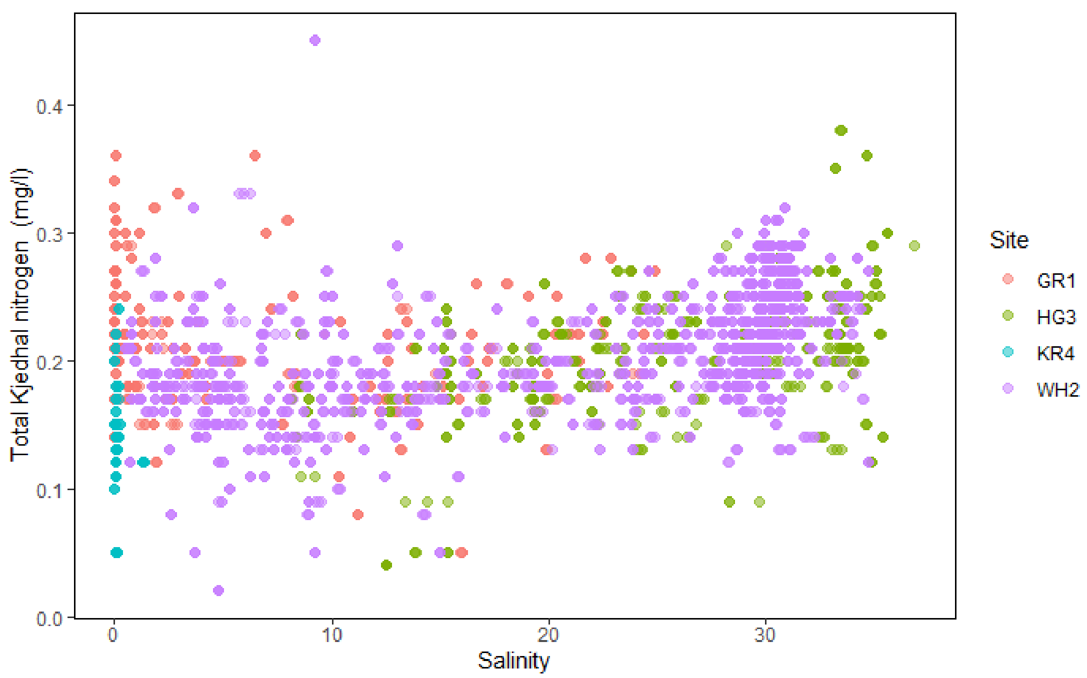


Figure 16 The relationship between salinity and total N at the entrance to Macquarie Harbour (HG3), the two main rivers (Gordon – GR1 and King – KR4) and one central MHBEMP site (WH2).

Nutrient conditions in Macquarie Harbour

The following describes the spatial and temporal variation of nutrient concentrations in Macquarie Harbour using available data. A more detailed analysis of the surface waters is undertaken within the phytoplankton analyses to specifically investigate how surface nutrient concentrations affect phytoplankton abundance and composition.

Ammonia

Ammonia concentrations varied throughout the water column, most likely due to the influence of stratification on vertical mixing (Figure 17). Concentrations at depths <10 m were higher and relatively consistent across sites. At depths >10 m, ammonia concentration was typically half the concentration of the surface waters.

An important component of analysing nutrient concentrations in this study is to identify spatial and temporal patterns of surface (2 m) nutrient concentrations as these could influence phytoplankton composition and abundance (Figure 17). Surface ammonia concentrations are highly variable throughout the year, with higher concentrations from late autumn to early winter and again in spring (Figure 18). Lower surface concentrations were observed in summer, and late winter. A decease in ammonia concentrations during summer may potentially be associated with a decrease in aquaculture feed inputs following the peak in spring (Figure 11), but it also may relate to uptake by primary producers while the photoperiod is at its longest. During winter there is also a reduction in aquaculture feed inputs; however lower concentrations of ammonia during these months may be associated with increased river flows, which reduces the residence time of surface waters.

Ammonia concentration in surface waters showed little spatial variation (Figure 19); however, slightly elevated concentrations were typically found in the central harbour (CHN, CHE, CH5, CH1 and PET3) and near the Gordon River (GR1 and GR2). This potentially reflects ammonia sourced from both rivers and aquaculture, as discussed in the previous section. The lowest concentrations were typically observed near the entrance of Macquarie Harbour suggesting oceanic waters contain low ammonia concentrations and are unlikely to contribute to ammonia loads in Macquarie Harbour.

Ammonia concentrations are highly variability in waters <10 m making longer term temporal trends difficult to discern (Figure 17). Nonetheless, there does appear to a downward trend in ammonia concentrations through time across most sites. Given that there is no discernible trend in catchment inputs over the same period, the reduction in nitrogen inputs from aquaculture in surface waters over recent years may help explain the trend in surface waters. At depth (>10 m) ammonia concentrations are lower and less variable, but again there is evidence of a downward trend at some of the sites (Figure 19). This may also reflect the reduction in the inputs from farming, either directly through inputs of ammonia via excretion or indirectly via inputs of ammonia following the remineralisation particulate waste inputs in the sediments. Nonetheless, the comparison of the more recent EPA data with that collected in the mid-1990s indicates that ammonia concentrations at 0 – 5 m, and to a lesser extent 5-10m are higher now at the three sites for which data are available (Figure 20). However, given the paucity of data between these data sets, these trends and their interpretation should be treated with caution.

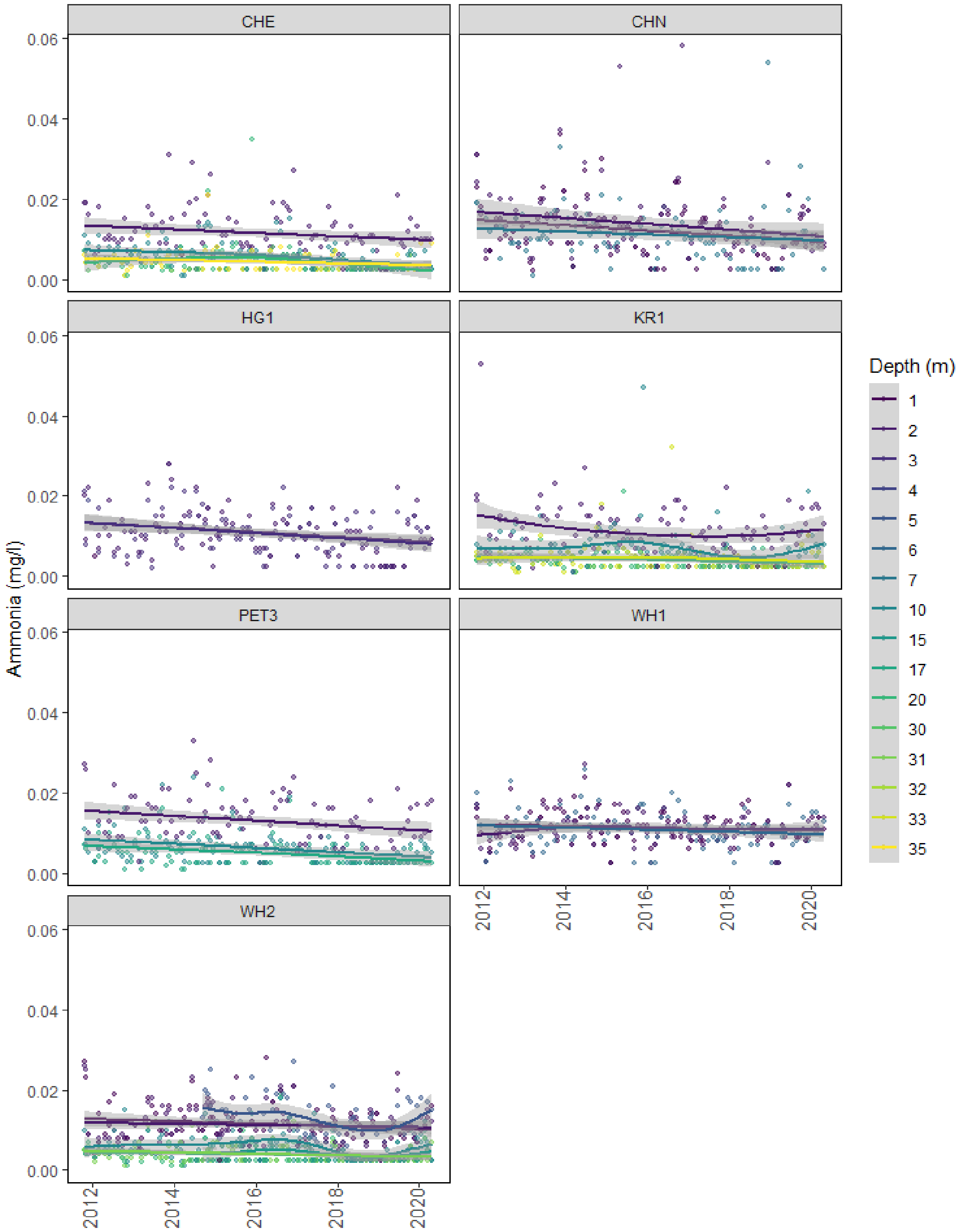


Figure 17 GAMs displaying temporal trends in ammonia concentration at the various depths it is measured in MHBEMP sampling. Note: A single outlier of 0.094 mg/L measured at KR1 was omitted from this figure as it distorted the trend of the remaining data.

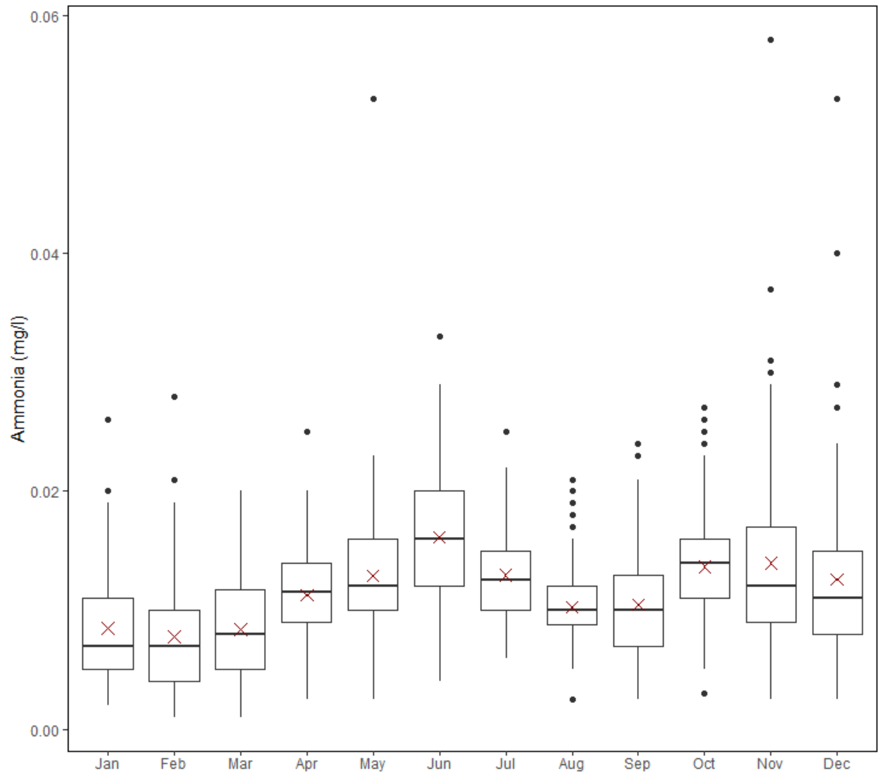


Figure 18 Monthly variation in the concentration of ammonia (all MHBEMP sites) in surface waters (2 m) in Macquarie Harbour. Red crosses represent the mean.

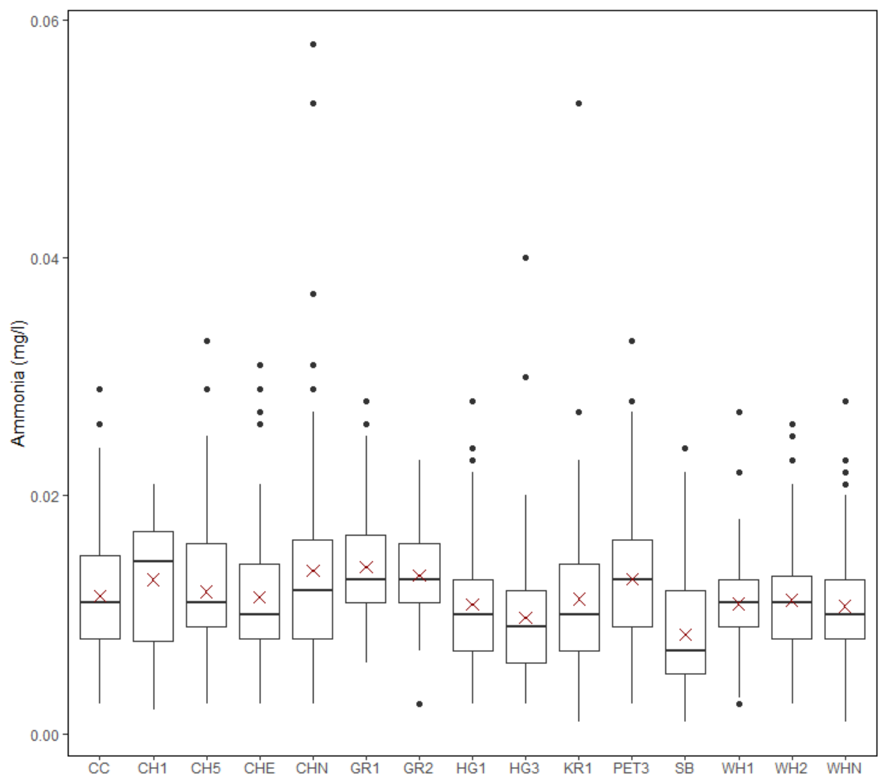


Figure 19 Concentration of ammonia in surface waters (2 m) at each MHBEMP site (all months) in Macquarie Harbour. Red crosses represent the mean.

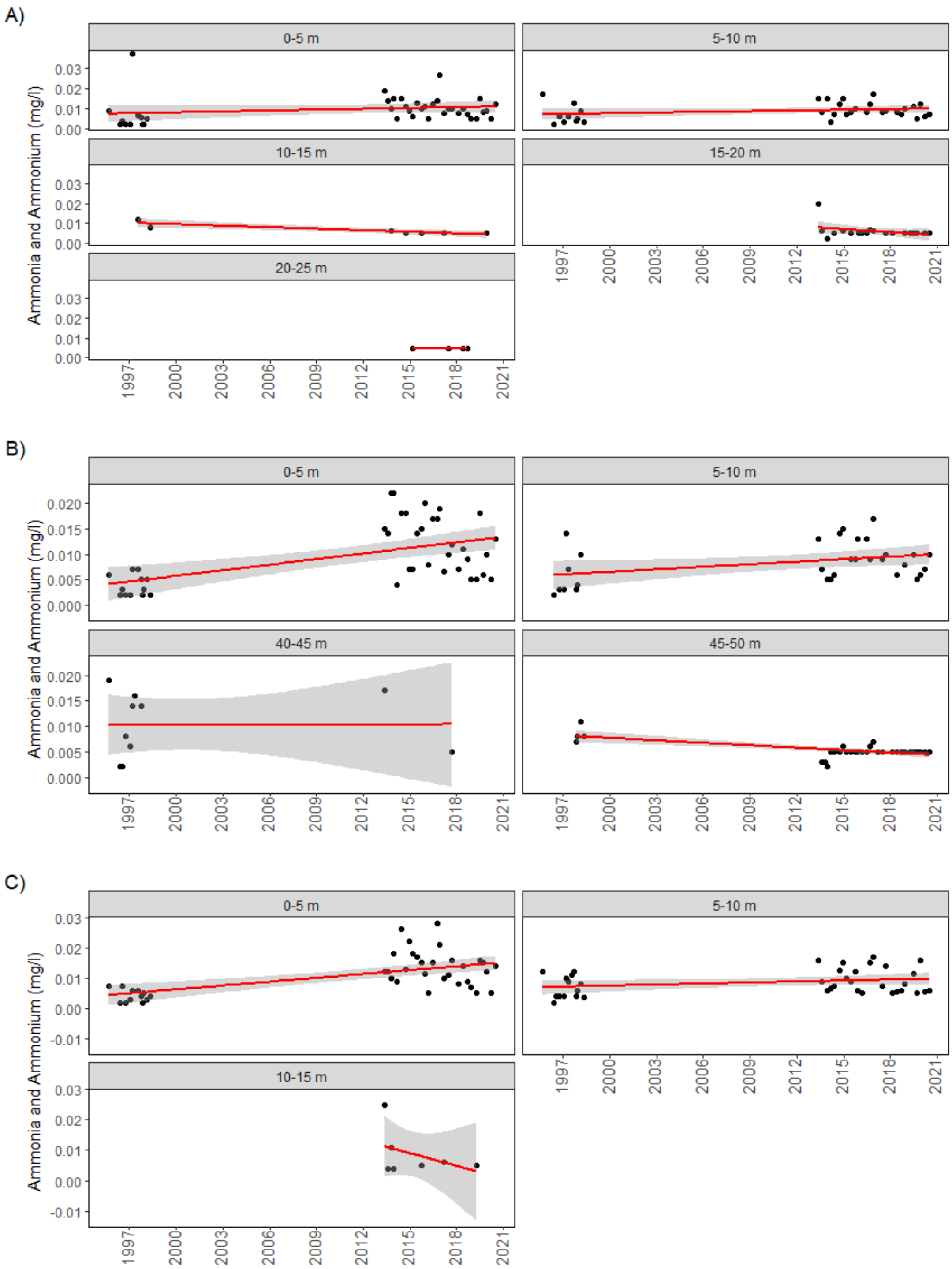


Figure 20 Ammonia concentration at EPA monitoring sites MH11 (A), MH12 (B) and MH14 (C). Red line is a linear model, and the shaded area represents 95% confidence intervals of the model.

Nitrate

Water column patterns in nitrate are for the most part opposite to that described for ammonia, with the highest concentrations occurring at depths >10 m (Figure 21) rather than in surface waters. Although there was no clear temporal trend in nitrate concentrations across the full MHEMP time series, both surface and bottom water concentrations were clearly at their highest in 2015 (Figure 21).

Seasonally, surface nitrate concentrations increase from late summer and peak in autumn (Figure 22). The seasonality is difficult to explain given aquaculture inputs peak in the preceding months (i.e. late spring to early summer). Although the dilution plots described above indicate that the major sources of nitrate that explain the high nitrate concentrations observed at depth are from within the system, this doesn't preclude the possibility that there isn't a significant contribution from the ocean and river, particularly when considering concentrations in surface waters. The lower nitrate concentrations in surface waters from spring through summer may also reflect increased uptake by primary producers.

The comparison of EPA data collected since 2014 with that collected in the 1990s indicates an increase in nitrate concentrations at most sites and in most depths. Nitrate concentrations at 5 – 10 m approximately doubled during this period as did 45 – 50 m at site 12 (the only site with data at this depth) (Figure 24). However, since 2017, concentrations appear to be declining. Given the paucity of data from the mid-1990s, the interpretation should be treated with caution.

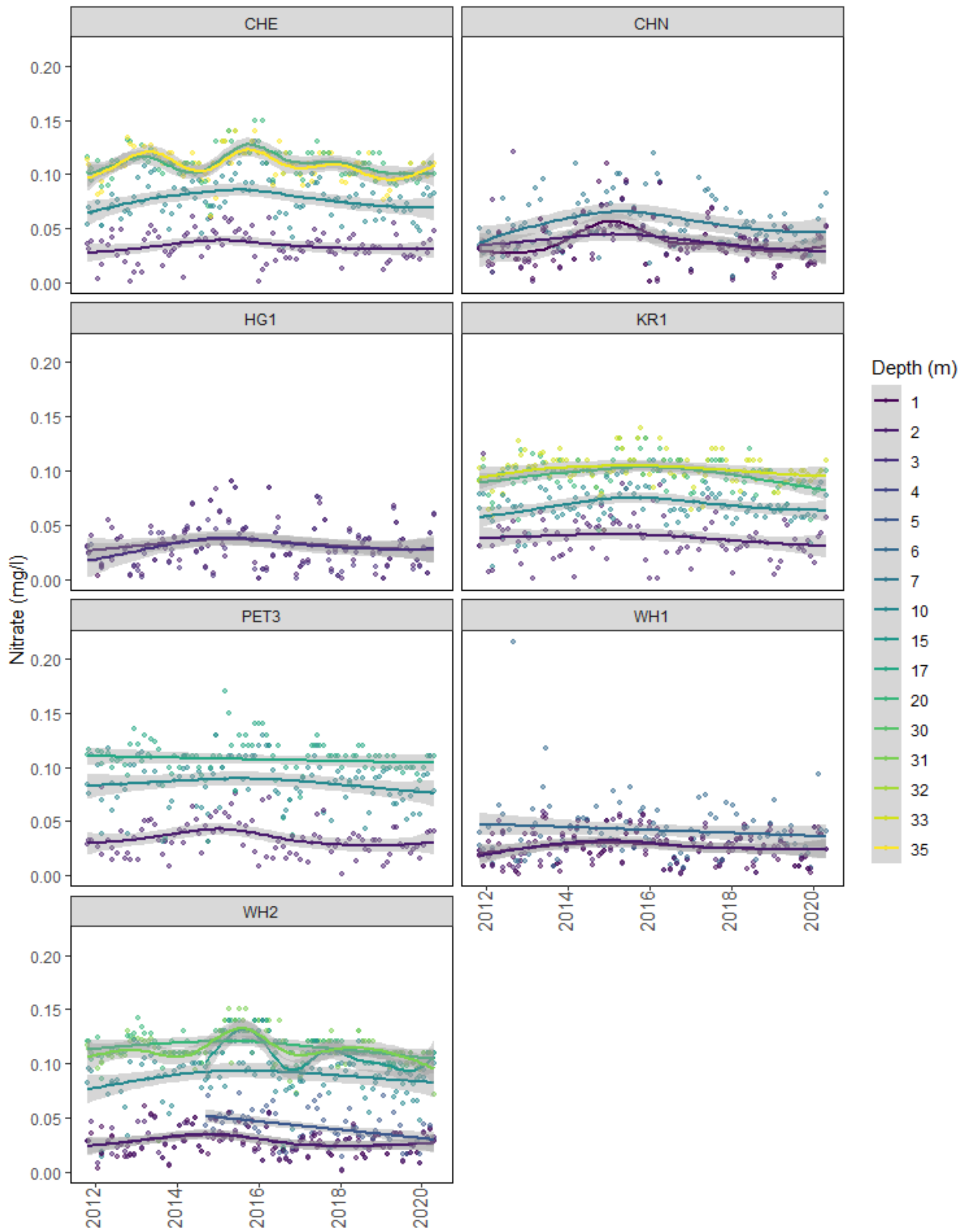


Figure 21 GAMs displaying temporal trends in nitrate concentration at the various depths it is measured in MHBEMP sampling.

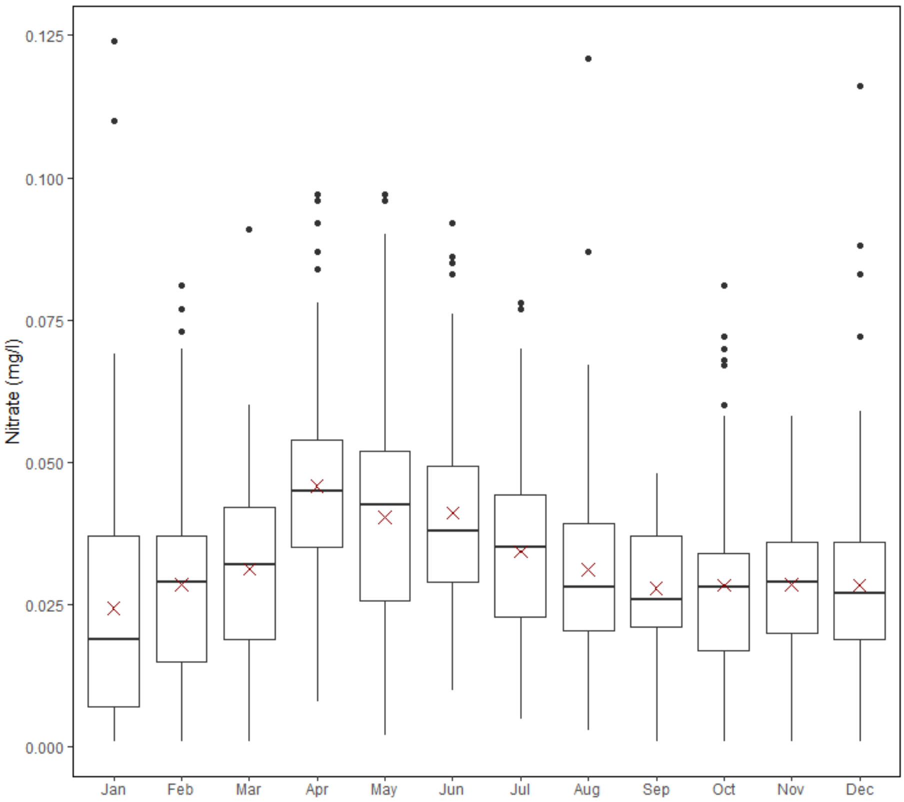


Figure 22 Monthly variation in the concentration of nitrate (all MHBEMP sites) in surface waters (2 m) in Macquarie Harbour. Red crosses represent the mean.

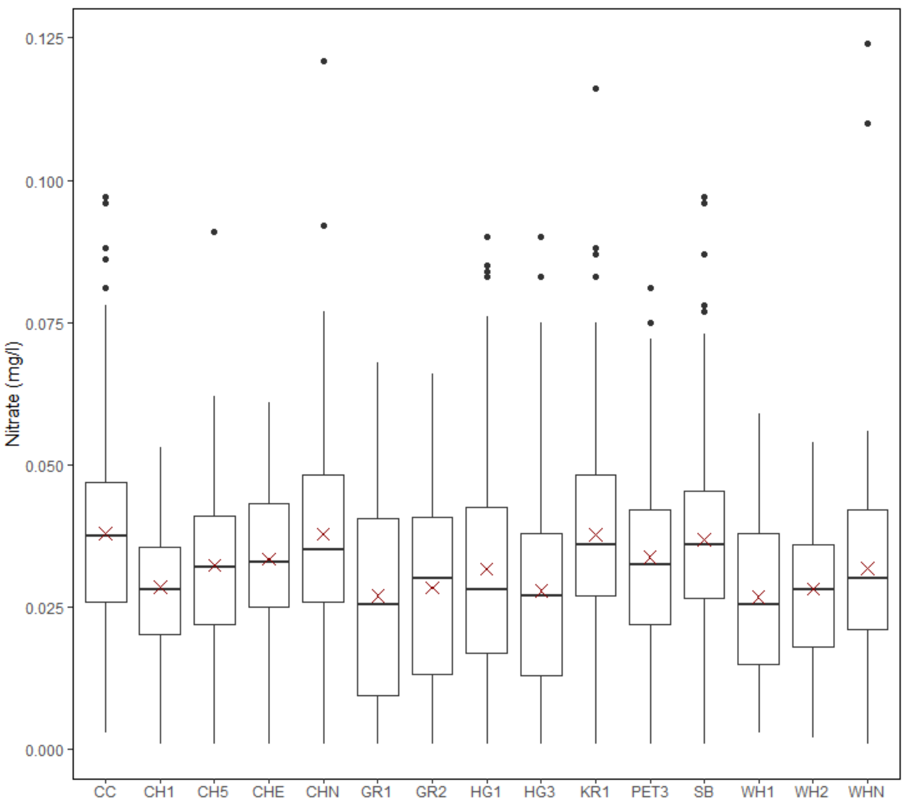


Figure 23 Concentration of nitrate in surface waters (2 m) at each MHBEMP site (all months) in Macquarie Harbour. Red crosses represent the mean.

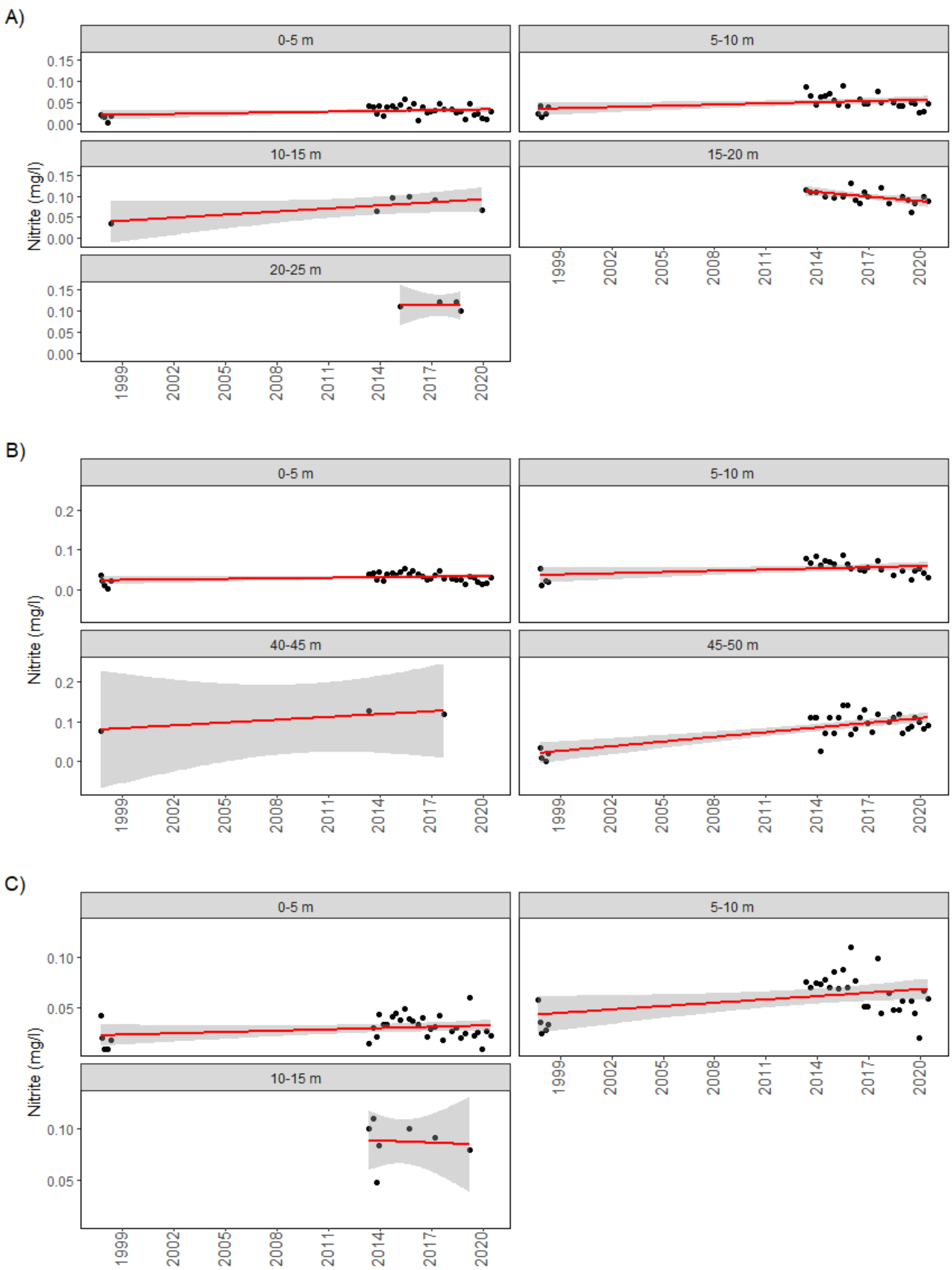


Figure 24 Nitrate concentration at EPA monitoring sites 11 (A), 12 (B) and 14 (C). Red line is a linear model, and the shaded area represents 95% confidence intervals of the model.

Total Nitrogen

Total Kjeldahl Nitrogen (filtered) and Total Nitrogen are measured at fewer sites and depths than ammonia and nitrate. For TKN (filtered), there is a consistent trend of increasing concentration up to 2017 exists at all sites and depths, followed by a decrease from 2018-2020 (Figure 25). This is best illustrated at WH2, where TKN (filtered) is measured at the most depths and in the deepest waters. In the deeper waters at WH2, TKN (filtered) increased by ~30% between December 2011 and December 2017 and subsequently returned to 2011 levels by 2020, with the shallower waters following a similar but less pronounced pattern. TKN (filtered) is highest in deeper waters and, as it does not include nitrate, the high concentrations, and increasing trend, suggests an increase in dissolved organic N, particularly because ammonia, which is included in TKN (filtered), has a relatively low concentration in deeper waters. Although the data period for TN is shorter, a similar, but less pronounced was apparent; TN concentrations are higher at depth, and they appear to increase from 2014- 2017/18 before returning to 2014 levels by 2020. A comparison of the TKN (filtered; ~ 0.2 mg N/l) and TN concentrations (~ 0.2 mg N/l) also indicate that dissolved organic nitrogen makes up a greater fraction of the organic nitrogen pool than particulate organic nitrogen.

There is no historical data available for either TKN (filtered) or TN within the EPA data set for comparison.

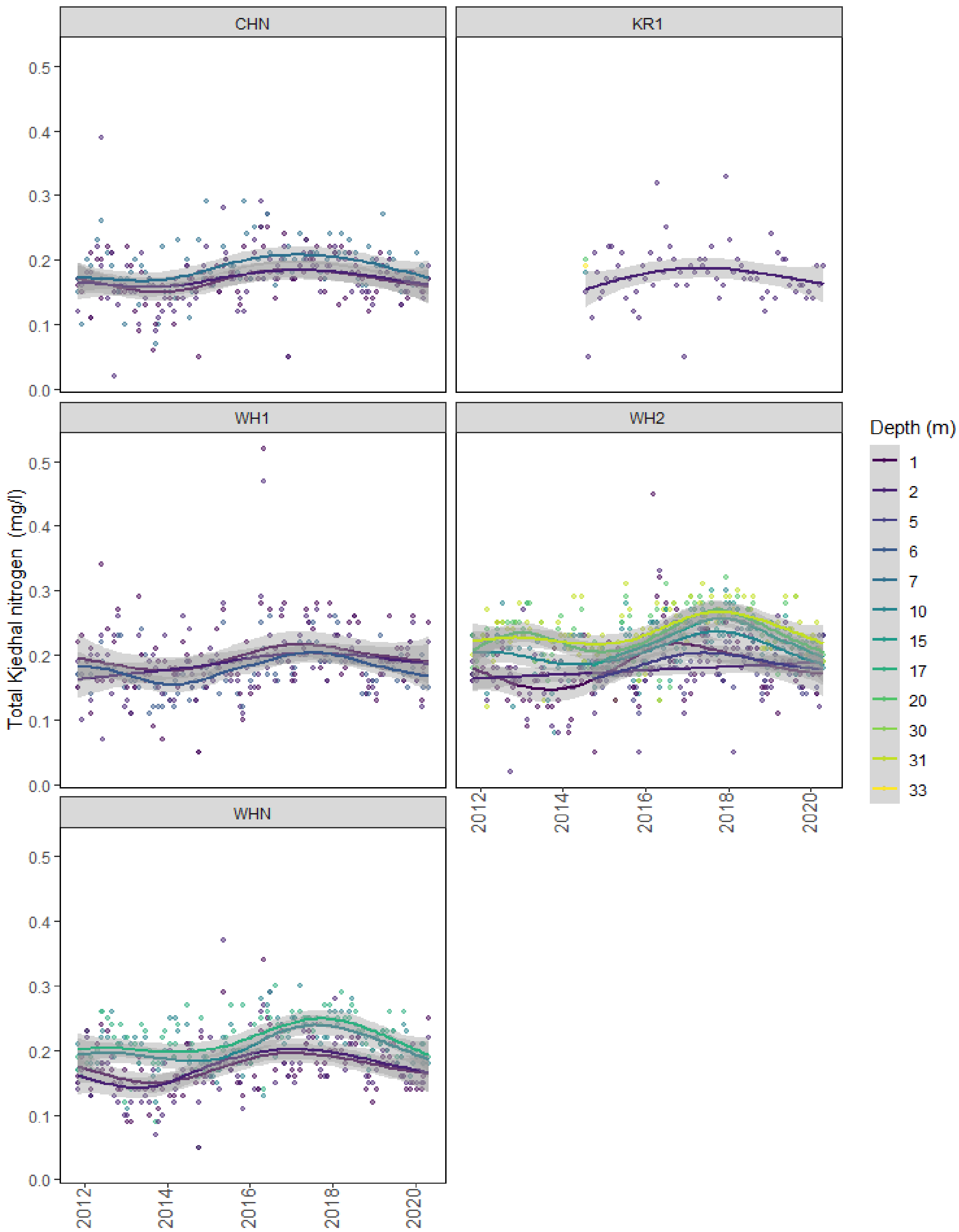


Figure 25 GAMs displaying temporal trends in total Kjeldahl nitrogen (filtered) concentration at the various depths it is measured in MHBEMP sampling.

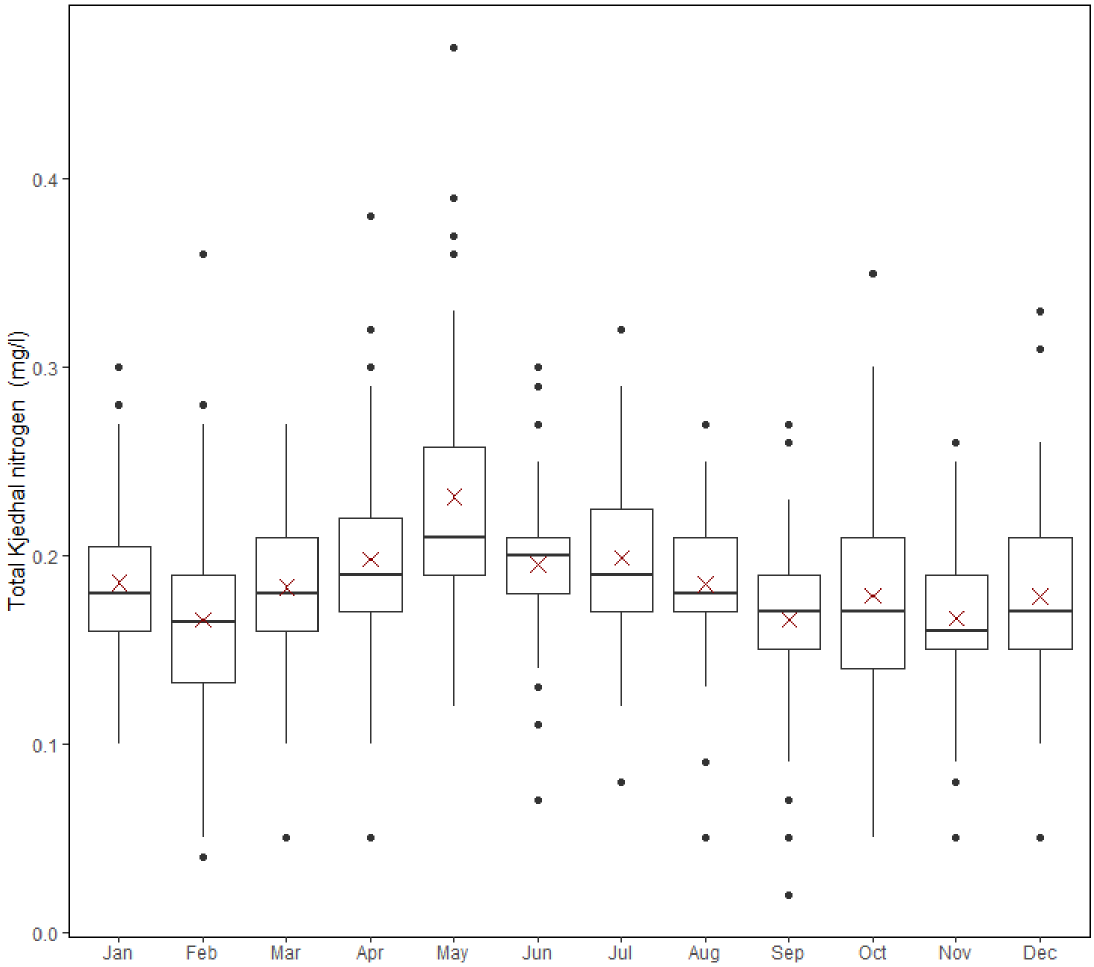


Figure 26 Monthly variation in the concentration of total Kjeldahl nitrogen (filtered) (all MHBEMP sites) in surface waters (2 m) in Macquarie Harbour. Red crosses represent the mean.

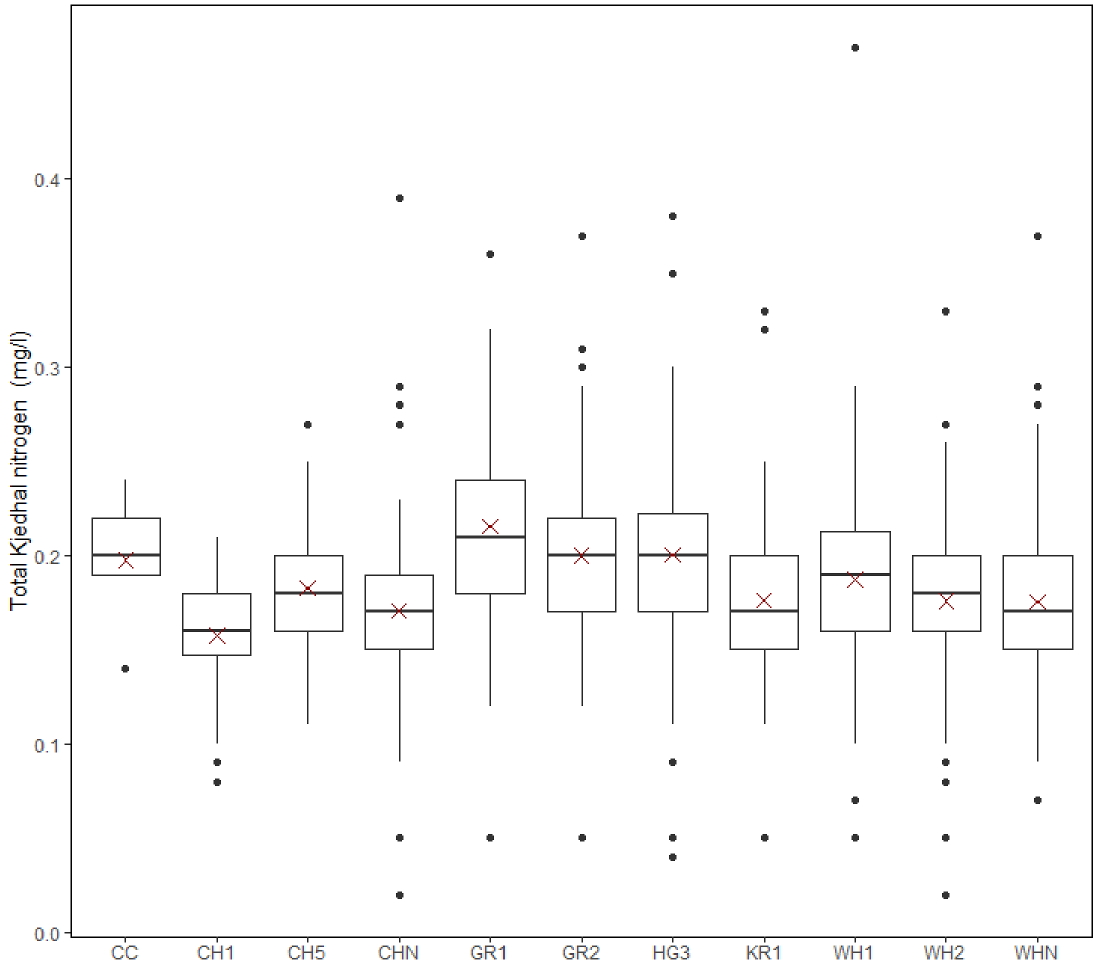


Figure 27 Concentration of total Kjeldahl nitrogen (filtered) in surface waters (2 m) at each MHBEMP site (all months) in Macquarie Harbour. Red crosses represent the mean.

Phosphorus

Dissolved phosphorus is not measured in the MHEMP sampling program, and as such the assessment is limited to data collected by the EPA. There is no indication that dissolved phosphorus concentration has increased in the surface waters of Macquarie Harbour, remaining relatively stable between the 1990s and the last few years (Figure 28). At the one site where dissolved phosphorus was measured in deeper waters (40-45m and 45-50m at EPA site 12), there appears to have been an increase between the 1990s and recent years, although this is based on a small amount of data.

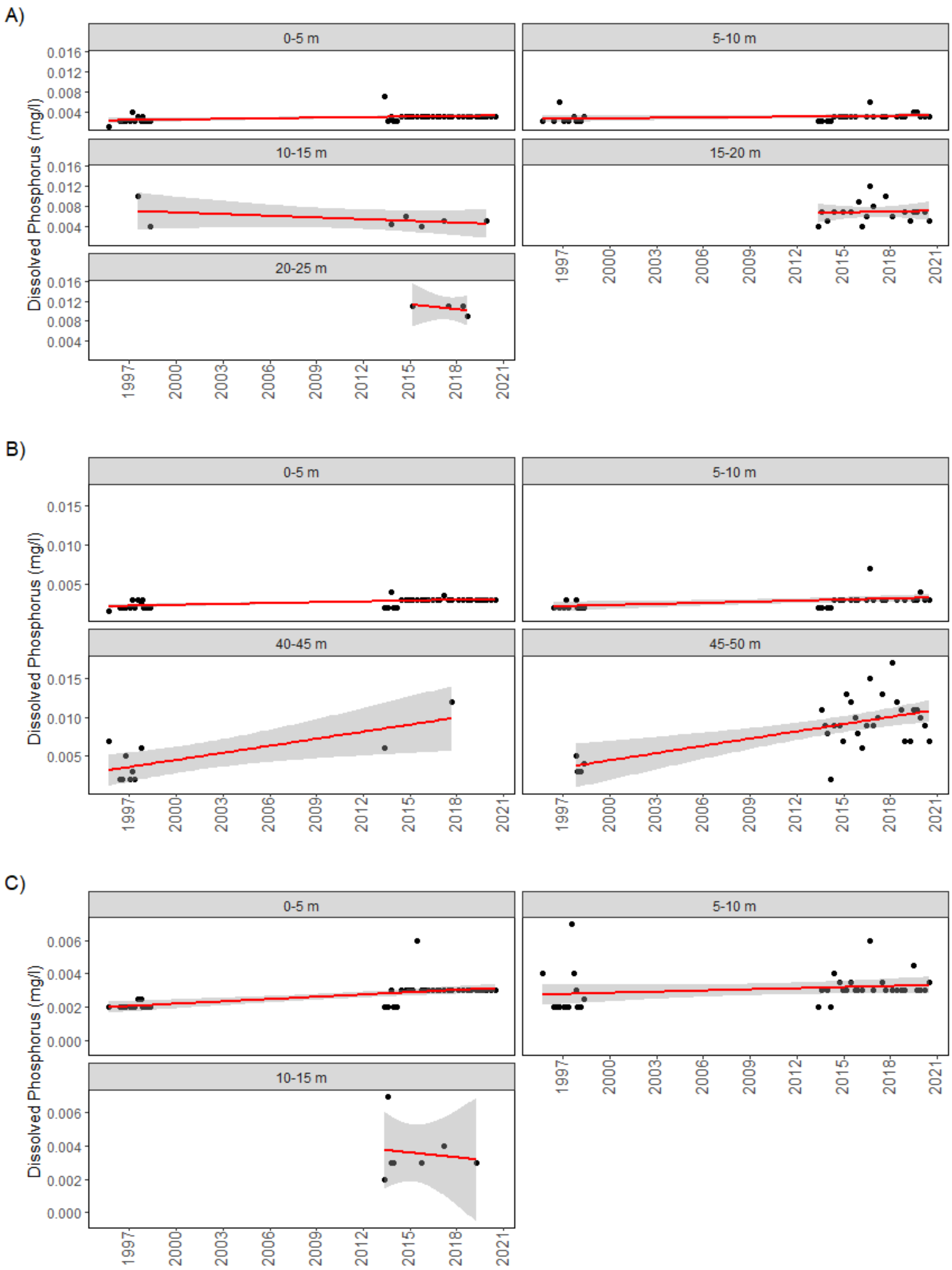


Figure 28 Dissolved phosphorus concentration at EPA monitoring sites 11 (A), 12 (B) and 14 (C). Red line is a linear model, and the shaded area represents 95% confidence intervals of the model.

Dissolved nitrogen: dissolved phosphorus ratio

To determine whether nutrients are potentially limiting primary productivity in Macquarie Harbour, the ratio between dissolved nitrogen species (ammonia, nitrate and nitrite) and dissolved phosphorus (dissolved reactive phosphorus) was investigated in surface waters (<5 m) using available EPA data.

Nitrogen: phosphorus ratios in the 1990s were variable, fluctuating above and below the Redfield ratio of 16:1 at all three EPA sites (Figure 30). From 2012 to 2017 the ratio has also been variable but at sites 12 and 14 it has more frequently been above the Redfield ratio potentially indicating phosphorus limitation. From 2018 to 2020 values at all three sites have been predominantly below the 16:1 Redfield ratio. Given the variability and limited data available, more concurrent dissolved N and P measurements are required to understand the potential importance of nutrient limitation of primary productivity in Macquarie Harbour.

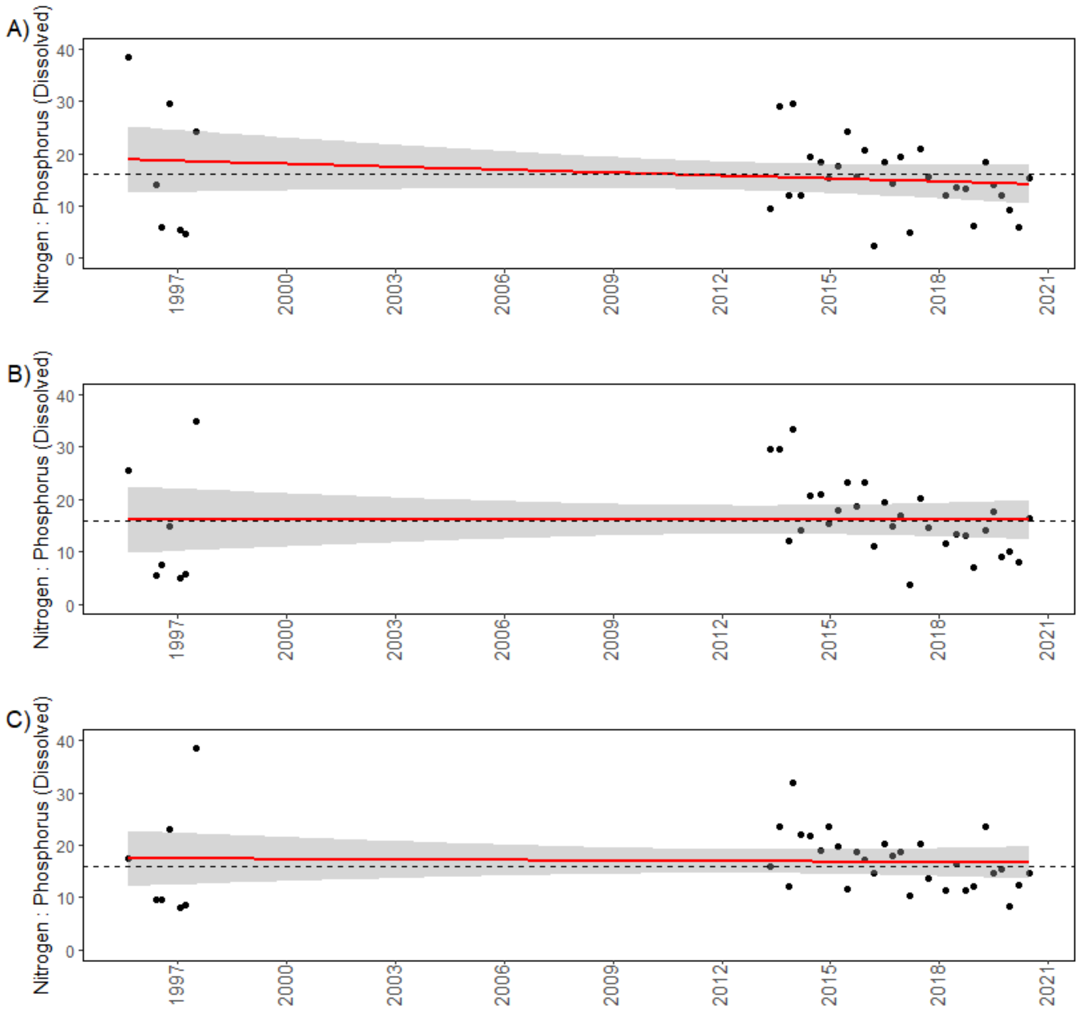


Figure 29 Surface dissolved nitrogen: dissolved phosphorus ratio at EPA monitoring sites 11 (A), 12 (B) and 14 (C). Red line is a linear model and the shaded area represents 95% confidence intervals of the model. Horizontal dashed line is the Redfield ratio of 16:1.

Organic carbon

Total organic carbon concentration (measured as NPOC) has been measured at sites WH2, C10, GR1, HG3 and KR4 since July 2014; here we present the data from WH2. It is highly seasonal in the shallow waters (1, 2 and 5 m) with the highest concentrations occurring in winter and lowest occurring during summer/autumn (Figure 30). In deeper waters (10, 20, 31 m), it is relatively stable with a seasonal influence (i.e. increase in winter) only observable in the winter of 2016 at 10 and 20 m depth. The above trend indicates that most of the organic carbon entering Macquarie Harbour is from riverine sources because concentrations are highest above the halocline in fresh water and the seasonal peaks occur during the cooler months when riverine inputs are greatest. This is consistent with estimates that aquaculture inputs would account for a relatively small fraction of carbon loading in the system; however, the fraction of the respective inputs that is labile and bioavailable is significantly higher for aquaculture inputs.

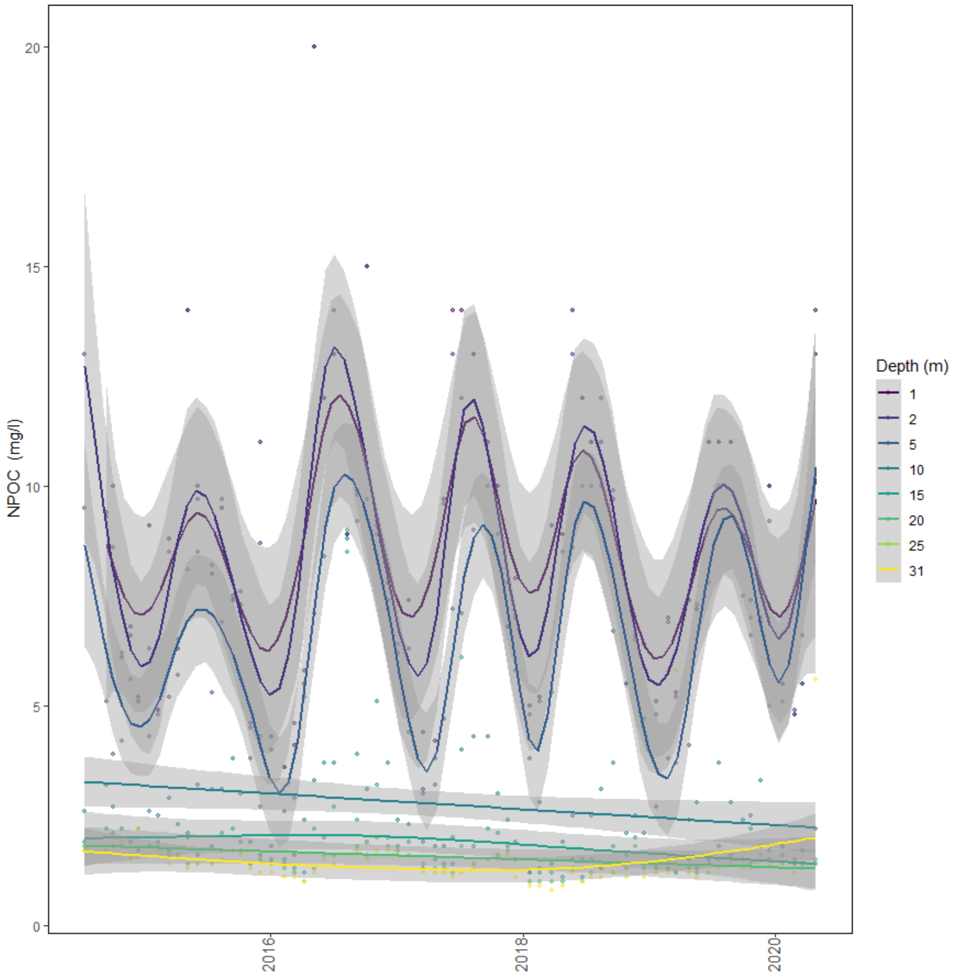


Figure 30 GAMs displaying temporal trends in NPOC concentration at the various depths it is measured in MHBEMP sampling at WH2. Gam smoother fitted using a tensor product smooth with k=22 to highlight the seasonal pattern in the data.

Summary of nutrient conditions in Macquarie Harbour

There is evidence that nutrient concentrations have increased in Macquarie Harbour, particularly from the years 2012-2017 (nitrate, TKN filtered and TN); however, nutrient concentrations appear to have declined since ~2018. Nutrient concentrations remain elevated, but these data (particularly ammonia) need to be treated with caution given the paucity of data from the late 1990s until the MHEMP began in 2011. The decrease in ammonia and increase in nitrate at depth (>10 m) at WH2 indicates that ammonia is rapidly nitrified to nitrate in Macquarie Harbour. The increase in TKN (filtered) and to a lesser extent TN, suggests that there was an increase in the organic N pool within the system that is now beginning to decline.

It was not possible to analyse the influence of phosphorus, or in particular, dissolved forms such as phosphate, in detail in the present study as these are not measured in the MHBEMP and the available data from the EPA sampling is limited and does not align with the more detailed MHBEMP sampling. Lakes and rivers are typically phosphorus limited, whereas oceanic waters are typically N limited (Correll, 1999). Estuaries are typically transition zones, which may be limited by either N or phosphorus (or other elements such as carbon or silica), or may vary in different regions of the estuary depending on the hydrodynamics of the system (Correll, 1999). However, in estuaries and coastal waters that are influenced by large rivers with high N concentrations (i.e. Macquarie Harbour), phosphorus is typically limiting (Harrison et al., 1990). Due to the above, it is recommended that dissolved reactive phosphorus (i.e. phosphate) be added to the MHBEMP sampling regime given that it may be limiting primary production in the harbour.

Silicate is fundamental to diatom proliferation (Egge and Aksnes, 1992) and hence its relative concentration can dictate diatom abundance. Silicate concentration is not measured in the MHBEMP, unlike in the southeast, so it was not possible to determine whether silicate limitation is responsible for limiting the abundance of diatoms. This lineage represents only a minor component of the Macquarie Harbour phytoplankton community (discussed in the phytoplankton section), unlike in the southeast (Bell et al., 2017) and other marine ecosystems throughout the world (Levinton, 1995), as such, future inclusion of silicate measurement in the MHBEMP would help further understand the factors determining phytoplankton community composition.

Physico-chemical conditions in Macquarie Harbour

Temperature

Temperature of the water column in Macquarie Harbour is quite consistent between sites. In the surface waters (<5 m) there is a strong seasonal pattern with temperatures approaching 21 °C in summer and 8 °C in winter (Figure 31). The seasonal variation is also evident in waters 5 – 10 m (~10 – 18 °C). Seasonal variation deeper in the water column (>10 m) is greatly reduced, with differences of ~ 2 °C between summer and winter. This is likely due to a combination of factors, including the shorter residence time of surface waters, the presence of a strong halocline at ~ 10 m, and the heavy tannin content of the riverine water. All these combined create an insulating layer and reduced mixing between surface and deep waters, which is responsible for the more stable conditions at depth.

Analysis of the longer-term EPA data shows that there has been an increase of ~1.5 - 2 °C in the mean temperature of deeper waters from 1993 to 2020 (Figure 31). The interannual temperature range has remained consistent through this period (~ 2 °C difference), but the summer maxima and winter minima have increased by ~1.5 -2 °C. Monthly data from the main MHEMP sites corroborates the increase in temperatures at depths > 10 m across the harbour (Figure 32).

Surface temperature data (<10 m) does not show a similar increase in average temperature across any of the sites. Likewise, the increase is not evident at the riverine endmember sites (Figure 33). Conversely, long-term temperature data (single-sensor multi-satellite data from IMOS 1992-2020) measured ~ 500 m north of the harbour entrance, shows a similar mean increase of ~1.5 -2 °C in sea surface temperature (SST) since 1993 (Figure 34). Deep water temperature inside the harbour was compared with SST outside the harbour from 1993 to 2020, with the analysis further refined to show the correlation by site (EPA), depth bin (5 m) and time of the year (spring and summer vs autumn and winter). There was a clear positive relationship between the SST from outside the harbour and temperature measured inside the harbour at all depths, but the pattern was strongest during the spring and summer months (Figure 35), when oceanic recharge is most common. This suggests that water temperature at depth in Macquarie Harbour is mostly influenced by oceanic recharges rather than from top-down warming and the influence of the river on surface waters.

It is unclear if the observed warming is the result of broader climate change patterns or part of natural long-term oscillations. However, these observations suggest that Macquarie Harbour has a unique dynamic that is distinct from most estuaries, where deep waters form a stable thermal environment with little seasonal variability but are susceptible to long-term changes in external SST. Temperature can affect aerobic performance, energetic demands, fitness, other metabolic processes in organisms and importantly, oxygen solubility. If temperatures in Macquarie harbour continue to increase, such increases are likely to have synergistic effect on all other stressors, including the oxygen dynamics of the harbour. Therefore, monitoring temperature, both internal and external (SST) is critical for informing ongoing management of the harbour.

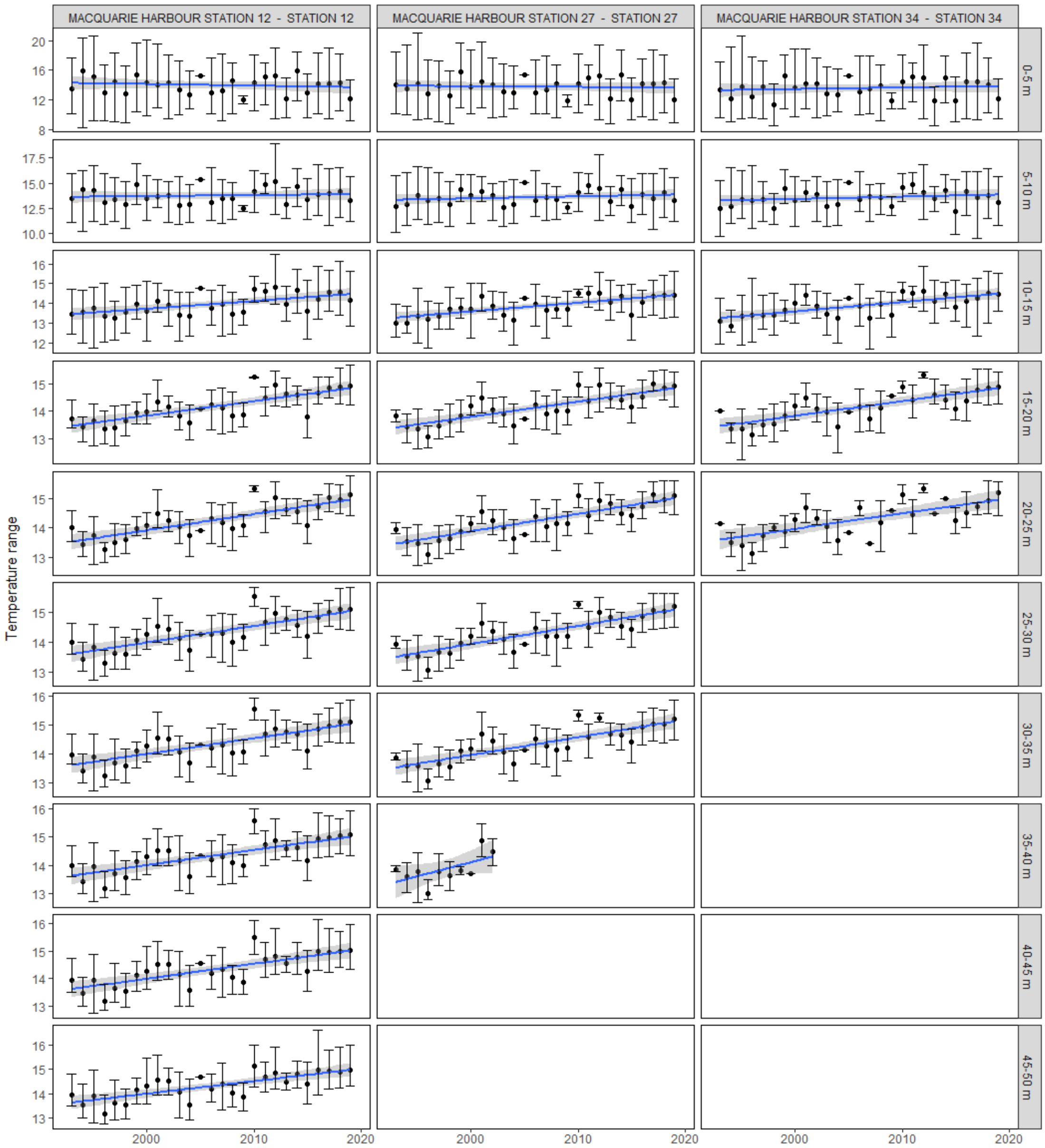


Figure 31 Long term annual temperature range in Macquarie Harbour at EPA sites 27 (A) 12 (B) and 34 (C). Whiskers are annual min and max temperatures, and the linear model is fitted to the mean annual temperatures.

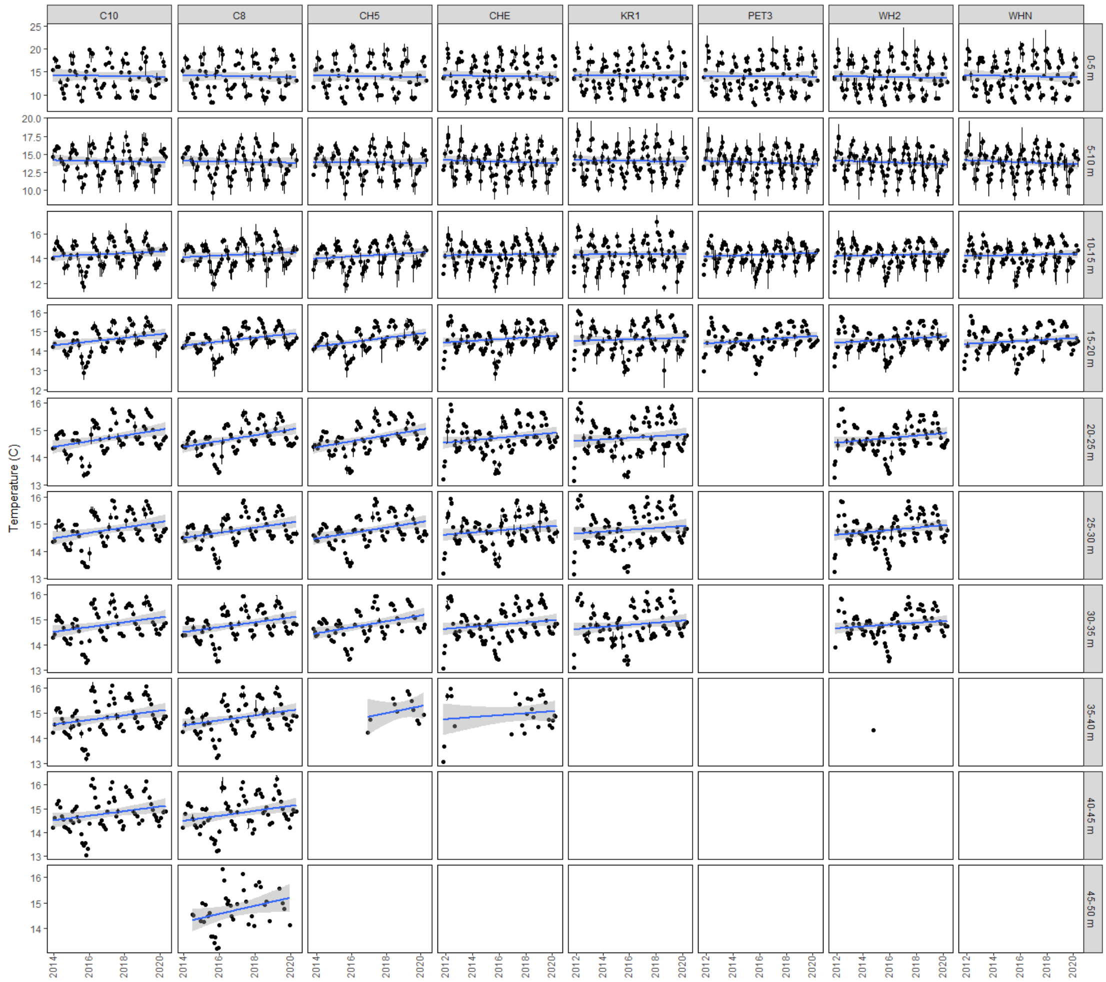


Figure 32 Long term annual temperature range in Macquarie Harbour at the main MHEMP sites. Whiskers are annual min and max temperatures, and the linear model is fitted to the mean annual temperatures

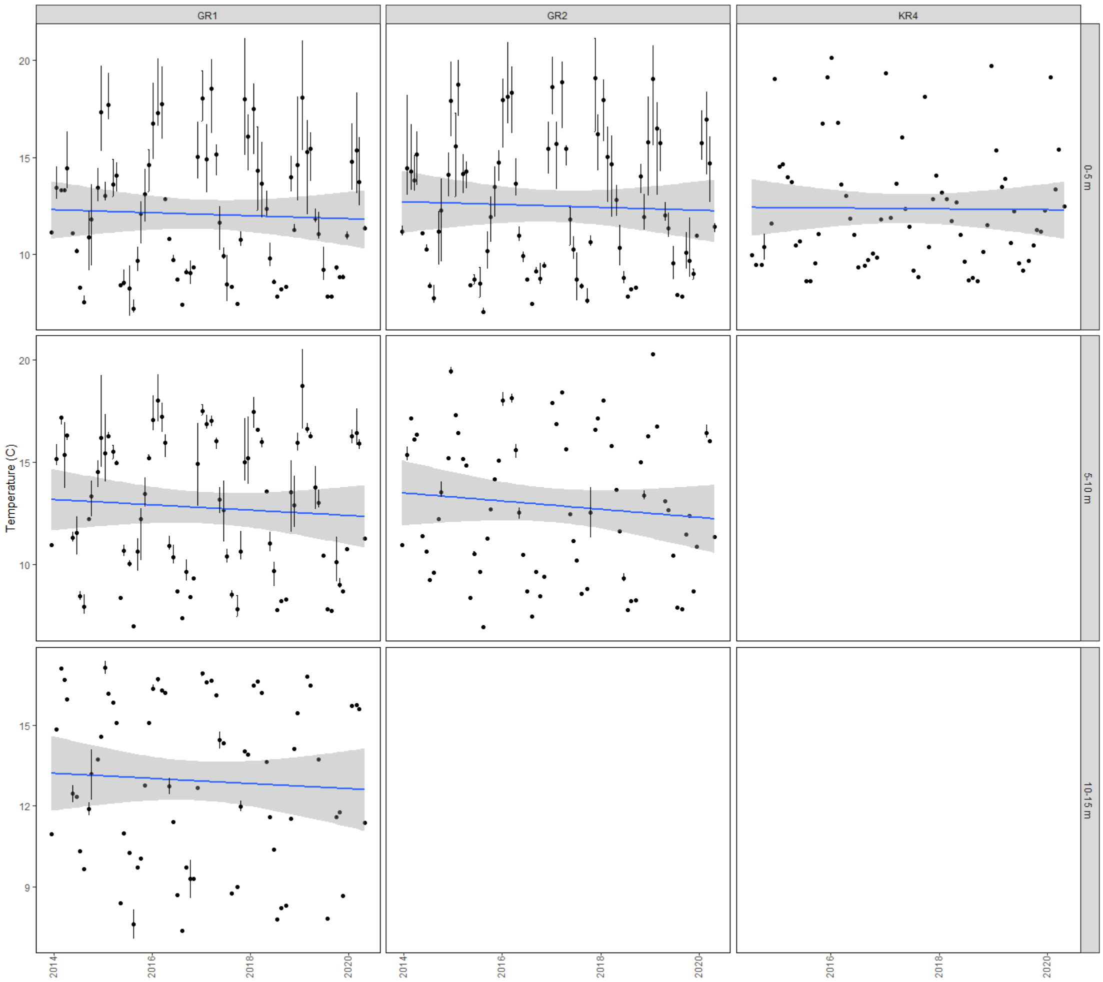


Figure 33 Long term annual temperature range in Macquarie Harbour at the MHEMP riverine endmember sites. Whiskers are annual min and max temperatures, and the linear model is fitted to the mean annual temperatures

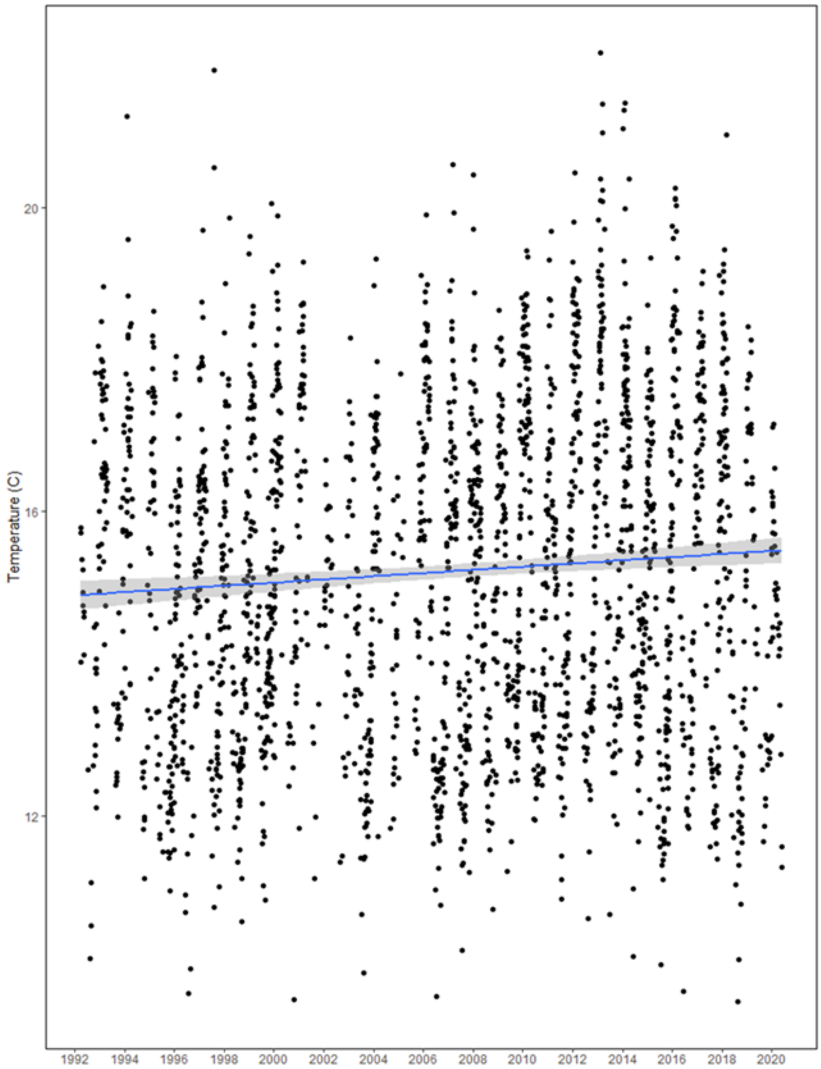


Figure 34 Long term IMOS satellite data showing sea-surface temperature measurements outside Macquarie Harbour.

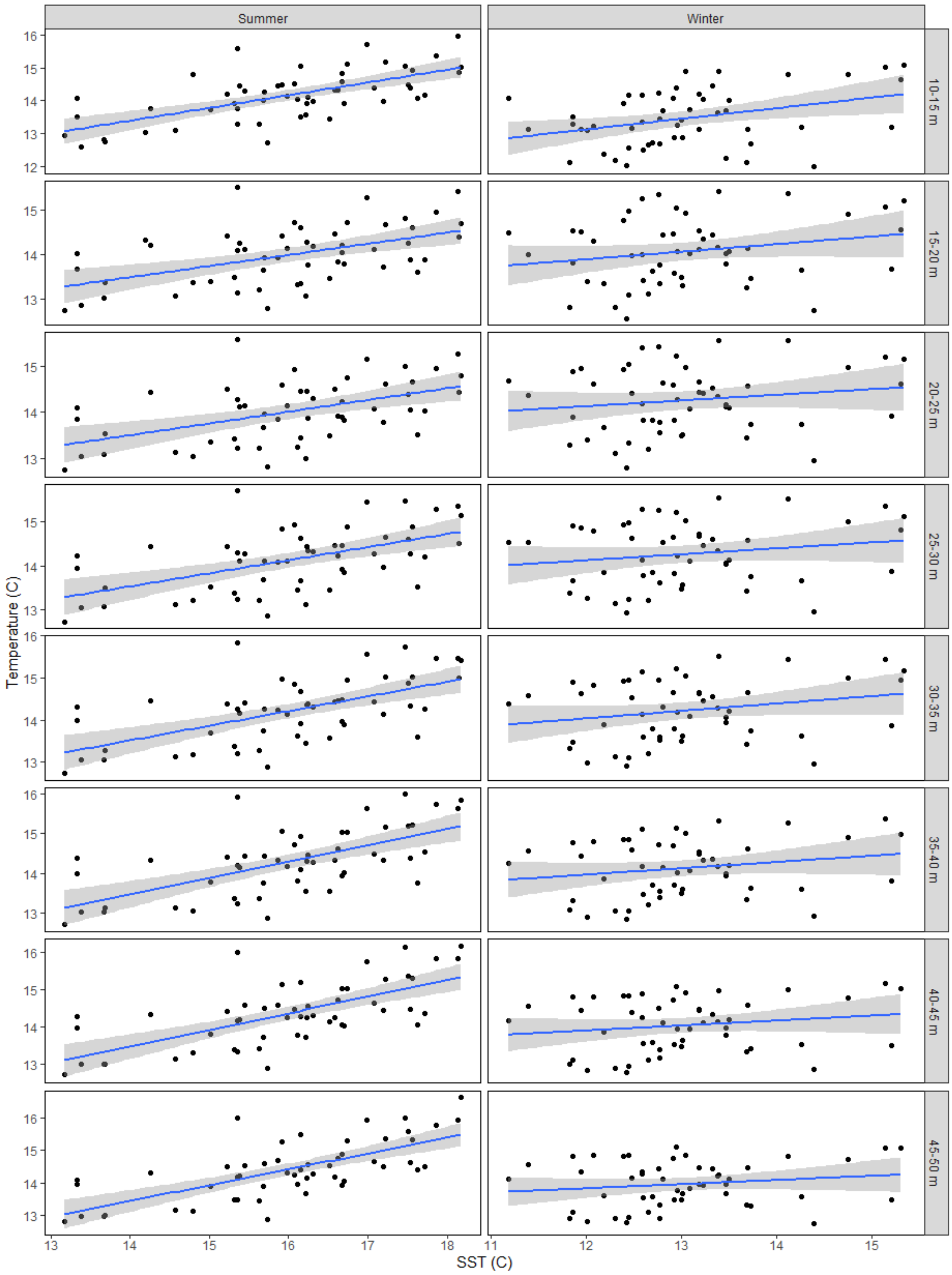


Figure 35 Long term (1993-2020) monthly temperature at depth in Macquarie Harbour (EPA sites 12, 27 and 34) against sea surface temperature outside the harbour separated into periods of high (summer) and low (winter) oceanic influx.

Salinity

The salinity of Macquarie Harbour is highly variable with shallow waters <10 m showing a strong seasonal pattern (Figure 36), ranging from ~0-20 ppt at <5 m and 10-30 ppt at 5-10 m. Deeper waters are far more stable, rarely fluctuating outside of

25 – 34 ppt, though on several occasions lower salinity water has penetrated to greater depths. For example, in 1995, waters >25 m declined to ~27 ppt and in 2014 water at 10 – 15 m depth at EPA site 34 declined to <10 ppt during a winter storm (Figure 36).

In the long-term EPA data, there does not appear to be any long-term trend in salinity (Figure 36). However, in the MHBEMP data, salinity in the deepest waters of Macquarie Harbour approached 35 ppt in the summer of 2011/12 before declining from late 2012 after which salinity was ~ 30 ppt through 2014- 15 (Figure 37). In the summer of 2015/16 salinity increased to ~32 ppt and it has since varied between 30 and 33 ppt. These changes correspond to periods of low and high river flow (Figure 6) and thus likely influence of river flows on the influx of high salinity oceanic waters.

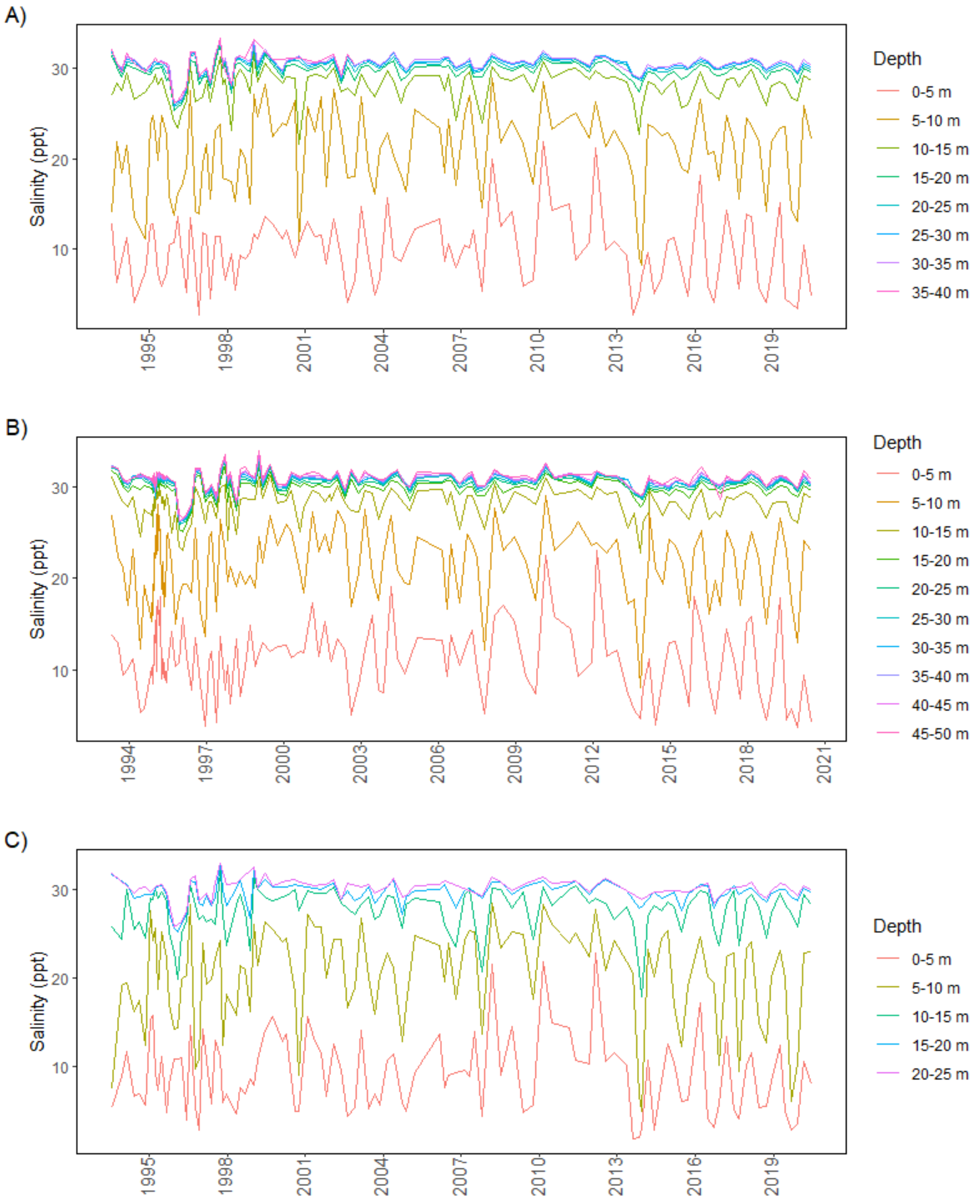


Figure 36 Long term trend in salinity with depth in Macquarie Harbour at EPA sites 27 (A) 12 (B) and 34 (C).

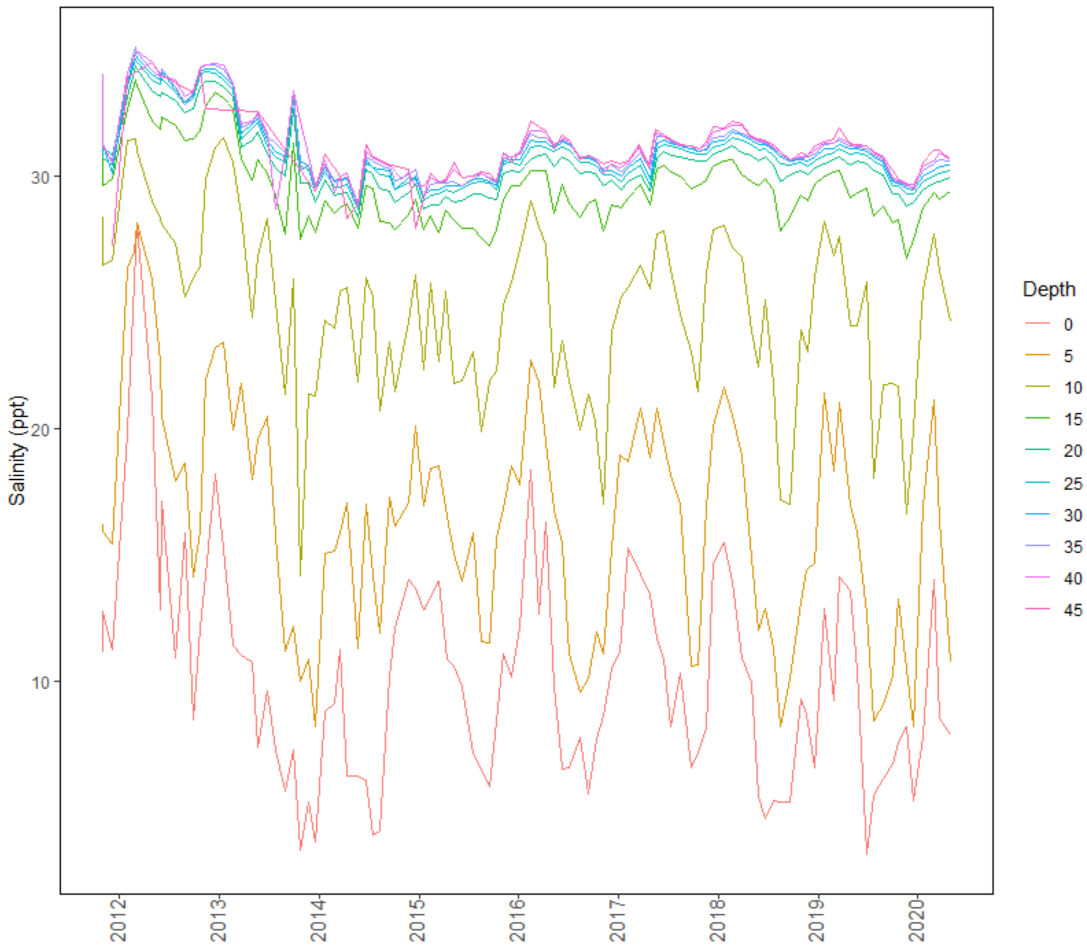


Figure 37 Average salinity across MHEMP sites from 2011-2020

Dissolved oxygen (DO)

The DO dynamics in Macquarie Harbour are complex and vary greatly with depth but show a relatively consistent pattern throughout the harbour (Figure 38 ,Figure 39). The general water column trend in both EPA and MHBEMP data is for surface waters (<5 m) to remain highly oxygenated (~100%) with DO levels decreasing with depth, but in waters >35 m, DO concentrations are often higher than mid-depths.

The longer-term EPA data shows a relatively consistent trend at all three sites from 1993 through until 2009 (Figure 38). From 2008/9 – 2013/14 there was a notable decline in DO at depths >20 m. The latter part of this decline are best explored using the MHBEMP and Sense-T data sets (Figure 39, Figure 41), which have greater spatial and temporal resolution.

The DO of surface waters (0 – 5 m) has remained high (>90% saturation) and relatively stable (Figure 38). At 5 – 10 m depth, DO is variable between 70 – 90% and the GAMs converged to a linear model at all sites indicating a slightly declining trend (Figure 38). At 10 – 15 m the trend is like that of 5 – 10 m but DO is typically ~50%, except for KR1, which is notably higher than all the other sites (~60%). The depth categories from 15 – 20 m through to 30 – 35 m displayed a similar trend at all sites throughout the period investigated: a decline from the start of the data set (late 2011) through until mid-2014 when concentrations were in the 10 – 20% range, followed by an increase due to a recharge of deep waters that occurred in late July 2014 (detailed below) in which concentrations increased to >30% in most instances. This was followed by a slow upwards trend throughout the remaining period, which includes periodic recharges, particularly in summer, and periods of decline through winter and spring.

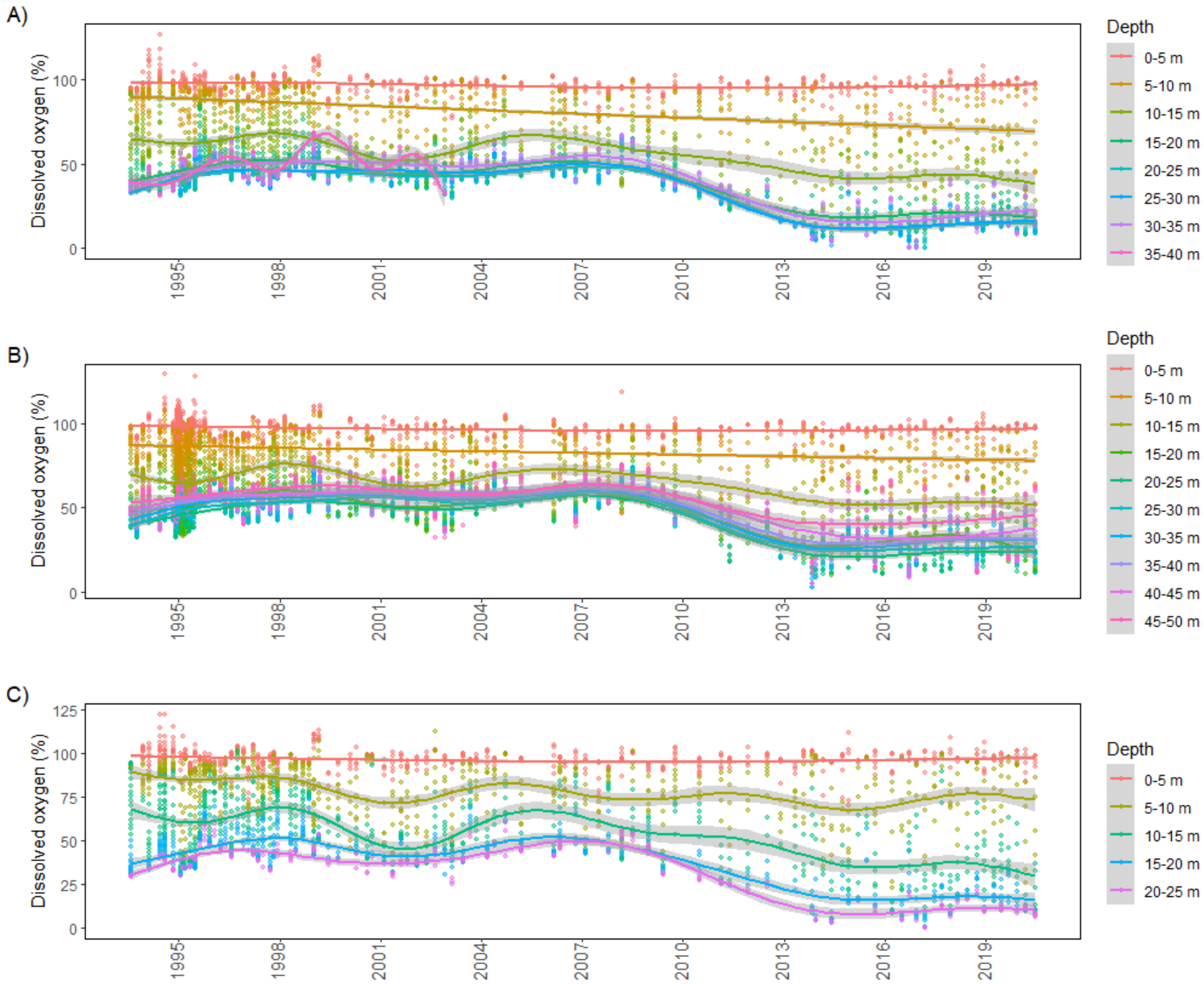


Figure 38 GAMs of dissolved oxygen concentration using the long-term EPA time series from May 1993 to June 2020. Site 27 (A) is in the centre of the harbour between Lease 266 and 267, Site 12 (B) is mid harbour to the north of Liberty Point, and Site 34 (C) is in the southeast of the harbour approximately 1 km south of Farm Cove. See EPA (2017) for exact EPA sampling locations.

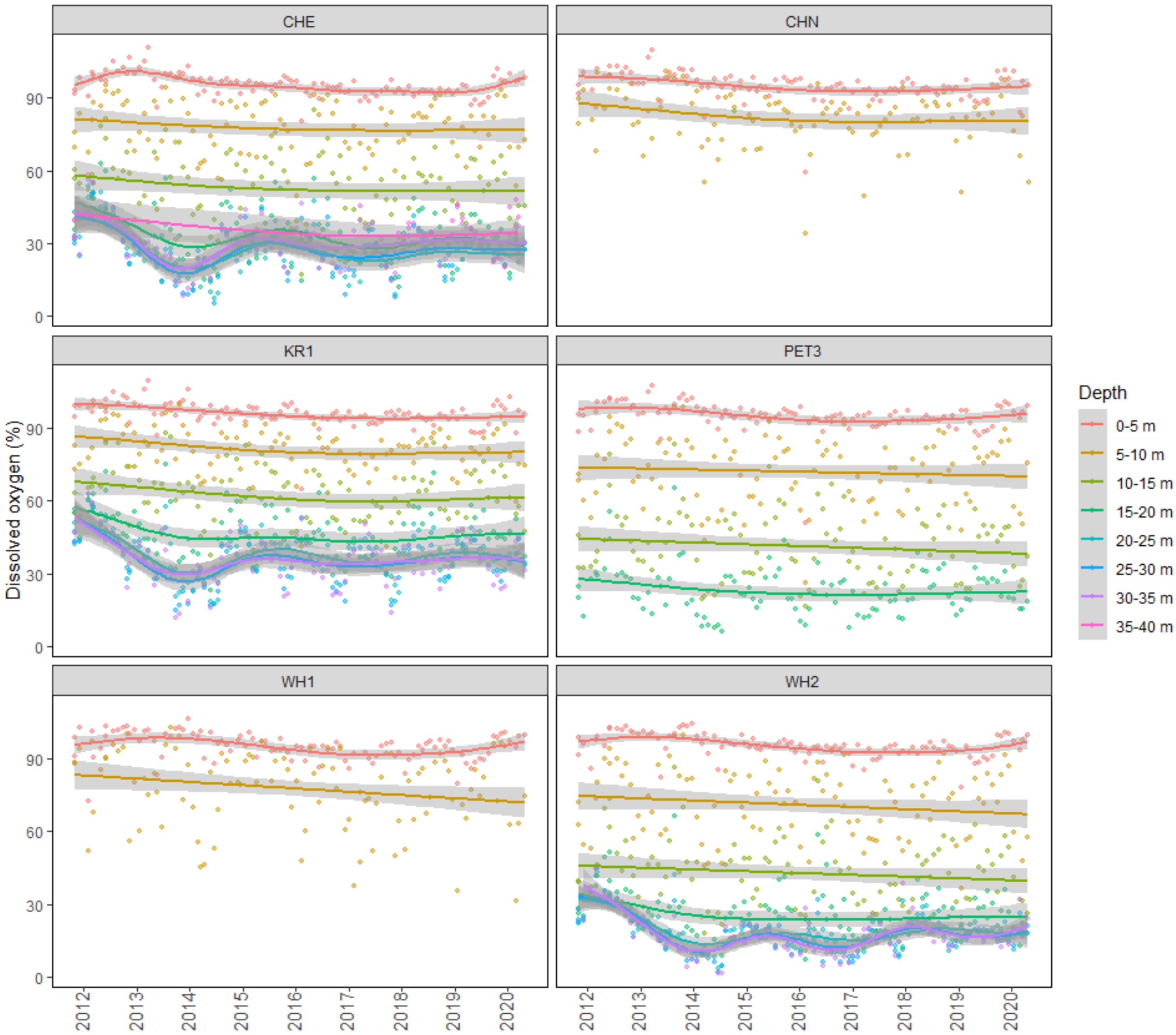


Figure 39 DO concentration (percent saturation) through time in 5 m depth classes at the main MHBEMP sites. Trends are smoothed using GAMs.

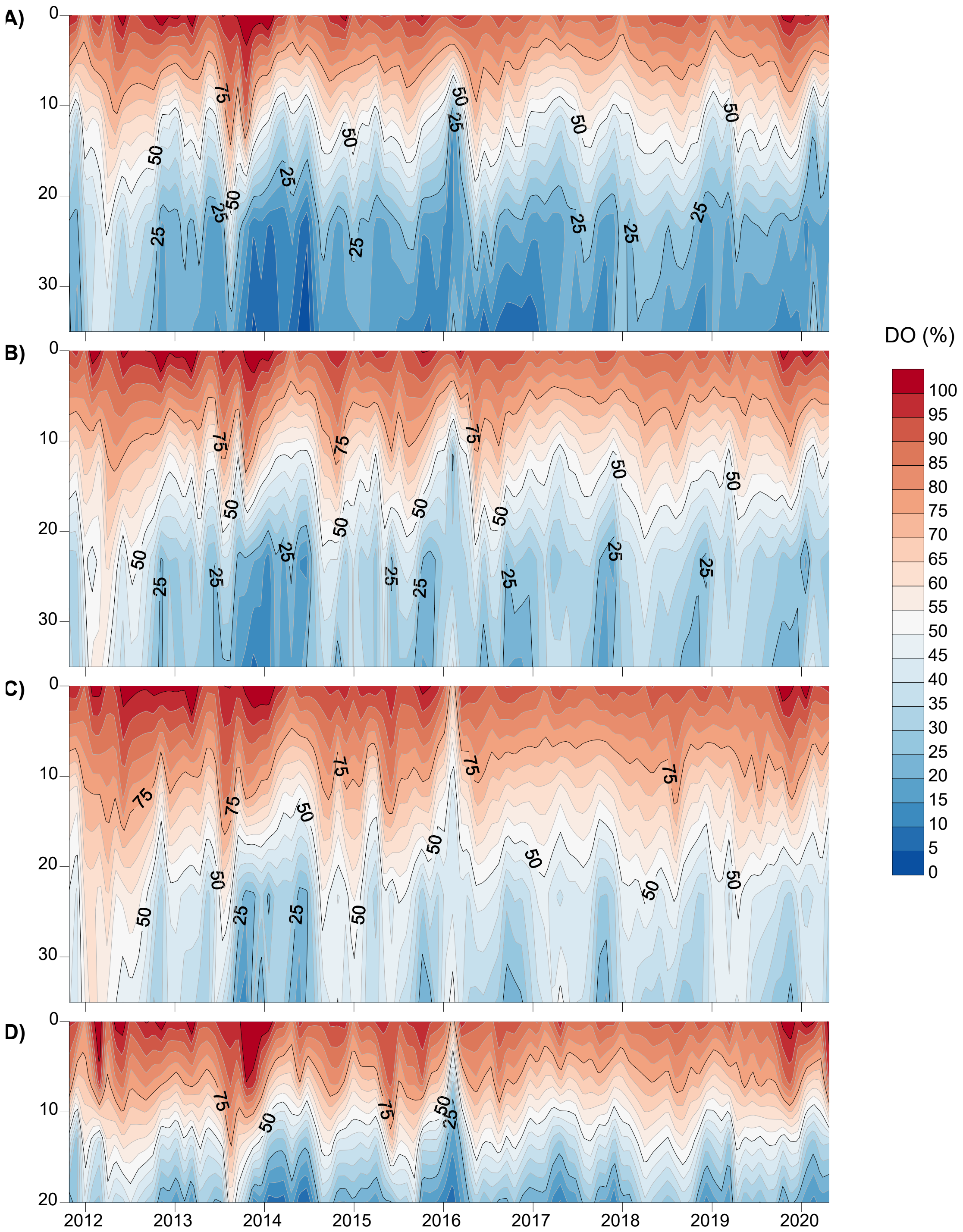


Figure 40 Contour plots of dissolved oxygen concentration (percent saturation) through time at the main MHBEMP sites (A=WH2, B=CHE, C=KR1, D=PET3).

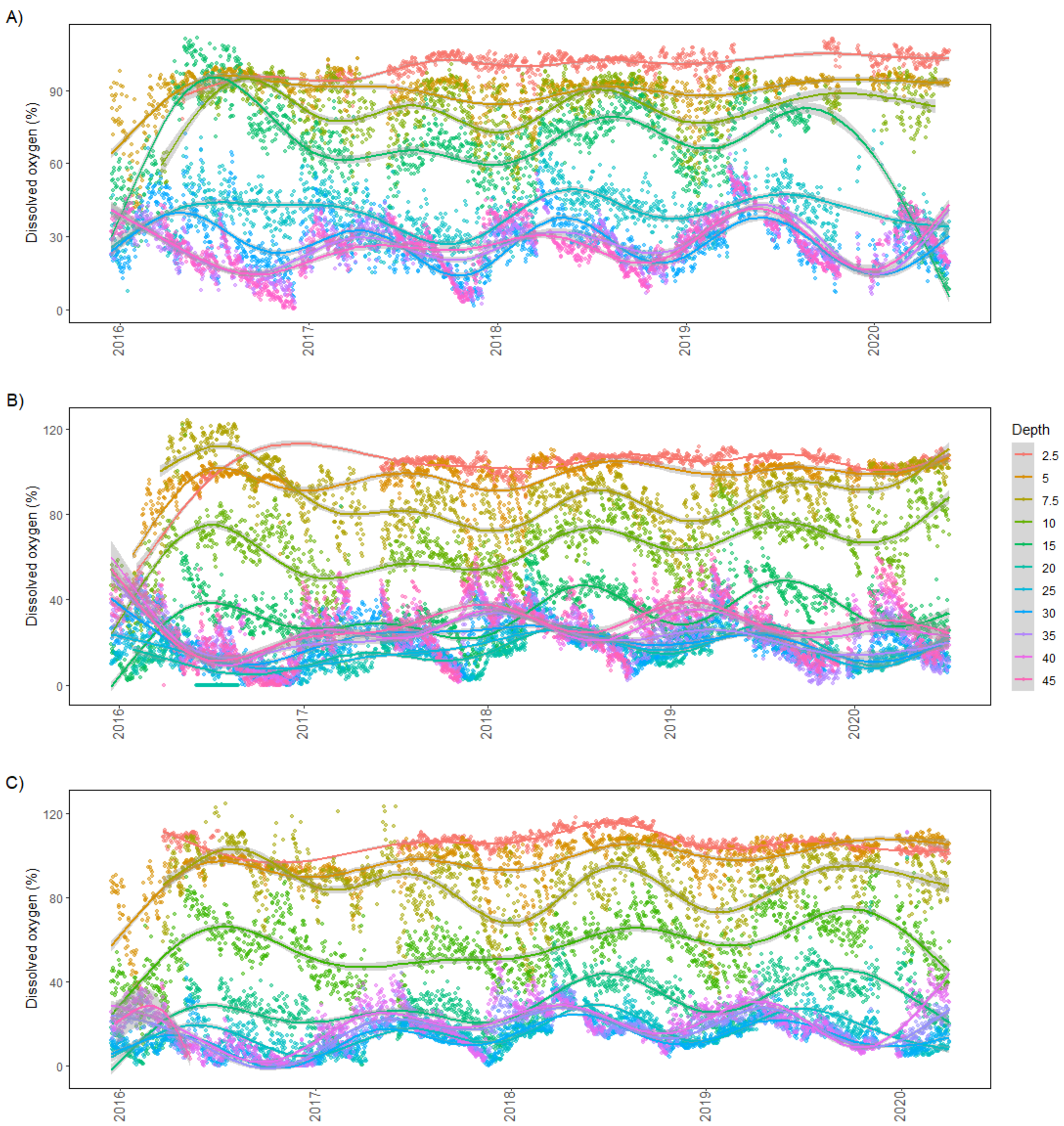


Figure 41 GAMs of dissolved oxygen concentration at various depths as measured by the Sense-T acoustic monitoring strings, Table Head Central (A), Strahan (B), and Franklin (C). See Figure 1 for the location of the strings.

Notable oxygen recharge events

July 2014

A major oxygen recharge event during the time period investigated occurred on the 31st July 2014, the exact timing and evolution of which was detailed by (MHDOWG, 2015, Bell et al., 2016). Unlike many of the minor recharge events that influence only a small proportion of the water column, this event increased dissolved oxygen at all depths throughout Macquarie Harbour (see Figure 40). Here we explore a range of the conditions associated with the recharge events that are considered to be most influential (see MHDOWG 2015).

On the 31st July 2014, and during the four days previous, a prolonged period of low pressure (Figure 45) associated with a series of cold fronts and troughs crossed Tasmania (Figure 42). Notably, these fronts directed strong north-westerly airflows across the state with north-westerly winds at Cape Sorell of >25 knots (at times >30 knots) for several days (Figure 43; Appendix i shows the entire MHBEMP time period to signify the rarity of winds such as these). While north-westerly winds are not uncommon in Macquarie Harbour, wind speeds of this magnitude are relatively rare (Figure 44). North-westerly winds blow the length of Macquarie Harbour and are thought to facilitate oxygenation of the system due to two processes: 1) they reach the maximum fetch in the system (other than south-easterly winds that are rare and typically gentle), causing increased turbulence and vertical mixing and 2) this wind direction forces water to accumulate in the upper reaches of the estuary, thereby decreasing the water elevation near the entrance and enabling more oceanic water to enter the system. In contrast, the extreme low pressure (Figure 45) will likely lead to higher sea levels outside the entrance, thereby increasing the gradient in water height between the ocean and the harbour and thus, the propensity of oxygen rich oceanic water to flow into the harbour (Appendix ii shows the air pressure for the entire MHBEMP time period to signify the rarity of such low air pressure coupled with north-westerly winds). Additionally, this weather pattern resulted in very high maximum (8 – 12 m) and significant[2] (~ 5 – 7.5 m) wave heights offshore from the 28th July to the 1st August. These were the highest of 2014 (Figure 46) and were the fifth highest since the wave rider buoy was deployed off Cape Sorell in January 1998 (Figure 47). The resulting storm surge and wind direction is likely to have further increased water elevation outside of the harbour, further increasing the gradient in water height between the ocean and harbour and subsequent influx of oceanic water. Unfortunately, the water elevation gauge at Strahan was non-operational during this event (see water elevation section below) so it is not possible to determine the exact influence these climatic conditions had on water elevation within Macquarie Harbour.

Under normal circumstances, high river flows result in a physical barrier that restricts oceanic water ingress at the harbour entrance. However, high intensity North-westerly winds may negate this effect by pushing freshwater back into the harbour, and consequently deepening the halocline. This, coupled with the influx of oxygen rich oceanic water, generates turbulent downward mixing that results in a recharge of dissolved oxygen levels as seen in this event.

The above weather conditions, in combination, appear to be responsible for the recharge of DO. It is worth noting that poor weather, in particular cold fronts, in

[2] Significant wave height is the average wave height, from trough to crest, of the highest one-third of waves

southern Australia are typically associated with south-westerly winds with north westerlies preceding cold fronts. In the entire data set analysed, there was no other period when such strong north-westerly winds occurred for such an extended period (Appendix I has daily wind speed and direction from 2012-17). Nor were they typically associated with very large ocean swell. This suggests that fairly unusual weather situations are required to generate a system wide recharge such as this.

z

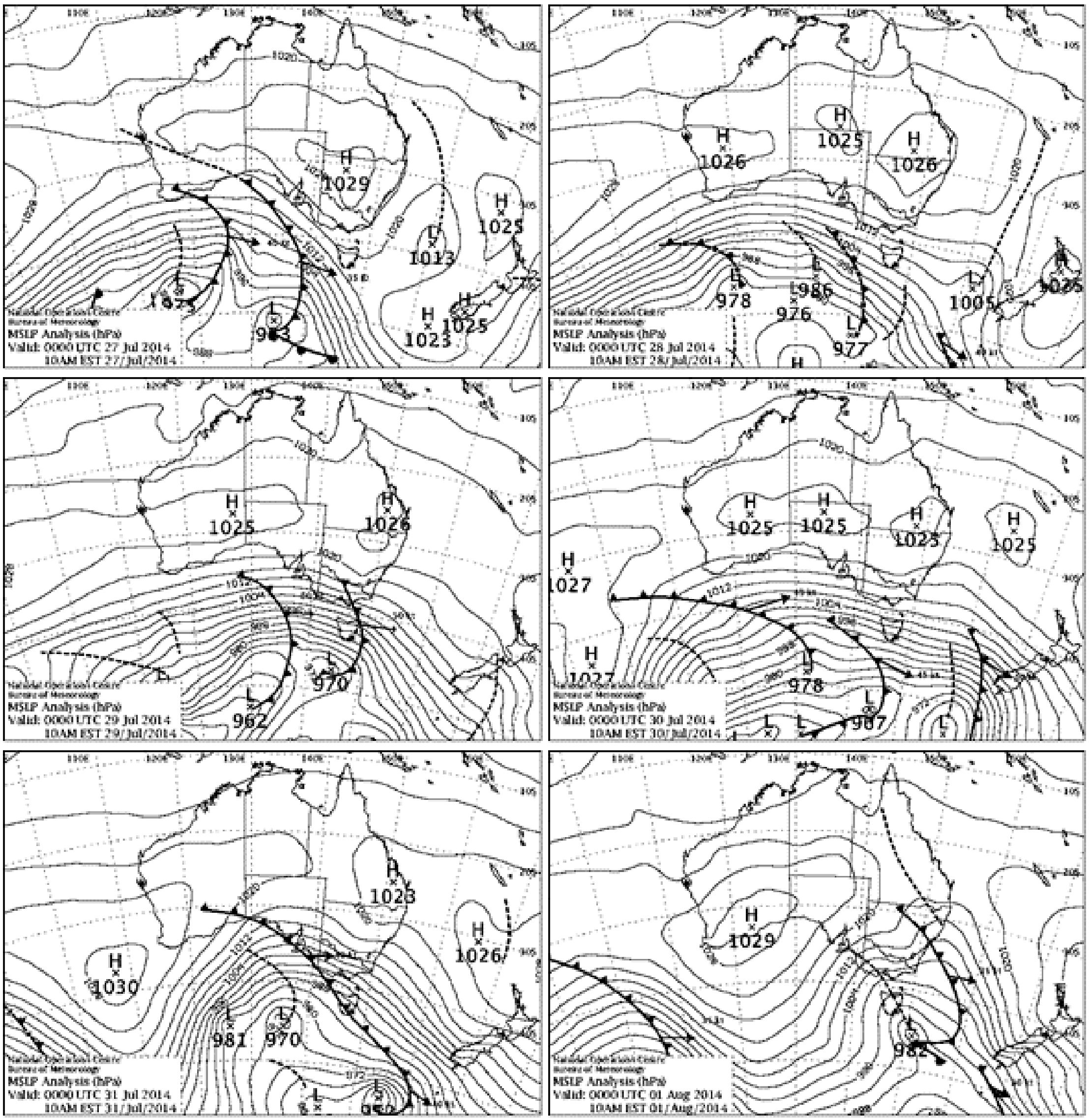


Figure 42 Barometric pressure (hPa) chart of Australia from the 27th July 2014 to 1st August 2014. Source: Bureau of Meteorology. For details on how to interpret barometric pressure charts see http://www.bom.gov.au/australia/charts/4day_col.shtml.

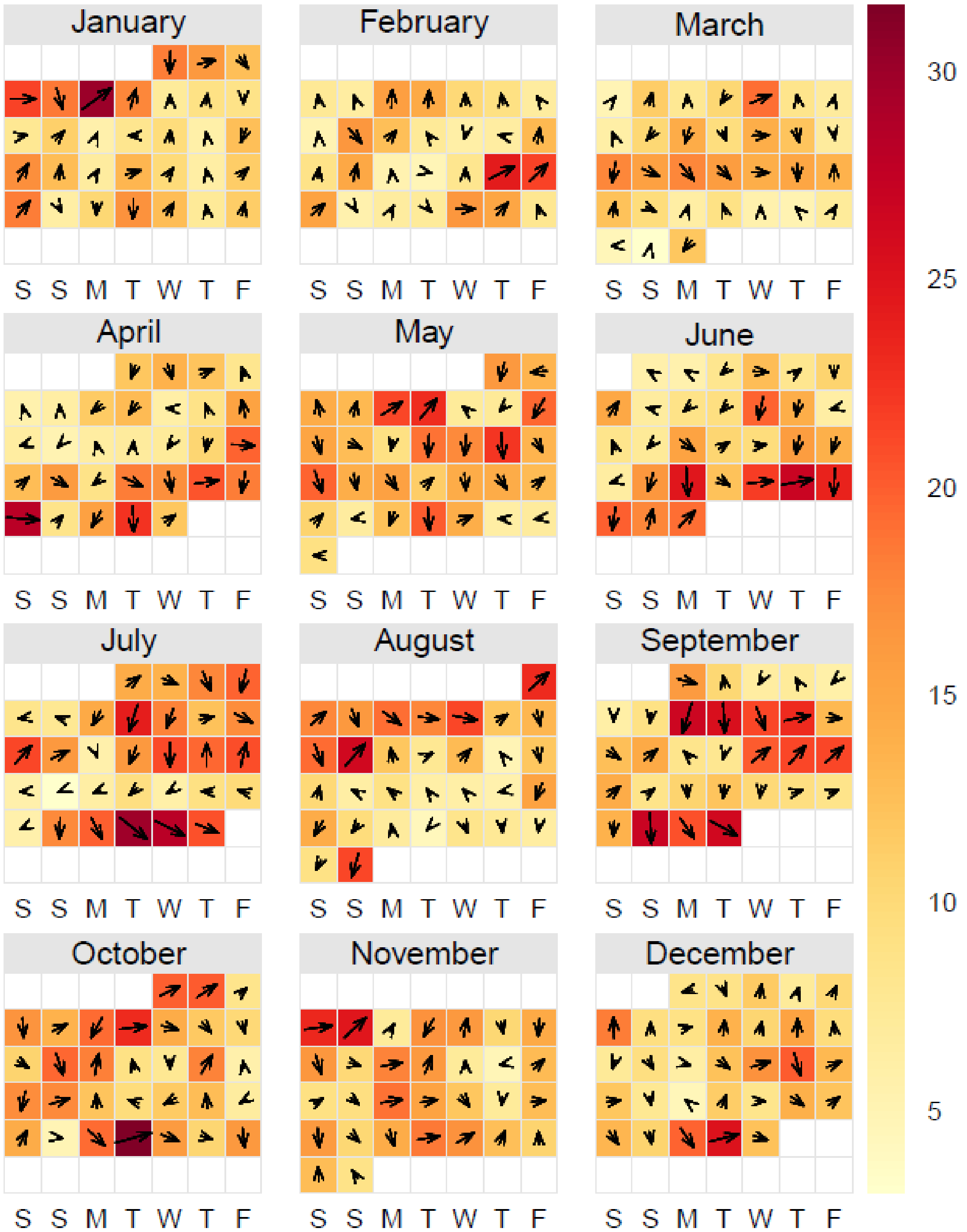


Figure 43 Calendar plot of wind speed and wind direction throughout 2014. Figure 47 can be used in conjunction with this Figure to specify dates.

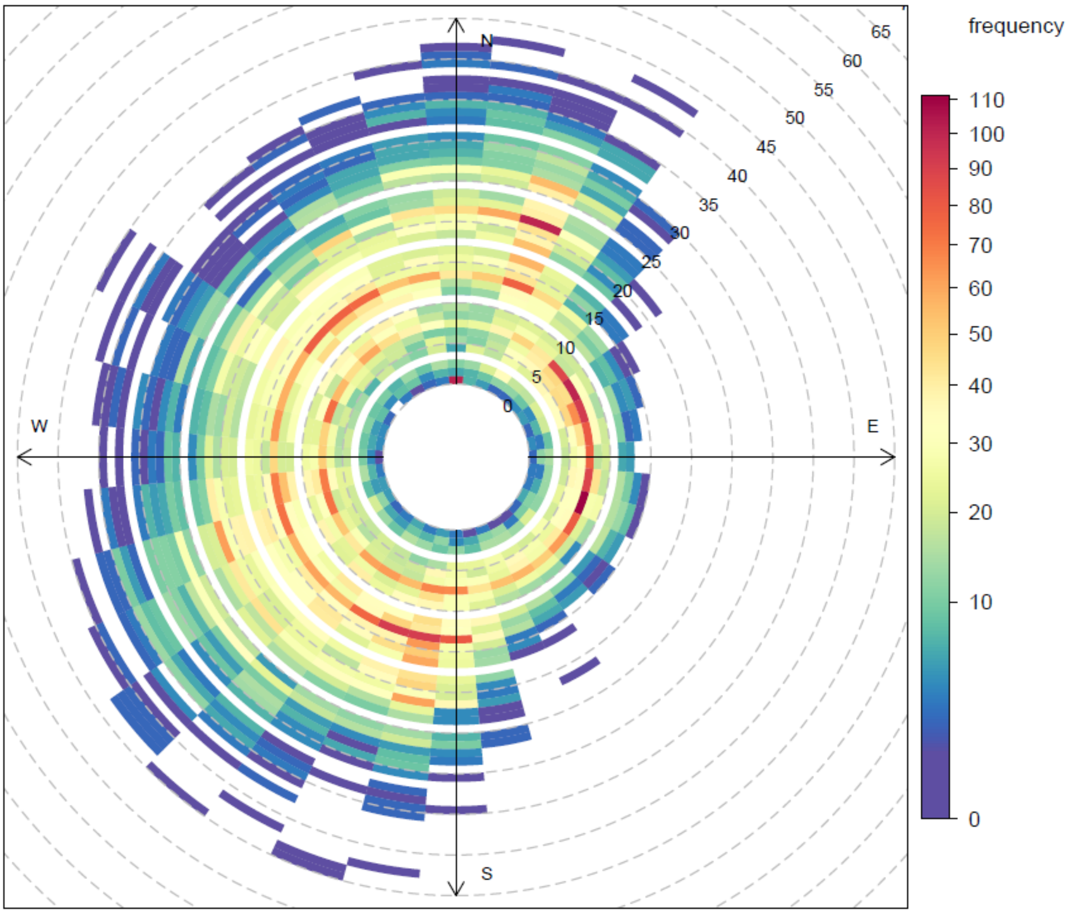


Figure 44 Wind speed and direction from 2012 to 2017 at Cape Sorell.

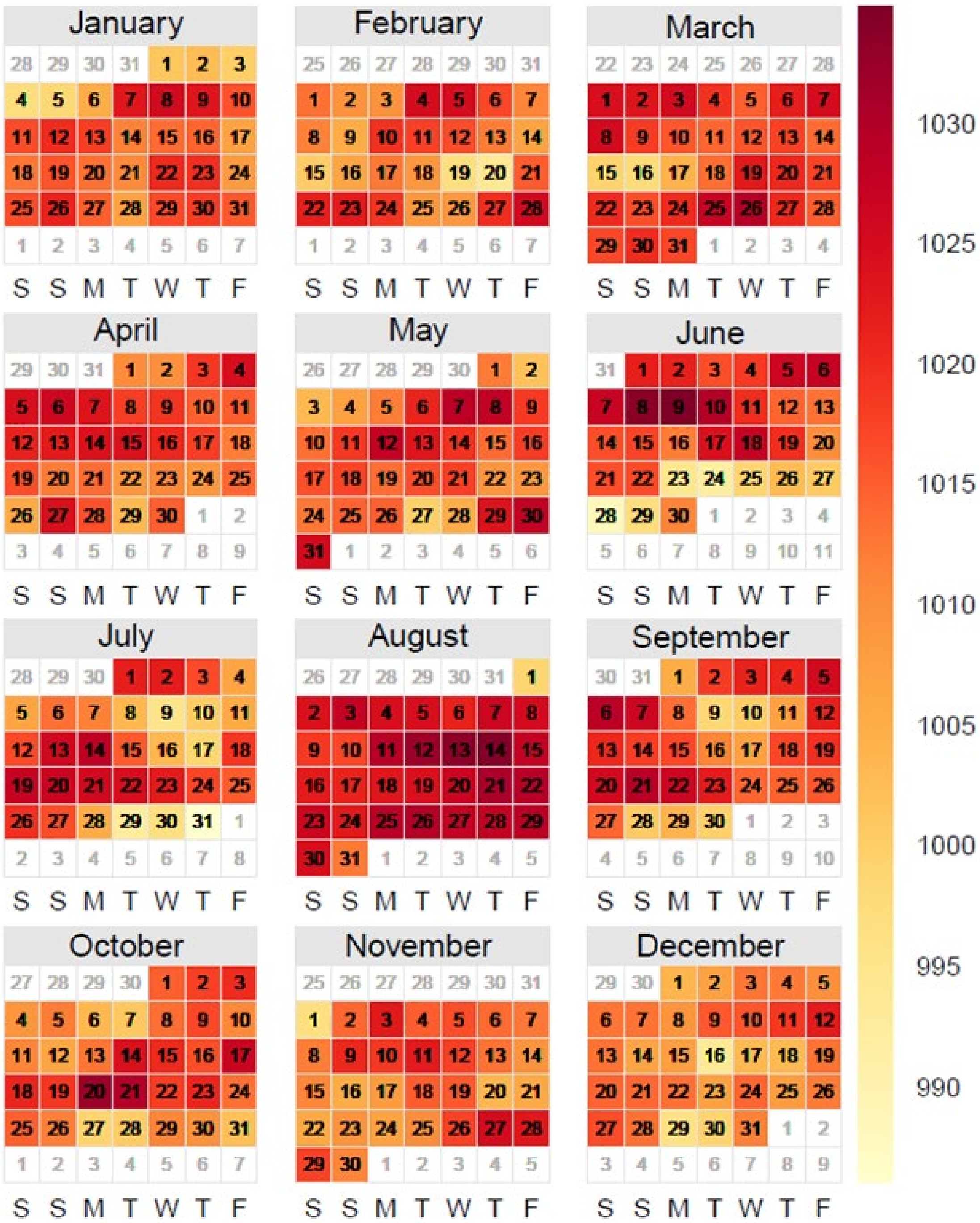


Figure 45 Calendar plot of barometric pressure (hPa) throughout 2014.

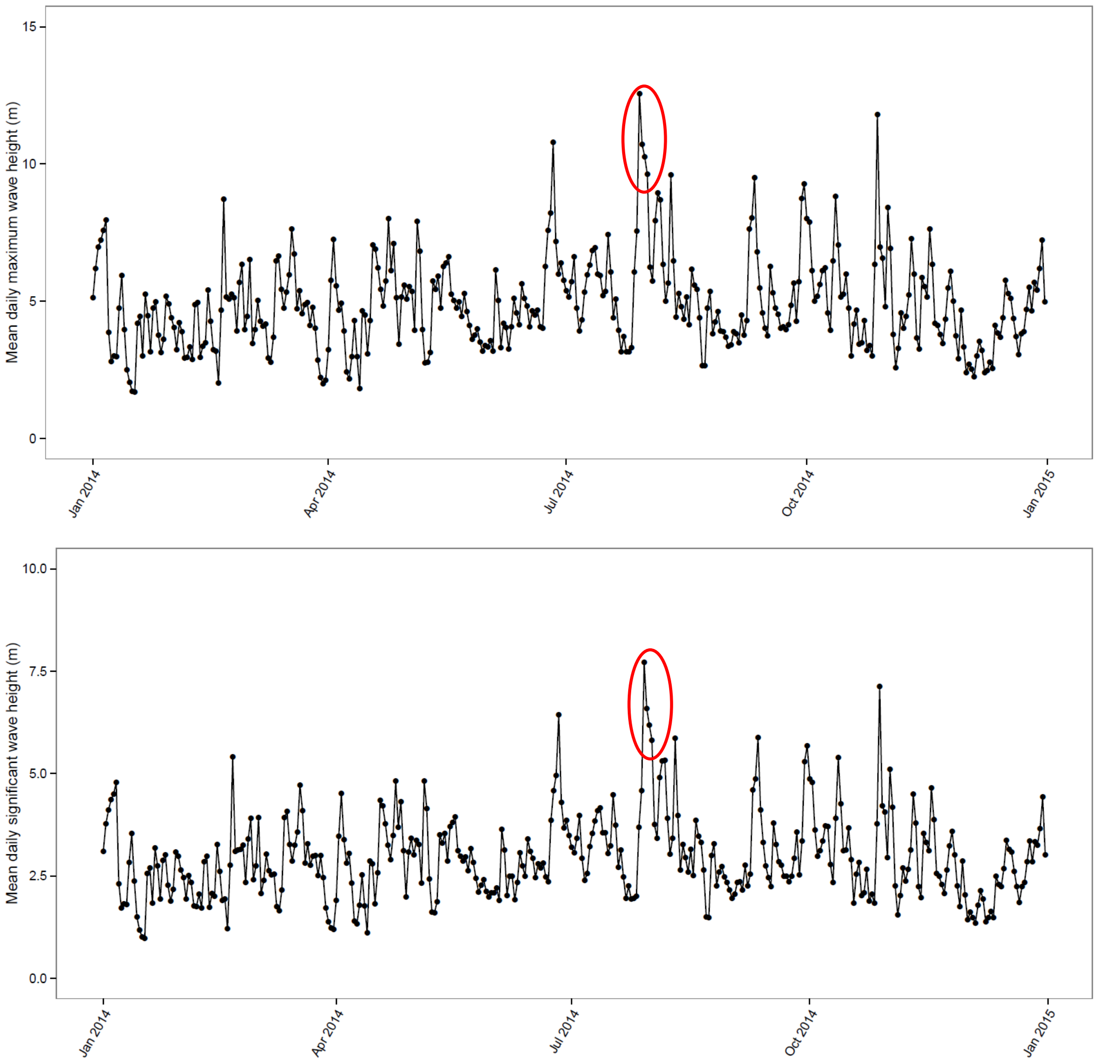


Figure 46 Mean daily significant wave height (top) and mean daily maximum wave heights (bottom) measured by the Cape Sorell Wave rider buoy during 2014.

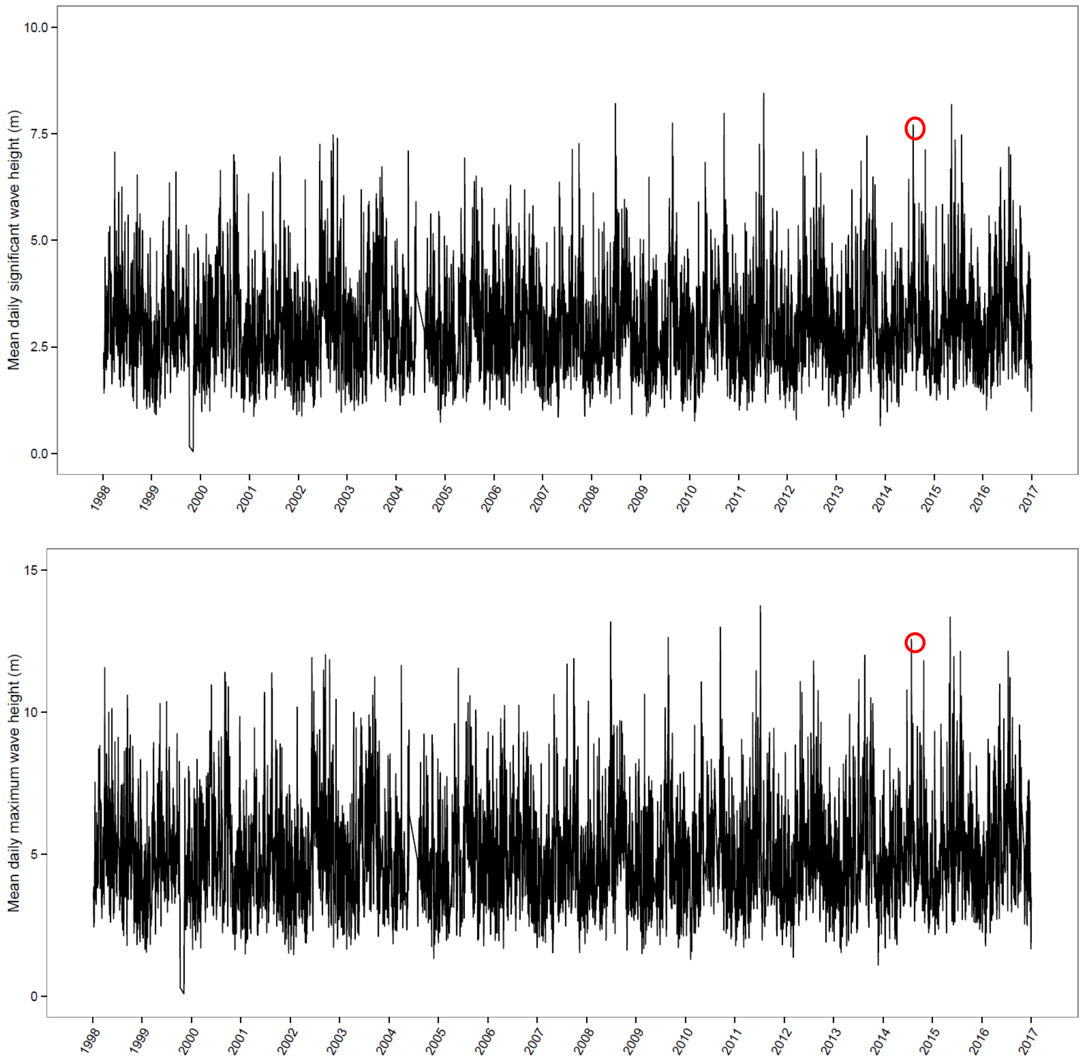


Figure 47 Mean daily significant wave height (top) and mean daily maximum wave heights (bottom) measured by the Cape Sorell Wave rider buoy between 1998 and 2017.

November 2017

Another notable oxygenation event was observed in late 2017 (Figure 48, Figure 49), but the circumstances were quite different to the previous example. In this case conditions were quite calm and river flow was low. The recharge is best illustrated through the increase in salinity and decrease in temperature observed at the permanent logger close to the harbour entrance which is consistent with an influx of high salinity and cooler oceanic waters. Reports from industry representatives suggest that Macquarie Harbour water levels were particularly low at the time, both due to low spring tides and low river inflows (Figure 50). Again, it seems likely that the difference in water height between inside and outside the harbour facilitated the influx of oceanic water and recharge, but in this case due to a high-pressure system and low river flows rather than strong north westerly winds. In the preceding example, the strong and sustained north westerly winds enhanced vertical mixing and forced the higher river flows to accumulate in the upper reaches of the estuary, thereby decreasing the water elevation inside the entrance.

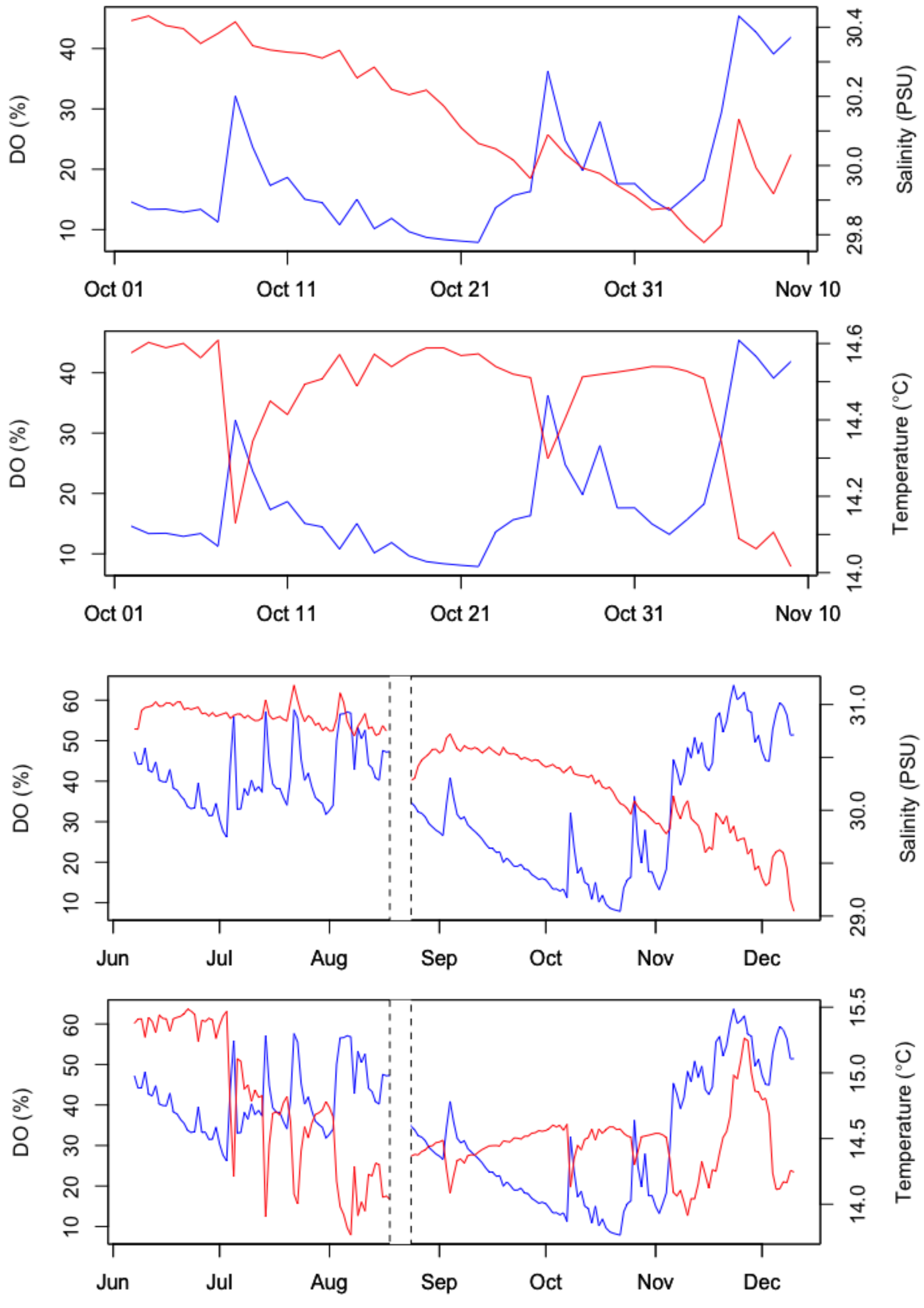


Figure 48 Dissolved oxygen (blue lines) and salinity/temperature (red lines) at the 40 m KR4 fixed monitoring station, which was the closest site to the entrance of Macquarie Harbour.

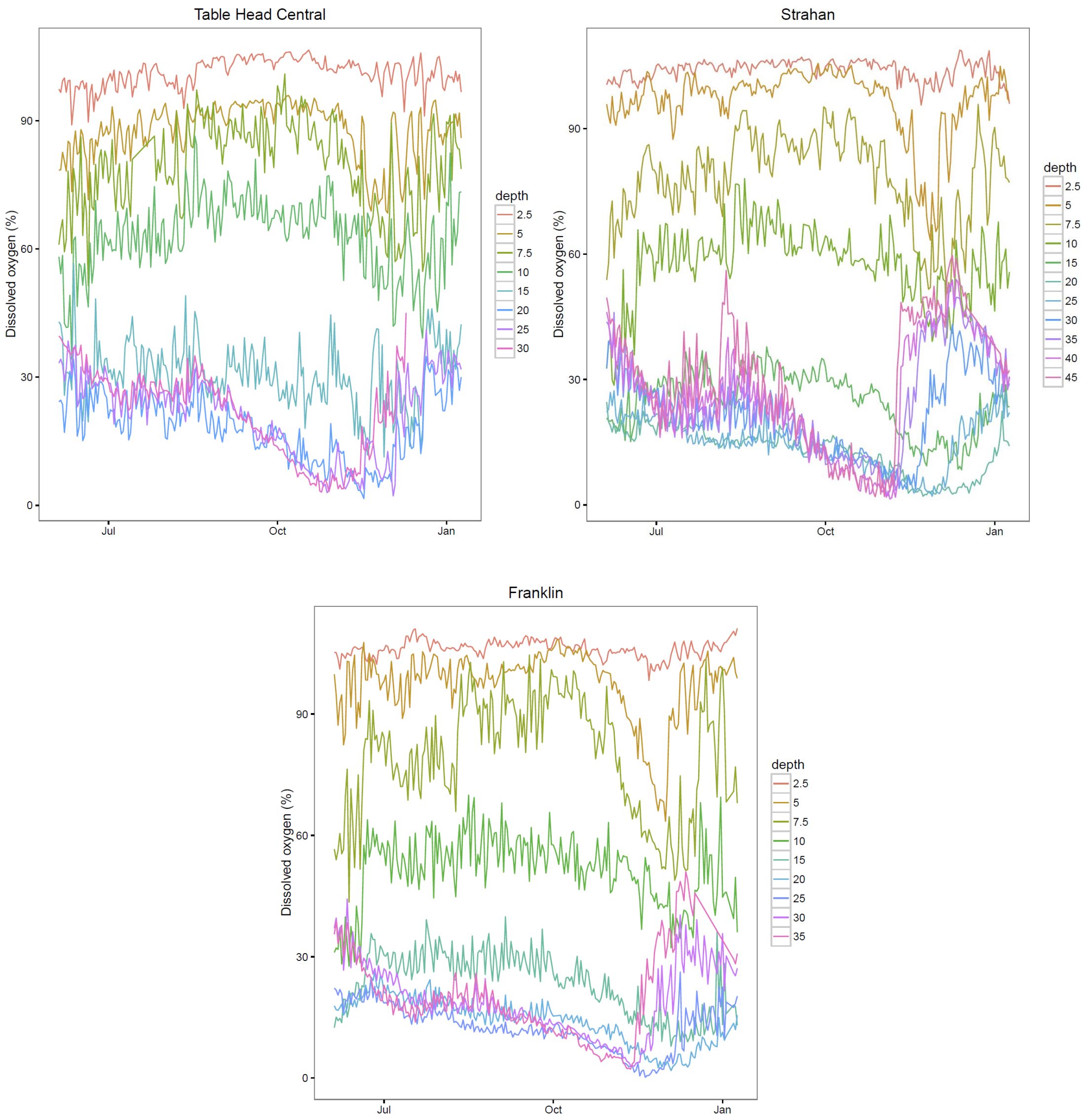


Figure 49 Daily mean DO (% saturation) levels at sensor depths from strings at Table Head Central, Franklin and the World Heritage Area over the period from the beginning of June 2017 to the 8th of January 2018.

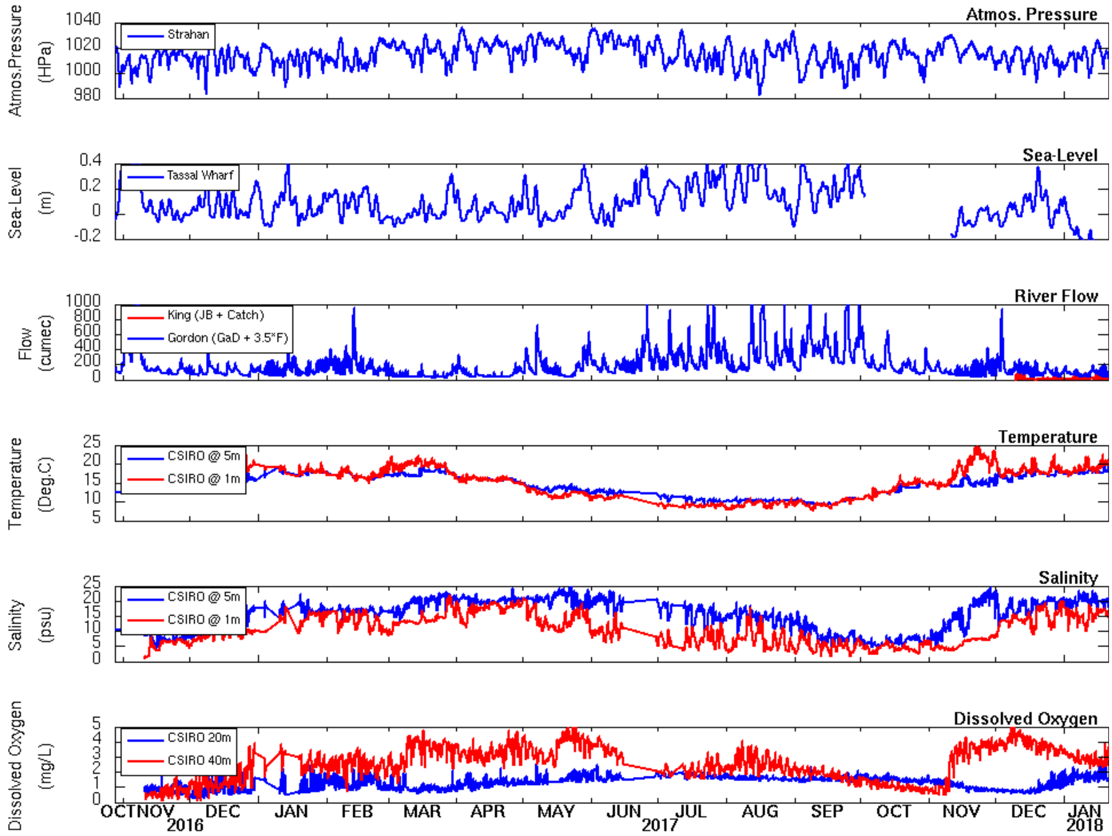


Figure 50 Time-series of concurrent environmental phenomena which may suggest favourable conditions for oxygen recharge (source Ross & Macleod 2018; data and figure provided by CSIRO).

General summer recharge

During most summers, low catchment and riverine inputs decrease the depth of the halocline thereby enabling oceanic waters to enter the harbour over the sill (MHDOWG, 2015). This is evidenced by the consistent increase in DO in the deeper waters of the harbour (Figure 40) beginning near the entrance of the harbour and then spreading throughout. It is notable that during the summers of 2012/13 and 2013/14 when inputs from the Gordon River were very high, the salinity of the deep waters declined (Figure 51) and there was no obvious recharge of DO in the deep waters (Figure 40). It is possible that this played a role in the very low oxygen concentrations observed in the harbour in autumn and winter of 2014 until the major recharge in late July.

General winter recharge

During most winters, there is a general recharge of mid-depths because of mixing between the oxygenated surface waters and the lower oxygen mid-depths (MHDOWG 2015). This is due to both seasonally strong winds and high river inputs,

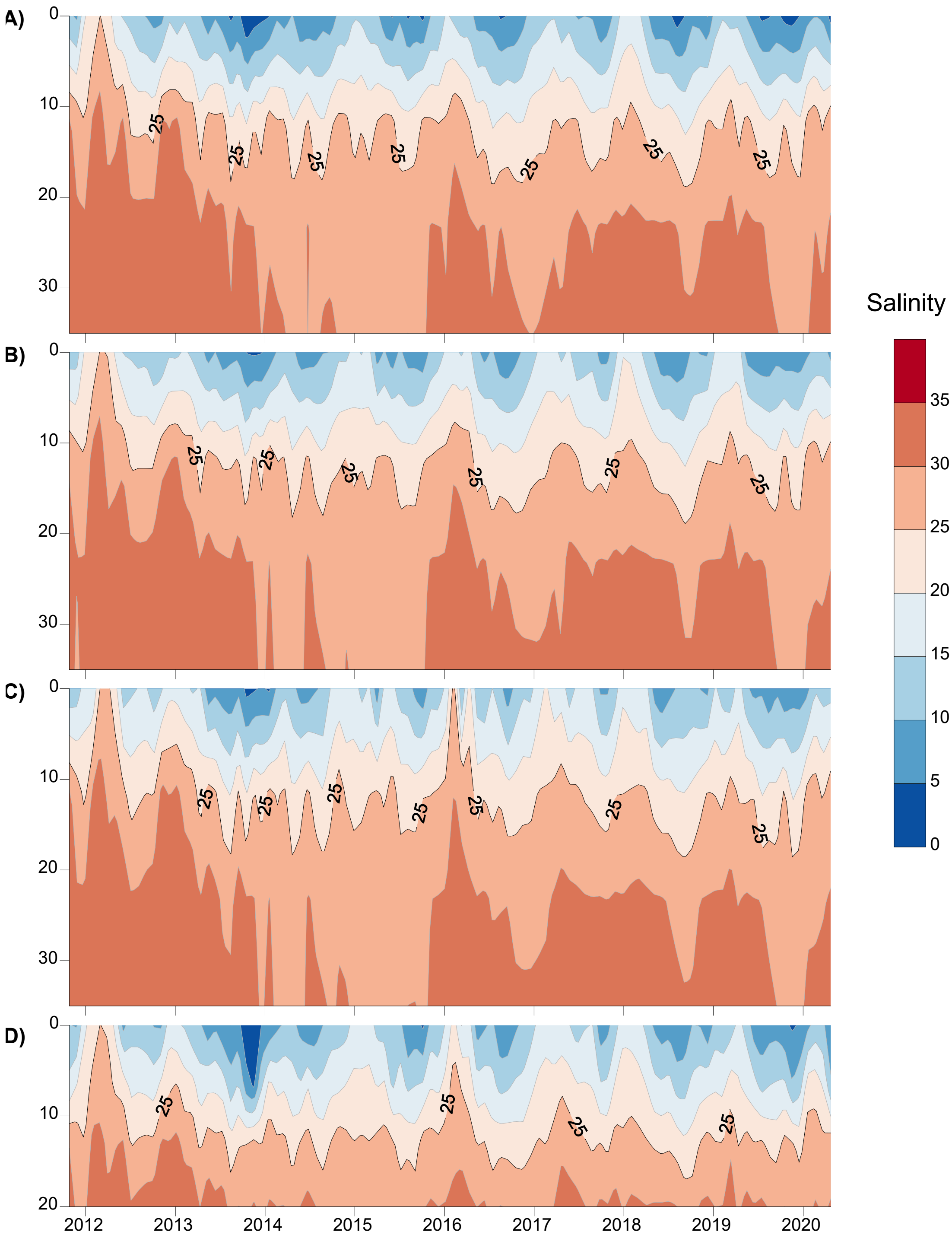


Figure 51 Contour plots of salinity at selected MHBEMP monitoring sites (A=WH2, B=CHE, C=KR1, D=PET3).

which both create turbulence in the water column resulting in vertical mixing, but high freshwater inputs also increase the depth of the halocline meaning mixing occurs at a greater depth than at other times of the year. The other factors that likely played a role in the July 2014 recharge event (i.e. low air pressure, large offshore wave height) are also more common during winter and likely play a role.

Phytoplankton composition and abundance

This section details spatial and temporal patterns in the abundance and composition of phytoplankton communities in Macquarie Harbour. Firstly, however, it is important to consider the factors that are likely to influence phytoplankton communities, notably surface nutrient concentrations and light penetration. Surface nutrient concentrations were explored in the earlier in the report and will only briefly be summarised here. Nitrate and ammonia concentrations were relatively similar across the harbour but with slightly elevated concentrations in the central region and northern arm. Nitrate and ammonia concentrations are highest through autumn and early winter, and for ammonia there appears to be a secondary peak in spring. As such, there is some indication that elevated nutrient concentrations in autumn, and to a lesser extent spring, could influence phytoplankton communities. However, dissolved nitrogen concentrations are relatively high throughout the year, so may not be limiting for phytoplankton growth.

Secchi depth

In terms of light availability, there was a significant difference in Secchi depth (December 2011 – July 2020) between MHBEMP sites (Kruskal-Wallis test: $\chi^2 = 50.2$, $df = 9$, $p = <0.001$). Post-hoc multiple pairwise comparisons suggest that the sites that differed significantly from most others were the Gordon River mouth (WH 1) and Hells Gates (HG 1). Light penetration was better (HG 1) or worse (WH 1) than most other sites, but the remaining sites were relatively similar. Changes in secchi depth in time also appeared to reflect river flow (Figure 53). For example, at the start of the MHEMP monitoring period (late 2011/2012) river flows were low and then through 2013/2014 river flow was high and this was reflected in a corresponding change in secchi depth/light penetration. Whilst light availability is clearly a limiting factor for phytoplankton growth in the harbour, these results suggest it is unlikely to be a factor dictating spatial variation in phytoplankton abundance or composition, other than at the extremities of the harbour (e.g. close to the harbour entrance and river inputs). However, temporal change in light penetration in response to river flow is likely to be one of several factors that can influence primary production.

Table 3 Pairwise Mann-Whitney test for spatial variation in Secchi depth in Macquarie Harbour from December 2011 – June 2020. Critical alpha value were corrected for multiple pairwise comparison using the method proposed by Benjamini and Yekutieli (2001).

Site	CC	CHE	CHN	HG1	KR1	PET3	SB	WH1	WH2
CHE	1	-	-	-	-	-	-	-	-
CHN	1	1	-	-	-	-	-	-	-
HG1	0.83	0.04	0.13	-	-	-	-	-	-
KR1	0.54	1	1	0.01	-	-	-	-	-
PET3	0.37	1	1	0.01	1	-	-	-	-
SB	1	1	1	0.18	1	1	-	-	-
WH1	<0.01	0.01	0.01	<0.01	0.12	0.29	0.01	-	-
WH2	0.06	0.59	0.46	<0.01	1	1	0.37	0.83	-
WHN	0.18	1	1	<0.01	1	1	0.83	0.34	1

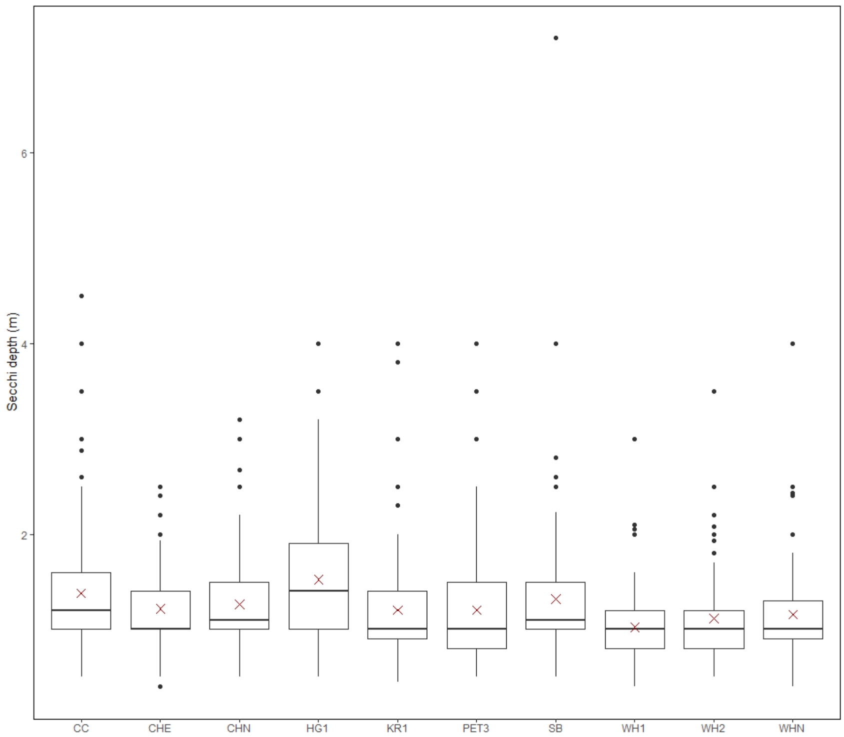


Figure 52 Boxplots of Secchi depth (m) at each MHBEMP site.

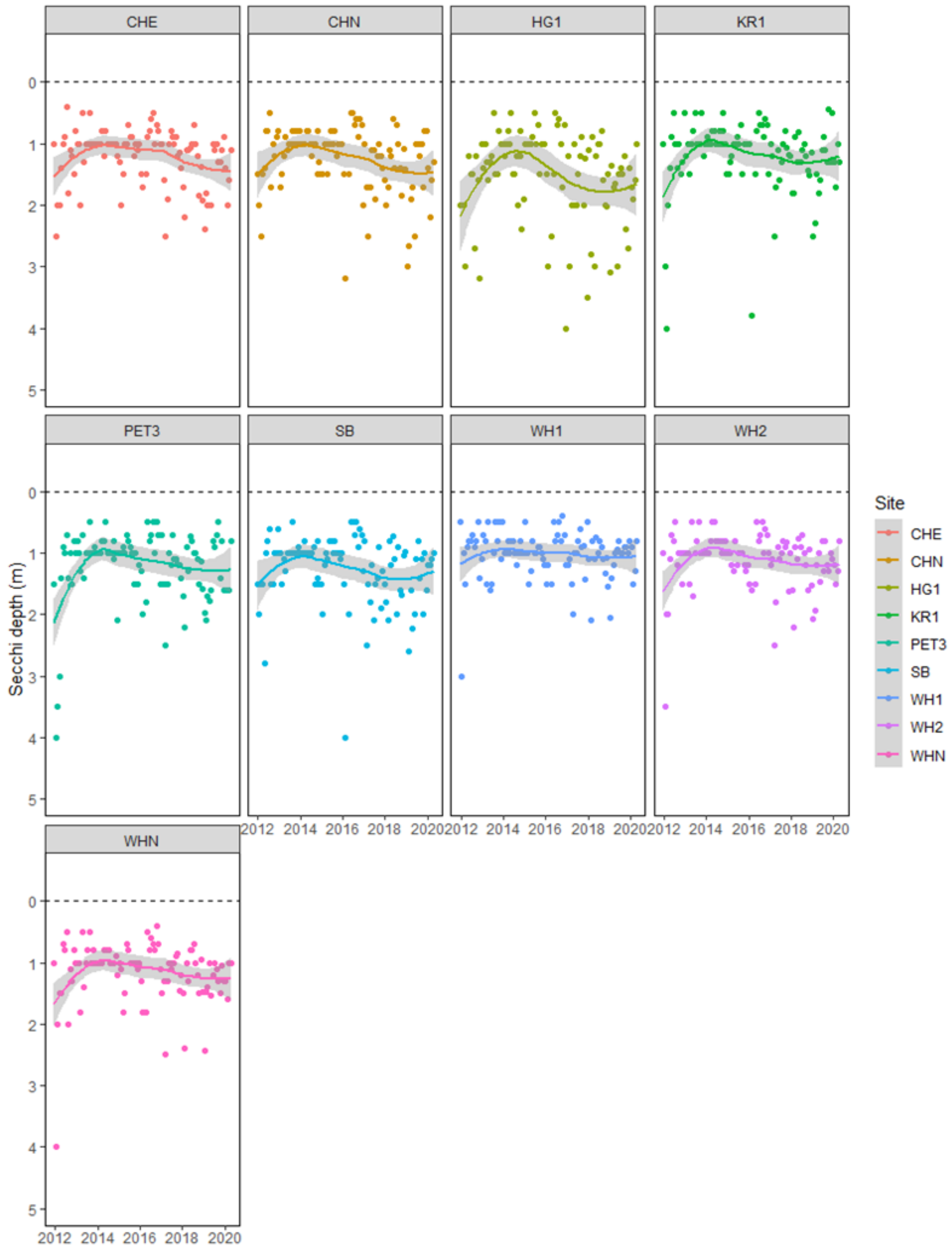


Figure 53 Secchi depth through time at the main MHBEMP sites. Trends are smoothed using GAMs.

Chlorophyll-*a*

Chlorophyll-*a* concentration is notably higher in the 2 m Niskin sample than in the 12 m integrated sample with the latter rarely exceeding the detection limit of 0.5 (Figure 54). As such, the 12 m integrated sample will not be discussed further. Chlorophyll-*a* displays a seasonal pattern in which concentrations are low from May to August before an increase in spring, reaching maximum concentrations from

November to March (Figure 54). Interestingly, there are several observations during late autumn and winter that also display elevated chlorophyll-*a* suggesting phytoplankton abundance can be high at any time of the year, though the frequency of high concentration is lower during the cooler months. There is some indication of spring and autumn blooms in September and March respectively, but this is less pronounced than in other Tasmanian coastal systems, for example the D'Entrecasteaux Channel (Bell et al., 2017). Additionally, chlorophyll-*a* concentrations in Macquarie Harbour were higher in summer (typically >2.5 – 4 mg/m^3), which is high compared to the D'Entrecasteaux Channel, which has a mean of <2.0 mg/m^3 in spring and summer (Bell et al., 2017). It is not clear whether these high chlorophyll-*a* concentrations are representative of the natural state of the harbour, or whether they were elevated when MHBEMP sampling began as aquaculture was already operating at a reasonable biomass prior to the commencement of the BEMP.

Mean chlorophyll-*a* concentrations across all MHEMP sites remained consistent throughout the observation period (2012-2020). However, the magnitude of seasonal peaks varied between years, with the summers of 2013 and 2016 showing the highest levels (Figure 55).

Chlorophyll-*a* concentrations at the river end member sites, Gordon River (GR1) and the King River (KR4) (Figure 55) were low, indicating the rivers are not major sources of chlorophyll-*a*. Additionally, other sites at the extremities of the harbour (GR2 and WH1 near the Gordon River mouth and HG1 at the entrance of the harbour) also had relatively lower concentrations compared with sites throughout the main body of the harbour. These observations indicate that the dynamics of chlorophyll-*a*, and hence phytoplankton production, within Macquarie Harbour are largely driven by the conditions within the harbour.

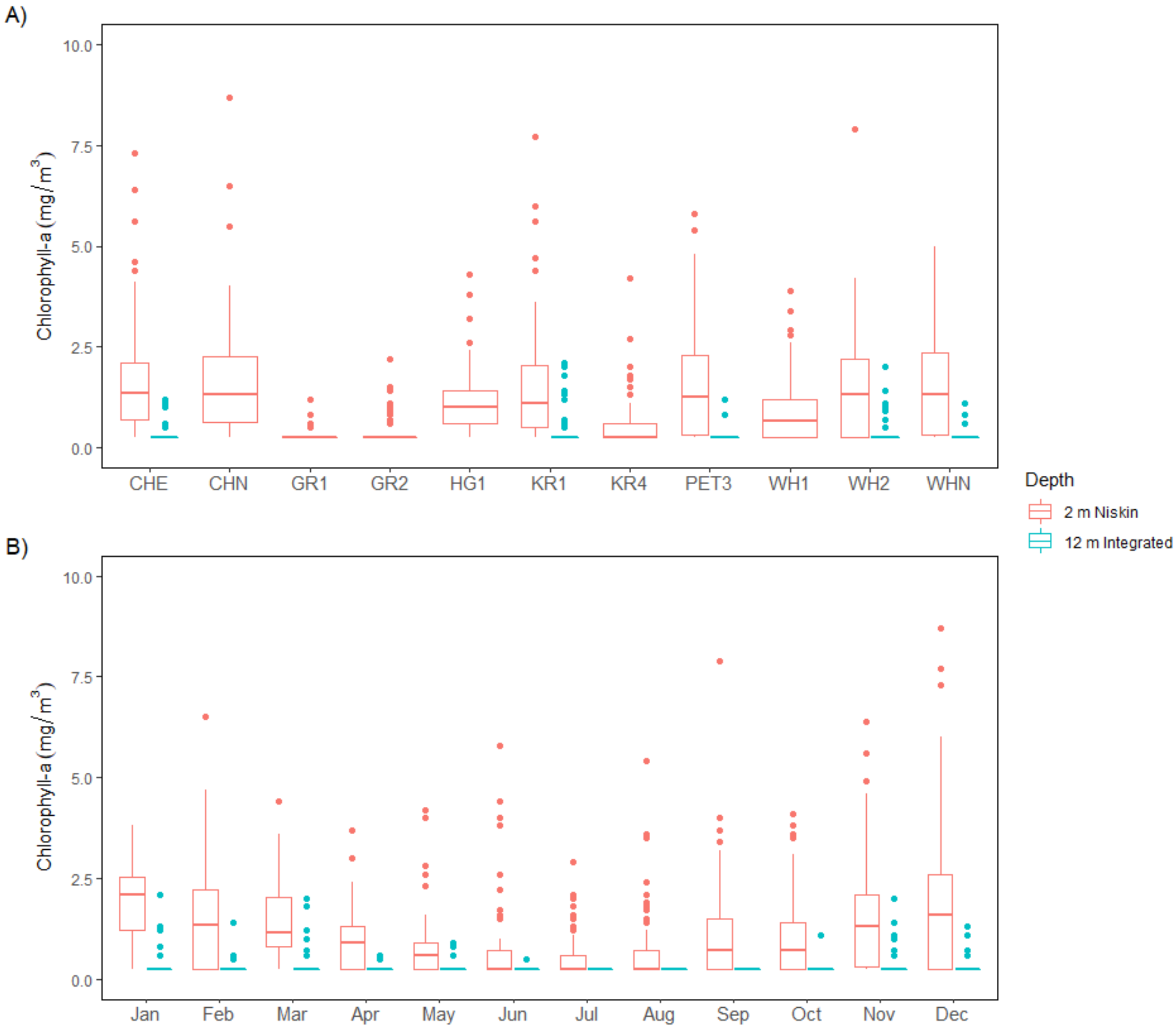


Figure 54 Chlorophyll-a concentration at each MHBEMP monitoring site (A) and during each month (B) from October 2011 to June 2020.

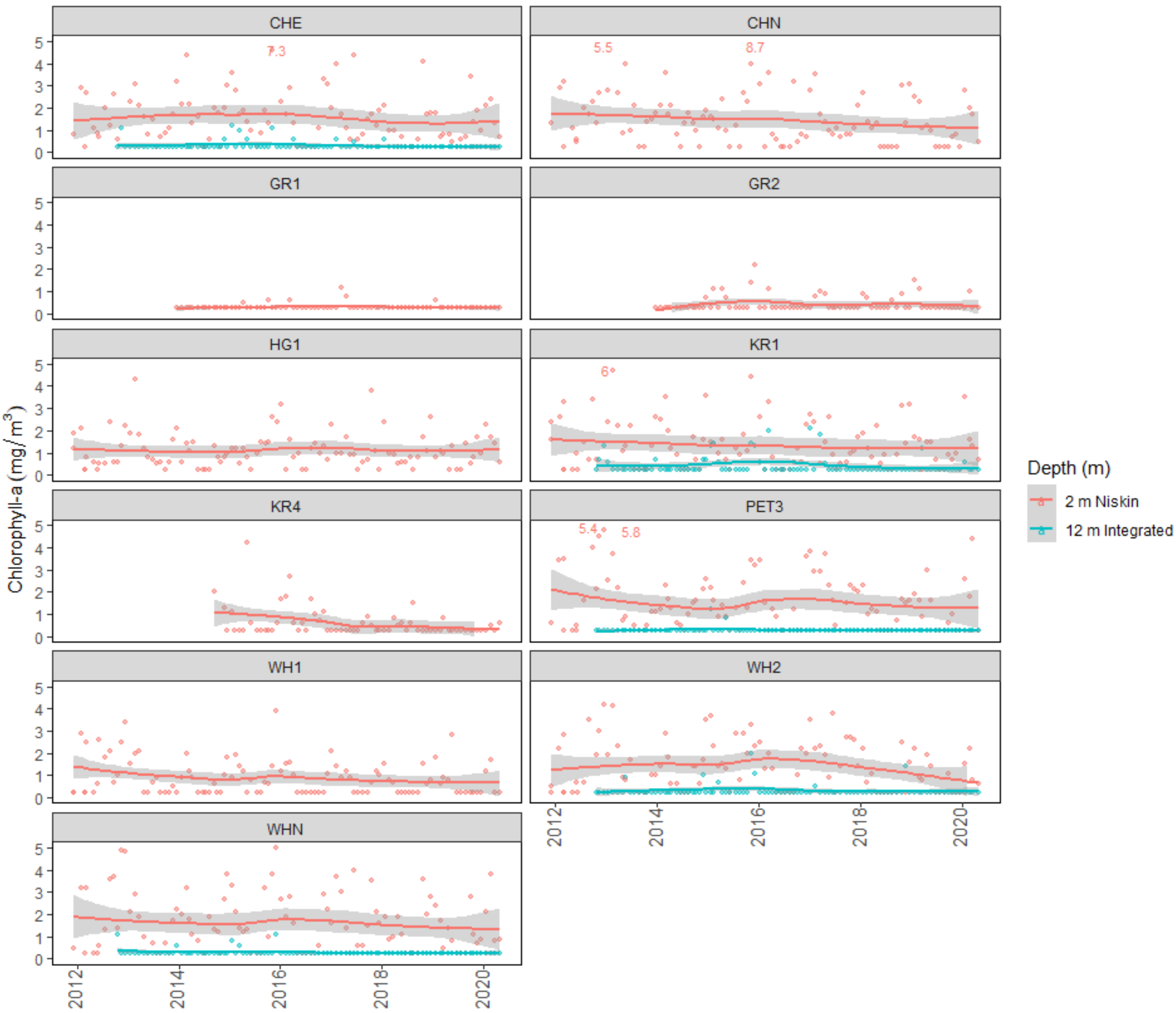


Figure 55 Loess regression displaying temporal trends in Chlorophyll-a concentration at the various depths it is measured in MHBEMP sampling. Numbers values represent observations that fell outside the y axis range. *Note: LOESS regression was used because convergence could not be achieved when fitting GAMs.

Phytoplankton community structure

Cryptophytes were the most abundant phylum group in all years except for 2014 and 2015, when the most abundant groups were unidentified Nanoflagellates and Bacillariophytes respectively. Chlorophytes and Prasinophytes were moderately abundant in all years (~4–20%) with the remaining groups being less common. Dinoflagellates, the group most frequently associated with harmful algal blooms, represented <15% of the total phytoplankton assemblage in all years.

Phytoplankton abundance was highest in the central basin and northern reaches of Macquarie Harbour (sites CH1, CHE, CHN, KR1, PET3, SB, WH1, WH2, WHN) and lowest near the entrance (sites HG1 and CC) (Figure 57). Given that phytoplankton abundance like chlorophyll-*a* concentrations were relatively low and the river and ocean end member sites, this indicates that phytoplankton productivity is predominately in the harbour. Phytoplankton counts across all sites peaked in 2015 and 2017 and were lowest in 2019.

Phytoplankton abundance follows a strong seasonal pattern whereby abundance is low in winter followed by a spring bloom that declines in November before a secondary increase in summer when the peak abundance is reached (Figure 59). Phytoplankton abundance then tails off from late summer through autumn.

The highest correlation between phytoplankton abundance and environmental and physico-chemical variables was with temperature, salinity, and the ammonia: nitrate ratio (Table 6). There were phylum specific responses to environmental covariates based on the SDM model (Figure 61). However, correlation in the bio-environmental model was relatively low (R^2 = 0.3229) and consistent among all combination of variables (0.23 – 0.32). Likewise, species specific coefficients were relatively low, particularly for unidentified nanoflagelates and Chrysophyta; suggesting other unmeasured factors are likely to also play a role in phytoplankton abundance.

Phosphorus concentration is not measured in Macquarie Harbour, and as such, it was not possible to determine whether this nutrient may be limiting primary productivity. In both marine and freshwaters, most frequently both nitrogen and phosphorus are limiting; however, phosphorus limits primary productivity more frequently in freshwater waters than it does in marine waters (Elser et al., 2007). As such, phosphorus, or potentially silicate in the case of diatoms, could be limiting productivity and measurement of these variables in future would aid in interpretation of the phytoplankton data.

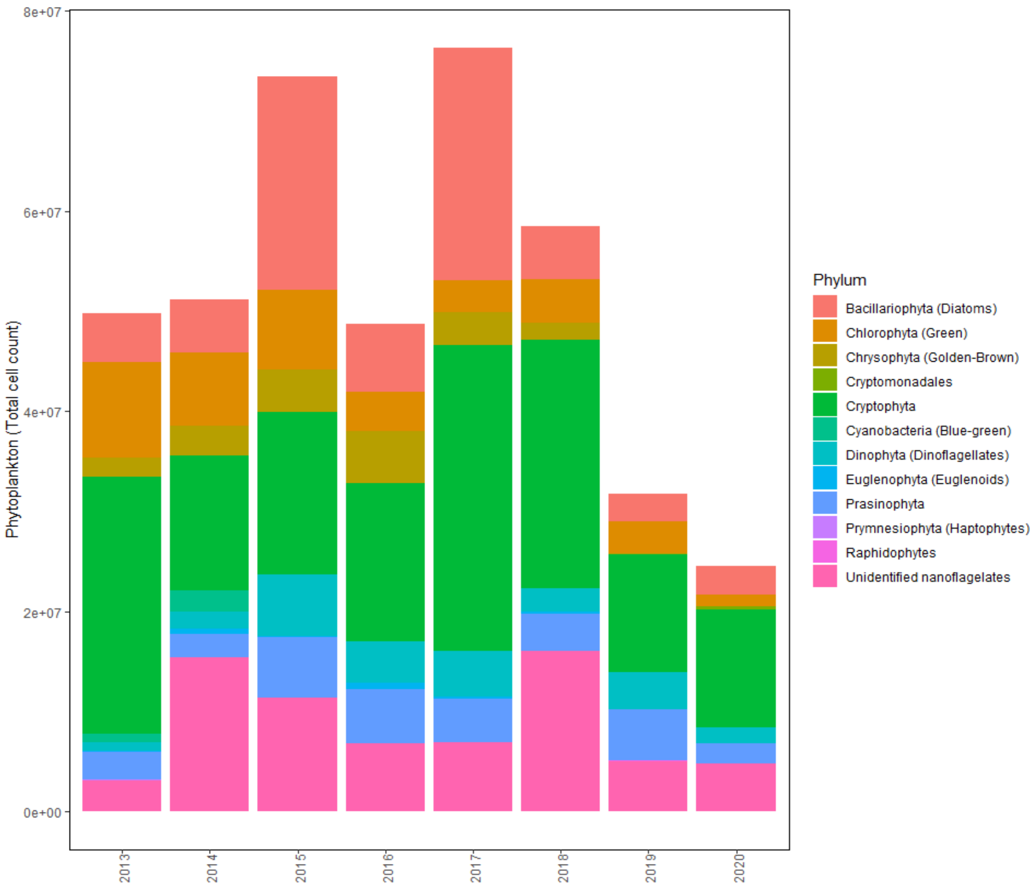


Figure 56 Annual total phytoplankton cell counts from all sites and months in Macquarie Harbour. The year 2012 was removed from this analysis as it only contained one month of sampling, year 2020 only represents 6 months of sampling.

Table 4 Minimum and maximum annual contribution of each phytoplankton phylum to the total phytoplankton community measured at all Macquarie Harbour BEMP sites between 2013 and 2020.

Phylum group	Minimum	Maximum
Bacillariophyta (Diatoms)	8.5	30.48
Chlorophyta (Green)	4.16	19.08
Chrysophyta (Golden-Brown)	0	10.49
Cryptophyta	22.08	51.54
Cyanobacteria (Blue-green)	0.1	4.16
Dinophyta (Dinoflagellates)	1.59	11.6
Euglenophyta (Euglenoids)	0	1.46
Prasinophyta	4.47	16.2
Prymnesiophyta (Haptophytes)	0.00	0.07
Raphidophytes	0.00	0.3
Unidentified nanoflagellates	6.24	30.12

Table 5 Percent annual contribution of each phytoplankton phylum to the total phytoplankton community measured at all Macquarie Harbour BEMP sites between 2013 and 2020.

Phylum	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
Bacillariophyta (Diatoms)	9.9	10.4	29.1	14	30.5	8.9	8.5	11.7
Chlorophyta (Green)	19.1	14.3	10.8	8.1	4.2	7.5	10	4.9
Chrysophyta (Golden-Brown)	3.8	5.8	5.9	10.5	4.4	2.9	0.4	0
Cryptophyta	51.5	26.3	22.1	32.5	40	42.5	37.3	48.3
Cyanobacteria (Blue-green)	1.8	4.1	0	0.1	0	0	0	0
Dinophyta (Dinoflagellates)	1.6	3.3	8.3	8.3	6	4.1	11.6	6.2
Euglenophyta (Euglenoids)	0.3	1.1	0.2	1.5	0.3	0.3	0	0
Prasinophyta	5.6	4.5	8.3	11.1	5.7	6.3	16.2	8.3
Prymnesiophyta (Haptophytes)	0.1	0	0	0	0	0	0	0
Raphidophytes	0	0	0	0	0.1	0	0.3	0
Unidentified nanoflagelates	6.2	30.1	15.4	13.9	9	27.5	15.6	19.5

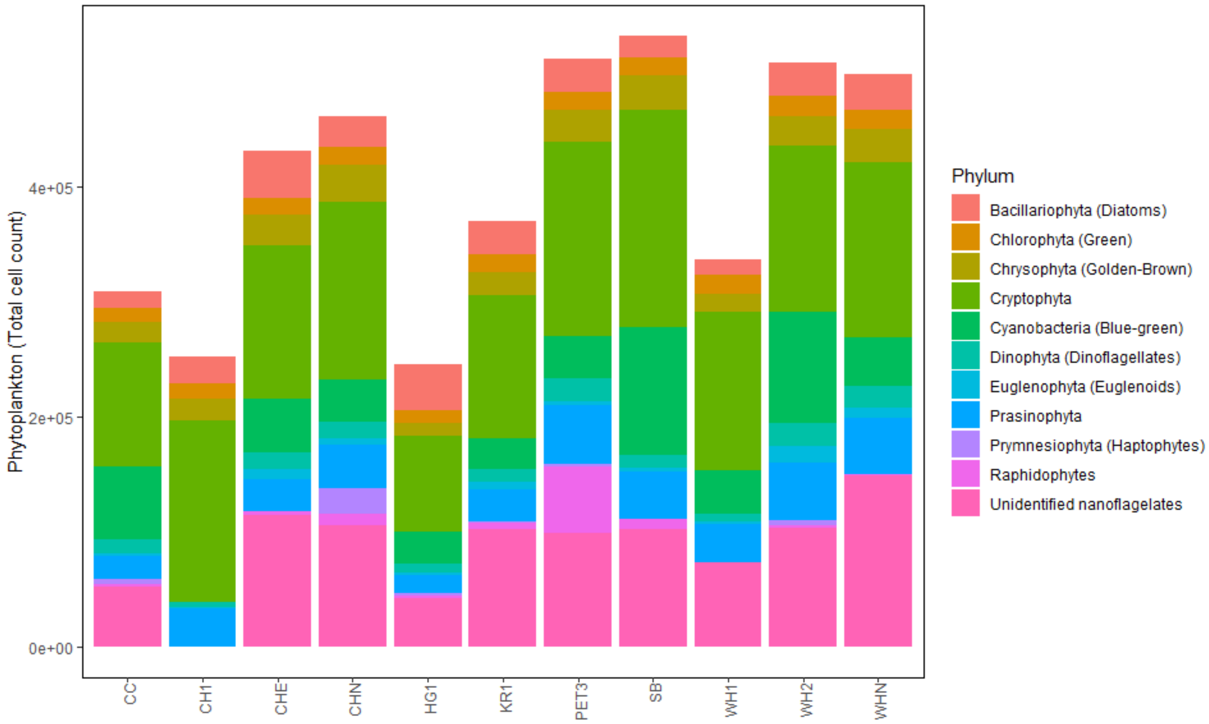


Figure 57 Mean contribution of the various phytoplankton phylum groups at each Macquarie Harbour BEMP site between December 2012 and June 2020.

Figure 58 Monthly mean contribution of each phylum group (all Macquarie Harbour BEMP sites) to the Macquarie Harbour phytoplankton assemblage.

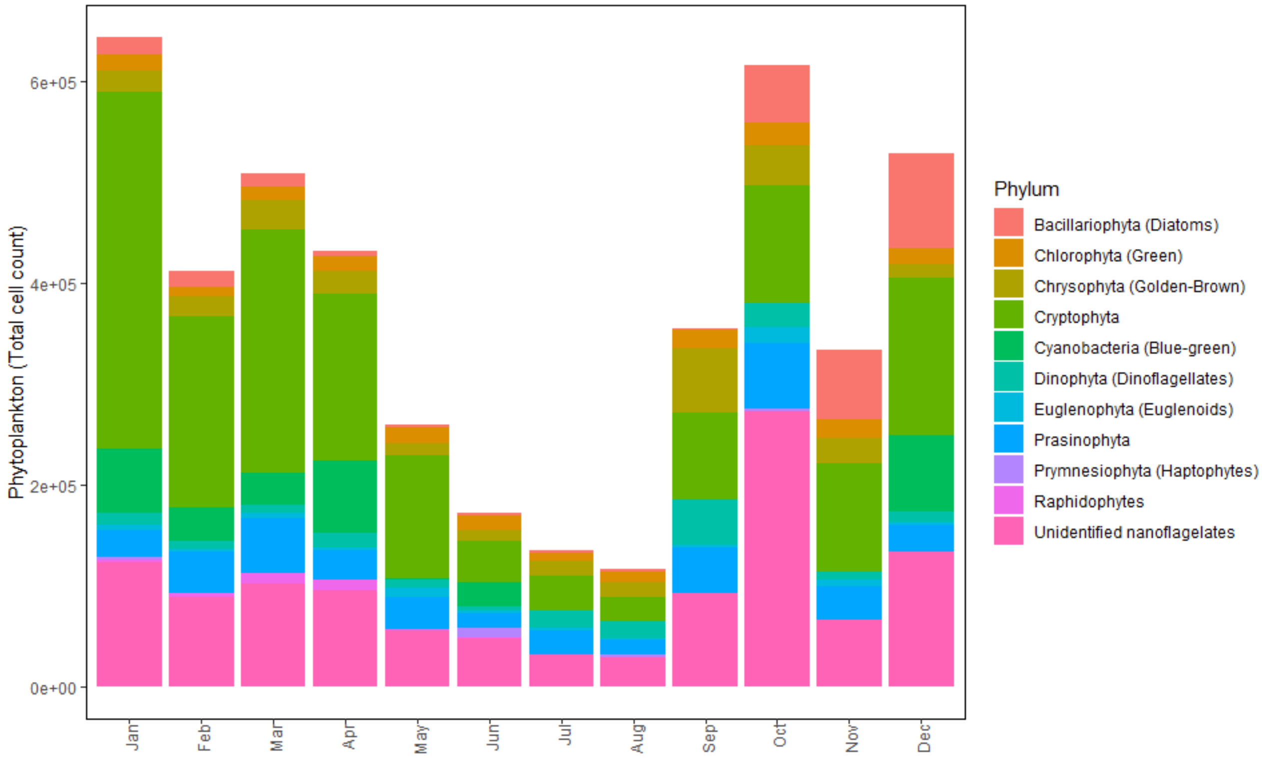


Figure 59 Monthly phytoplankton abundance in Macquarie Harbour (all sites).

Table 6 Bioenv analysis of the correlation between available environmental and physico-chemical variables and phytoplankton composition in Macquarie Harbour from December 2012 to June 2020.

Explanatory variables	Number of variables	Correlation
Temperature	1	0.2365
Temperature; ammonia:nitrate ratio	2	0.2931
Temperature; day length; ammonia:nitrate ratio	3	0.3229
Temperature; salinity; day length; ammonia:nitrate ratio	4	0.3142
Temperature; total nitrogen; salinity; day length; ammonia:nitrate ratio	5	0.3064
Temperature; nitrate; total nitrogen; salinity; day length; ammonia:nitrate ratio	6	0.3037
Temperature; Secchi depth; nitrate; total nitrogen; salinity; day length; ammonia:nitrate ratio	7	0.2971
Temperature; Ammonia; Secchi depth; nitrate; total nitrogen; salinity; day length; ammonia:nitrate ratio	8	0.2745

Temporal and spatial variation of the major phytoplankton groups

Cryptophytes

Cryptophytes are the most abundant lineage within Macquarie Harbour phytoplankton communities (Table 7), representing 36.8% of overall abundance and as high as 96.83% in one month. Blooms were common from December through to the end of summer, early autumn (Figure 59).

Cryptophyte densities were significantly higher in the 2 m sample than they were in the 12 m integrated sample (Figure 60) indicating they are predominantly comprised of freshwater species, or species that can inhabit a wide range of salinities and choose to remain in the freshwater surface waters. This is most likely because the euphotic zone is predominantly comprised of freshwater. All other phylum groups displayed a similar trend (Figure 60) so depth and salinity preference will not be discussed further unless there were examples that differed from this trend.

It has been shown that many Cryptophyte species are able to flourish in low light environments (Vesk and Jeffrey, 1977, Gervais, 1998, Hammer et al., 2002, Klaveness, 1988). Light penetration is poor in Macquarie Harbour due to the high tannin content of its tributaries. Analysis of spatial variation in Secchi depth demonstrated that light penetration is reasonably uniform throughout the harbour, thus, spatial variation in Cryptophyte abundance is unlikely to be due to variation in light penetration alone.

The most abundant phytoplankton species in the Macquarie Harbour system was the Cryptophyte, *Chroomonas* sp. which were ~8 times more abundant in the 2 m integrated sample than in the 12 m integrated sample indicating they are predominantly a freshwater species. Of nearly 30 Cryptophyte species investigated by Sandgren (1988), 3 of the top 6 with the largest niche width belonged to the *Chroomonas* genus indicating they are very versatile and likely to adapt to conditions that are not necessarily suitable for other species. This is because some species (e.g. *Chroomonas salina*) are known to be mixotrophic (Antia (1980) cited in Klaveness (1988)) and many species have been shown to flourish under very low light conditions (Vesk and Jeffrey, 1977, Gervais, 1998, Hammer et al., 2002, Klaveness, 1988). Additionally, bacterivory has been reported in some species (Tranvik et al., 1989, Marshall and Laybourn-Parry, 1989) and the difference in carbon: phosphorus ratio between bacteria and algae makes it possible for mixotrophic Cryptophytes to supplement their phosphorus demand by ingesting bacteria (Olrik, 1998). The above characteristics mean it is likely that *Chroomonas* sp. found to be abundant in Macquarie Harbour is particularly well suited to the conditions in Macquarie Harbour that include low light, high nutrient concentration, and high bacterial/archaeal abundance (Ross et al., 2016a). This, and their high abundance, make *Chroomonas* sp. likely to be a good bio-indicator in Macquarie Harbour.

In the only previous study we are aware of that investigated phytoplankton communities in Macquarie Harbour (O'Connor et al., 1996), Bacillariophytes were the most abundant lineage, however, only relative abundance was reported (an abundance index) so a direct comparison is not possible. *Chroomonas* sp. were identified by O'Connor et al. (1996), but in low numbers. This suggests that they were not incorrectly identified and the comparison of the two studies, at least in terms of lineage composition, is likely to be representative, noting, however, that the earlier study was conducted over a one-week period in late August to early September so was not comprehensive.

Table 7 Percent of the total phytoplankton community of each phylum group from December 2012 to June 2020 (Total) and the minimum and maximum monthly percentage contribution of each phylum group observed during the time frame. These data include both the 2 m and 12 m integrated samples.

Phylum	Total	Monthly minimum	Monthly maximum
Bacillariophyta (Diatoms)	11.2	0.1	70.8
Chlorophyta (Green)	13.3	0.5	55.8
Chrysophyta (Golden-Brown)	6.5	0	49.1
Cryptomonadales	3.1	3.1	3.1
Cryptophyta	36.8	2.6	96.8
Cyanobacteria (Blue-green)	6.5	0	14.2
Dinophyta (Dinoflagellates)	8.1	0.1	47.3
Euglenophyta (Euglenoids)	1.1	0	16.4
Miozoa	0.1	0	0.1
Prasinophyta	8.3	0.4	32.6
Prymnesiophyta (Haptophytes)	0.3	0	1.3
Raphidophytes	0.4	0	3.9
Unidentified nanoflagelates	19.2	0.3	69.9

Bacillariophytes

Bacillariophytes were the second most abundant group in the harbour during the period investigated (Table 5). The lowest concentrations were observed between January and September (<80 cells/mL) before an increase in October to December (Figure 59). The highest concentrations were confined to the northern arm of Macquarie Harbour and can reach ~1300 cells/mL, dominated by the non-toxic species *Chaetoceros pseudocrinitus* and *Cyclotella* sp. In 1995, two acidophilous Bacillariophytes, *Tabellaria flocculosa* and *T. fenestrate*, were abundant near the King River mouth (O'Connor et al., 1996) indicating they were potentially sourced from the riverine inputs. These species were not found in the present study.

Several diatoms (*Thalassiosira delicatula*, *Thalassiosira angulate*, *Synedra* sp., *Skeletonema* sp., *Pseudo-nitzschia delicatissima* (group), *Leptocylindrus minimus*, *Leptocylindrus danicus*, *Chaetoceros socialis*, *Chaetoceros affinis*) were considerably more abundant in the 12 m integrated samples than they were in the 2 m samples indicating they are potentially marine species and/or aggregate at the pycnocline. Some of these species are known to be sensitive to low salinity (e.g. *Chaetoceros socialis*; Shevchenko, 2008). Most of the other phytoplankton species in Macquarie Harbour were much more abundant in the 2 m sample indicating they are predominantly freshwater or euryhaline species.

In October 2017, *C. socialis* counts at sites HG3 and HG1 exceeded 10000 cells/mL at 2 m. These sites are at the entrance of the harbour and are more strongly influenced by marine conditions, explaining why an obligate stenohaline species (Shevchenko et al. 2008) was present in such high concentrations in the 2 m sample. Sites near the entrance but inside the harbour (CHN and CC) did not show a similar increase in Bacillariophytes, indicating that the bloom occurred in oceanic waters and did not affect Macquarie Harbour.

Dinoflagellates

Dinoflagellates did not represent a major component of the Macquarie Harbour phytoplankton assemblage (8.1% on average) during the period investigated (Table 5). Cell counts ranged between just 1–820 cells/mL with the highest concentrations observed in spring (Figure 59), peaking in September. A non-toxic *Katodinium* species had a recurrent presence and the only toxic species regularly observed, typically in summer/autumn (especially March) was the spirolid producing *Alexandrium ostenfeldii* (Cembella et al., 2000) but always in low concentrations (<2 cells/mL). Other toxic dinoflagellates were either not present, or very uncommon in Macquarie Harbour during the period investigated. The only historical study for temporal comparison was that by O'Connor et al. (1996) in which dinoflagellates were more widespread, and more abundant, than Cryptophytes.

Chlorophytes

Chlorophytes are not a major component of the phytoplankton community in Macquarie Harbour (Table 5). Monthly mean abundance reached a maximum of 470 cells/mL in spring (Figure 59). These higher cell concentrations in the estuary were typically in the north arm from September to November and near the Gordon River mouth in summer. Prasinophytes were the most spatially and temporally abundant Chlorophyte lineage.

Euglenoids

Euglenoids comprised only a small proportion of the phytoplankton assemblage (Table 5). This lineage was the most abundant phyla group in Macquarie Harbour in 1995, particularly near the entrance and in the southern most reaches (O'Connor et al., 1996) suggesting there may been a relatively major change in the phytoplankton composition of the harbour. However, sampling by O'Connor et al. (1996) only took place over a seven day period in late September to early October so it may not be completely representative of phytoplankton communities at the time. Nevertheless, Euglenoids did not represent a large proportion of the community in any month during the time series investigated (Figure 59) further supporting the assertion that there has been a change in phytoplankton composition, unless sampling by O'Connor *et al*., (1996) was undertaken during a particularly irregular time.

Unidentified nanoflagellates

Unidentified nanoflagellates now represent a relatively large proportion of the phytoplankton community in Macquarie Harbour (>50% of abundance in some months;Table 5). It is likely that these individuals belong to a variety of phyla and, if they were identified, may alter the relative abundance of several phyla groups within the overall Macquarie Harbour phytoplankton community. Interestingly, these nanoflagellates were absent until December 2013, a month in which they were the most abundant group in the system, and they have remained common ever since. In preparation of this study, we received confirmation from Analytical Services Tasmania that the increase in this group is real and not an artefact of a change in analytical technique or reporting (personal communication with Stephanie Fulton, AST). As a result, the increase in abundance of this group could represent a major change in community composition to the system.

Bacteria production is a major source of organic matter in Macquarie Harbour (Ross et al., 2016a) and in the correspondence with AST outlined above, it was noted that bacterial abundance has also increased over the same time frame, though these are not quantified. Nanoflagellate bacterivory has been shown to consume up to 40% of bacterial production (Christakill et al., 1999), thereby playing an important role in the dynamics of a system. As such, it is important that the nanoflagellates observed in Macquarie Harbour are formally identified, and their trophic level (i.e. autotroph, mixotroph or heterotroph) verified.

Summation of phytoplankton analyses

The Macquarie Harbour phytoplankton community was dominated by Cryptophytes (*Chroomonas* sp. in particular) with Bacillariophytes and unidentified nanoflagellates also being relatively abundant. Most other lineages were only found in low abundance, or only had sporadic blooms. There is no comprehensive historical data on which to compare phytoplankton composition or abundance. O'Connor et al. (1996) provided a measure of phytoplankton relative abundance during a one-week period in late August to early September in 1995, which enables some inferences to be made. For instance, it is possible that there has been a shift in the dominant groups present in Macquarie Harbour: the present study found that Cryptophytes (*Chroomonas* sp. in particular) were the most abundant lineage during most months of the study period, whereas O'Connor et al. (1996) found a dominance of Euglenoids and Bacillariophytes, the former being very rare now in Macquarie Harbour.

In December 2013 a bloom of unidentified nanoflagellates occurred in the system, dominating the phytoplankton assemblage and this group have remained relatively abundant ever since. This may represent an introduction to the harbour, or the system dynamics have changed to a state that is now particularly suitable for these species.

Bathurst Harbour is the only Australian estuary comparable to Macquarie Harbour; both are unique in having highly stratified, tannin-stained surface waters that prevent light penetration. Unlike Macquarie Harbour, Bathurst Harbour is dominated by Dinoflagellates (*Dinophysis acuminata* in particular) with very low numbers of most other species (Edgar and Cresswell, 1991). Macquarie Harbour had very low Dinoflagellate abundance in the present study and had the lowest number of Dinoflagellate species cysts, and the lowest abundance of cysts, of any of the 11 Tasmanian estuaries investigated by Bolch and Hallegraeff (1990). Interestingly, Bathurst Harbour was the second lowest in the above measures, suggesting that although Dinoflagellates are the most abundant group in Bathurst Harbour, the unique characteristics of both Bathurst and Macquarie Harbours are non-conducive to Dinoflagellates in general, possibly because Dinoflagellates tend to be k-strategists and the low residence time of surface waters in these estuaries is not conducive to long lived species.

Ammonia oxidizing bacteria and archaea were very abundant in Macquarie Harbour, and likely represent a major source of organic matter (Ross et al., 2016a). The most common phytoplankton genera and groups in Macquarie Harbour, *Chroomonas* sp. and the nanoflagellates are potentially mixotrophic or heterotrophic. These are yet to be formally identified meaning a large proportion of the trophic structure of the Harbour remains unexplained. As such, it is important that the species are identified, and their trophic role established.

Macquarie Harbour is relatively rich in all forms of nitrogenous nutrients when compared to other coastal areas where aquaculture is undertaken in Tasmania (e.g. the D'Entrecasteaux Channel; see Bell et al. (2017)). This is most probably due to a combination of high riverine nutrient inputs, the contributions from aquaculture and the long residence times in deeper waters that prevent nutrients leaving the system. Oceanic inputs of nitrate are also likely to contribute to the nutrient pool in the harbour but it appears less so than the other sources.

All phytoplankton lineages were more abundant at 2 m depth than they were in the 12 m integrated sample suggesting most species are freshwater, or euryhaline and they choose to stay in the freshwater surface layer. O'Connor et al. (1996) hypothesised that freshwater species may have a competitive advantage in Macquarie Harbour because of the humic substances in the surface layers, and the lower salinity, which mean the fresher surface layers have a greater copper complexing capacity (i.e. the ability of organic material to bind with copper cations) than does the deeper marine waters. Thereby making copper less toxic than when it is unbound. This is of particular importance because very high concentrations of copper are present in Macquarie Harbour and the copper complexing capacity of the harbour is about half of that necessary to cope with the total copper concentration (Carpenter et al., 1991). While the above may be true, freshwater species have a considerable competitive advantage simply because most, if not all, of the euphotic zone is comprised of freshwater in Macquarie Harbour. Additionally, bacteria are more abundant in the surface waters (Ross et al., 2016a) meaning bacterivorous species are also more likely to aggregate in surface waters.

The present study has highlighted several uncertainties regarding the phytoplankton communities of Macquarie Harbour and several recommendations are made below that could increase our knowledge of both the phytoplankton communities and the anthropogenic factors that may affect their distribution and abundance.

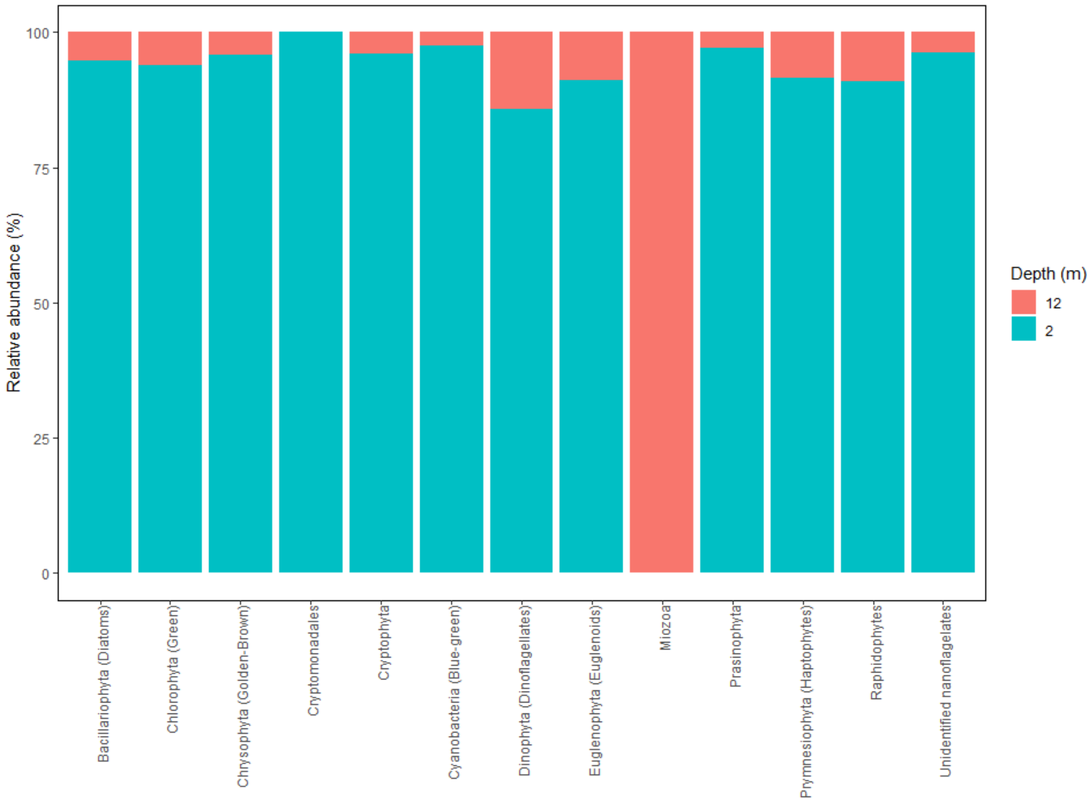


Figure 60 Relative abundance (%) of the various phytoplankton phylum groups in the 2 m and 12 m integrated samples.

Table 8 Welch t-test comparing the mean abundance of various phytoplankton abundance at 2 m and 12 m integrated depths. Haptophytes and Raphidophytes were not present frequently enough for meaningful statistical analyses.

Phylum group	t	df	p
Bacillariophytes (diatoms)	-4.6	2939.05	<0.001
Chlorophyta (Green)	-10.91	1111.63	<0.001
Chrysophyta (Golden-Brown)	-8.29	444.22	<0.001
Cryptophyta	-20.53	1172.12	<0.001
Cyanobacteria (Blue-green)	-4.45	74.51	<0.001
Dinophyta (Dinoflagellates)	-10.67	2093.23	<0.001
Prasinophyta	-3.74	409.52	<0.001
Euglenophyta (Euglenoids)	-8.52	868.86	<0.001
Unidentified nanoflagellates	-10.58	782.1	<0.001

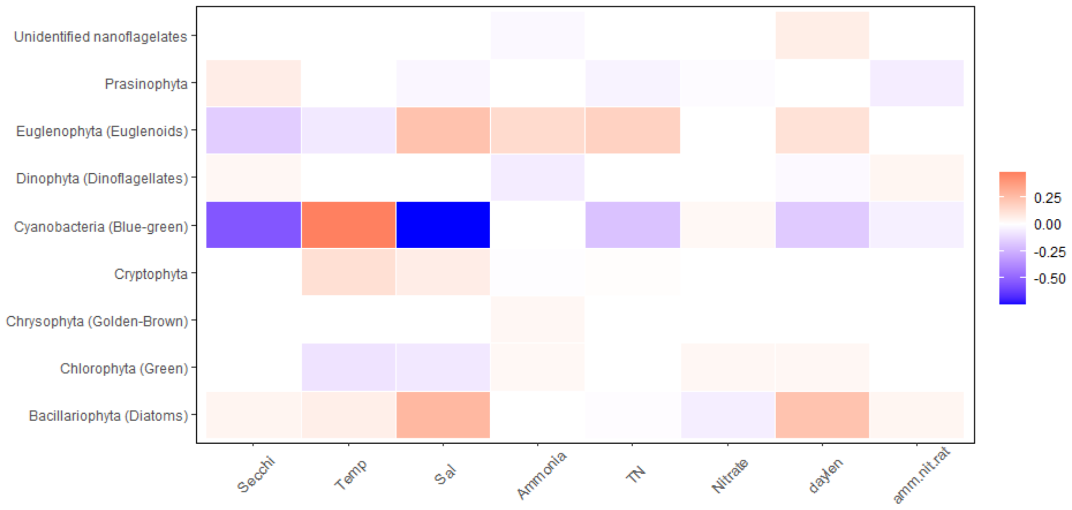


Figure 61 LASSO corrected coefficients (fourth corner) from the multivariate species distribution model. The matrix represents the signifficant environmental responses for the main phyla.

Macrofauna and Sediments

Abundance and species diversity and sediment variables

Like most estuarine systems on the west coast of Tasmania, the macrofaunal community in Macquarie Harbour is naturally depauperate with low species diversity and abundance. The lower macrofaunal biomass on the west coast in comparison to everywhere else in Tasmania is attributed to low concentrations of dissolved nutrients in rivers and dark tannin-stained waters, which greatly restricts algal photosynthesis and primary production (Edgar et al., 1999b). Heavy metal pollution and acid leaching from tailing dams into the harbour via rivers from historical mining activities may also contribute to the depauperate nature of macrofaunal communities in Macquarie Harbour (Talman et al., 1996). Despite low primary productivity and mining impacts, the macrofaunal community has responded to organic enrichment from salmon aquaculture. The faunal response to enrichment is evident out to 250-500 m from farmed cages, and there is a strong interaction with bottom water dissolved oxygen dynamics in the harbour (Ross et al., 2021, Ross et al., 2016b, Ross et al., 2017). Closer to the cages, surface deposit feeders (dorvilleids) were able to take advantage of the organic enrichment, and filter feeding sabellids and terebellids dominated the macrofaunal community at distances farther from the cage. Here we examine the macrofaunal communities and their relationship with the environment at the external sites (reference sites). Most sites were > 1km from an active cage.

In the 14 surveys conducted in Macquarie Harbour (Table 1), 16245 individuals were collected across 795 grab samples from 24 external sites, comprising 70 different taxa. Molluscs (bivalves and gastropods) were the most abundant group making up 44 % of the total abundance. Polychaetes were the second most abundant group (36 %), then crustaceans (16 %), echinoderms (3%) and other taxa (1 %, nemertean's, acorn worms and anemones). Forty percent of species were polychaetes, 36 % crustaceans, 13 % molluscs, 7 % other and 4% echinoderms.

Abundances were generally low at external sites averaging ~ 20 ind. per grab throughout the 14 surveys. Five of the 24 sites averaged > 40, 8 sites 10 – 30 and 11 sites < 10 ind. per grab (Table 9). Sites with the greatest abundances were relatively shallow (<15 m deep), situated near the mouth of the estuary or in the World Heritage Area and with oxygen saturation >50 % (Table 9). Sites with the fewest macrofauna were in the central harbour and in the proximity of the mouth of the King River. These sites were typically deeper with lower oxygen saturation (Table 9).

The number of species per grab was also relatively low at the external sites, again highlighting the naturally depauperate nature of the Harbour. Only site 49 averaged >10 species per grab, 6 sites averaged 5-7 species per grab, and the remaining sites <5 species per grab. The sites with the greatest number of species were in the lower third of the estuary near the mouth and at site 45 in the World Heritage Area. These sites were generally shallow (<15 m; except sites 1 & 60) with higher bottom oxygen saturation (Table 9). Sites with lower numbers of species per grab were generally located in the central harbour and in proximity to the mouth of the King River, although site 3, at the mouth of the Harbour also contained low number of species. Site 3 is situated in the channel near the entrance of the harbour, which experiences strong currents and shifting sediments. This factor may preclude some species from establishing populations if they prefer more stable sediments (Hall, 1994).

The decline in Macquarie Harbour bottom water oxygen levels since 2009 (see Figure 38) is well documented. Throughout the MHEMP monitoring period, concentrations have fluctuated between 10 and 30% saturation and occasionally lower. The timing of these fluctuations is difficult to predict given the variability in the environmental drivers of oxygen recharge; however, in recent years a seasonal pattern has become more apparent (Figure 41). Through winter and spring bottom oxygen levels decline when river flow is greatest and from late spring through summer ocean recharge events typically replenish bottom water oxygen levels. However, the duration, magnitude and spatial extent of the low oxygen period has varied annually. In Spring 2016, oxygen concentrations reached extremely low levels for several months; arguably the lowest levels on record over the past 30 years[3]. In spring 2017, similar low oxygen concentrations were experienced but the duration of the event was shorter (Ross et al., 2018). These events triggered a decline in benthic conditions, including a reduction in macrofaunal abundance and species diversity. An assessment of the external sites in the harbour wide surveys showed that the greatest decline in abundance and species numbers has occurred in the deeper central region of the harbour where oxygen levels reached lower levels, with relatively little change in the fauna in the shallower regions in the mid-harbour or to the north or south of the harbour.

Table 9 Characteristics at Macquarie Harbour sediment sites. Averages of environmental variables, and macrofaunal abundance and species per grab. Sites are listed in order by their location, from the World Heritage Area to the mouth of the Harbour.

Site	Depth	Temperature	Salinity	Oxygen (% saturation)	Redox	Abundance	Species
45	7.0	14.6	22.2	61.1	114.9	107.6	6.4
44	15.3	14.9	29.1	20.0	-26.7	20.0	3.7
43	24.2	14.8	30.5	14.1	-53.6	16.3	2.1
42	22.6	14.9	30.1	16.3	69.9	11.5	4.4
39	30.9	14.7	30.6	16.7	-5.9	10.6	2.7
41	17.0	14.5	30.0	24.7	24.0	1.8	1.4
37	18.0	14.8	29.5	15.8	47.6	5.3	2.9
28	14.9	14.8	29.0	23.1	89.7	4.7	2.9
26	37.1	14.8	30.7	23.3	-20.5	2.3	1.4
21	20.6	14.6	29.2	14.8	-5.6	4.9	2.0
6	17.9	15.0	28.9	17.6	-19.7	3.9	1.7
16	44.0	14.9	30.9	34.5	-13.2	7.2	4.5
9	42.7	15.1	31.0	42.8	-31.1	6.9	4.0
11	13.3	14.5	28.1	35.2	95.4	26.5	7.6
10	41.2	15.1	30.8	41.9	-15.6	4.2	2.8
15	6.6	14.8	25.9	47.5	-8.2	16.8	1.1
60	37.4	15.0	31.1	46.6	-48.6	10.4	5.7
52	33.3	15.1	30.6	40.4	-25.9	3.9	2.3
1	43.8	15.2	31.0	43.9	-15.3	9.9	6.0
49	14.2	14.6	28.8	49.1	98.9	43.2	11.7
12	8.3	15.2	25.2	77.8	287.6	16.4	7.6
2	1.7	14.7	11.1	96.3	328.8	69.1	6.3
14	3.3	14.9	16.9	96.9	309.4	64.9	4.9
3	3.6	14.7	19.4	98.6	267.8	52.6	2.7

[3] The extremely low DO event in spring 2016 was most evident from the high temporal frequency real time monitoring strings that were installed in late 2015. It is difficult to say for certain that DO hasn't been as low before because the longer-term data sets (MHEMP monthly and EPA quarterly) collect data less regularly.

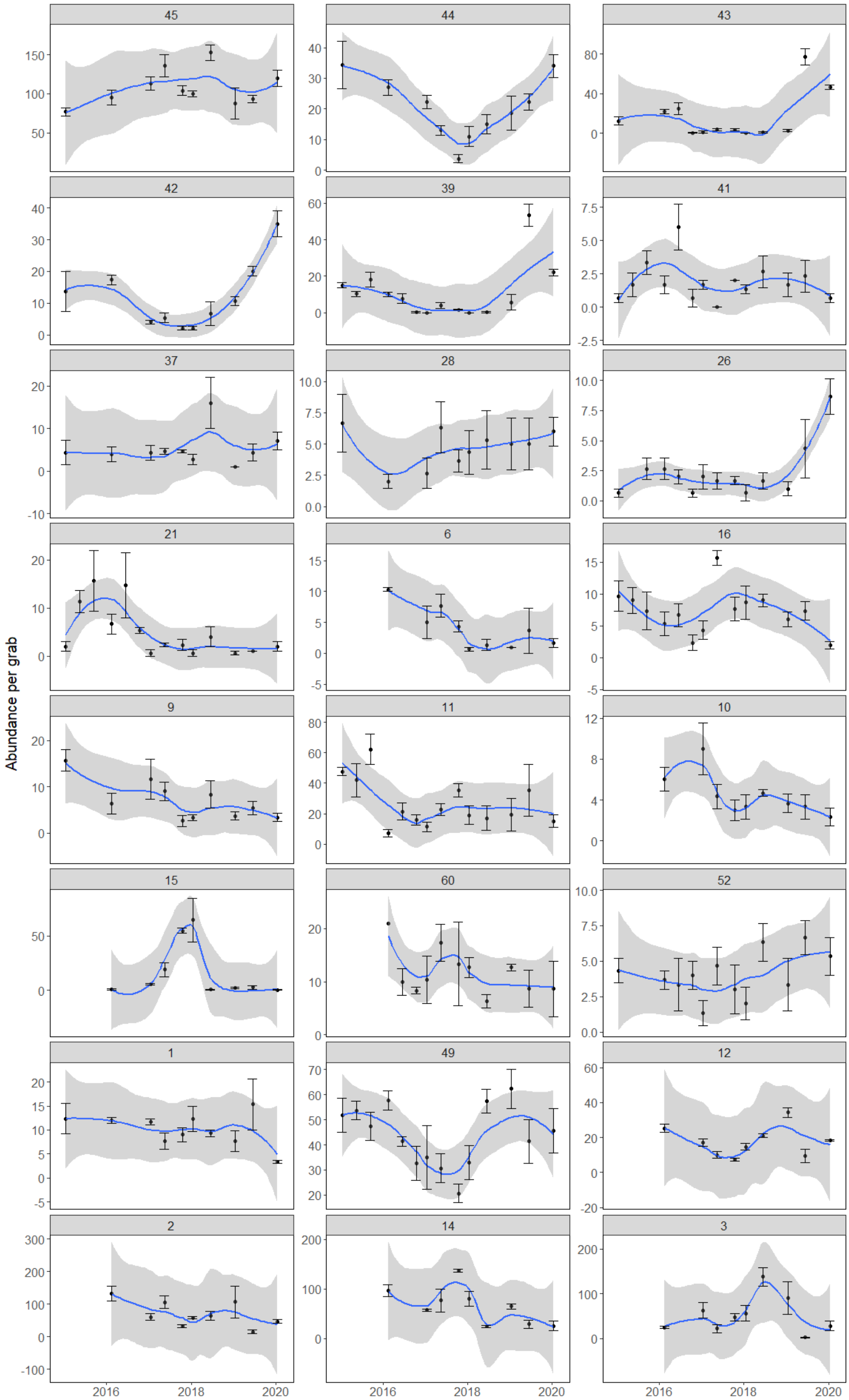


Figure 62 Average abundances (±SE) per grab of macrofauna at external sites in Macquarie Harbour during the study. Note that not all sites were visited during each survey.

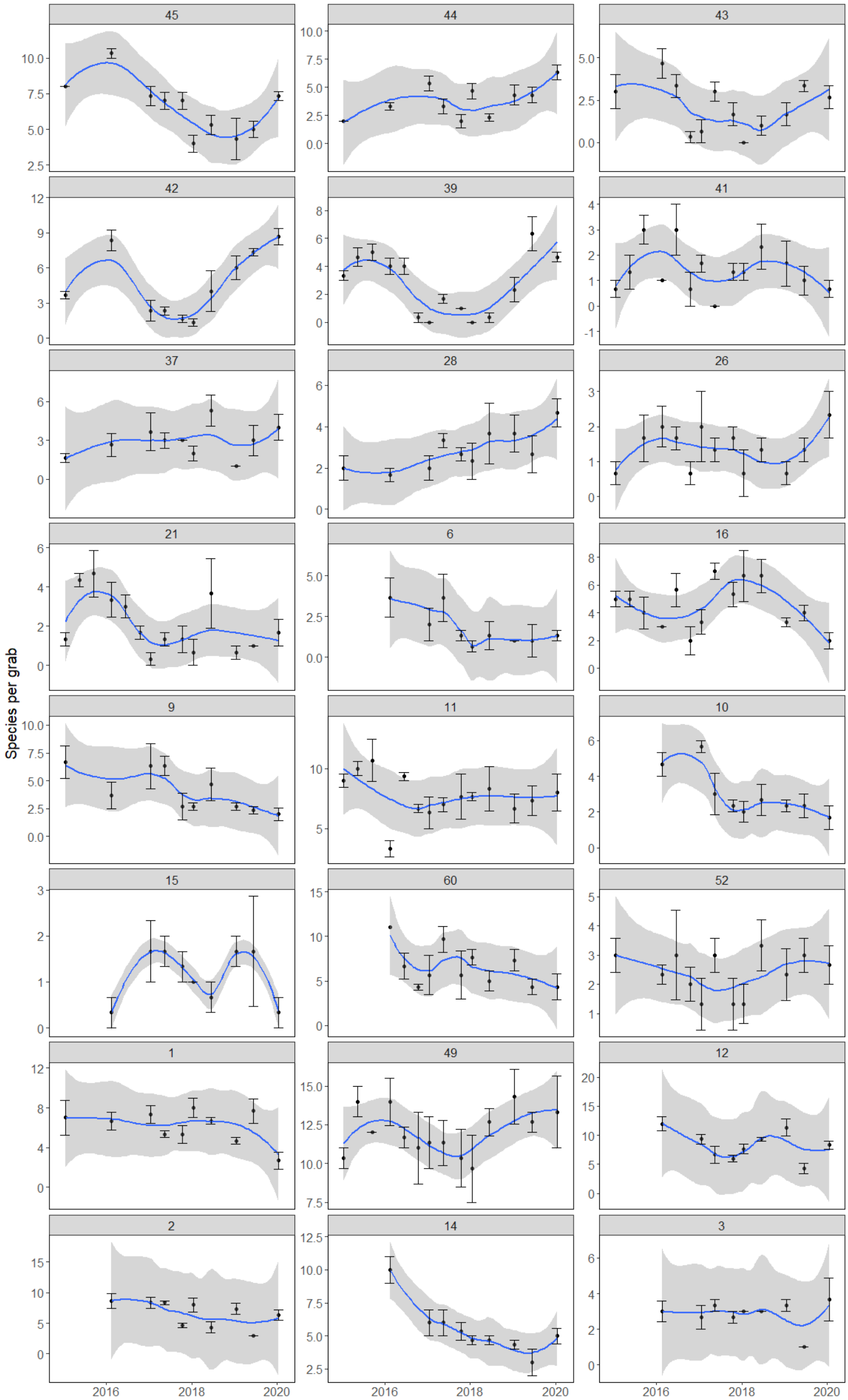


Figure 63 Average number of species (±SE) per grab of macrofauna at external sites in Macquarie Harbour during the study. Note that not all sites were visited during each survey.

At the four southernmost sites in the deeper central basin (sites 39, 41, 43, 44) where the decline was the greatest, faunal abundance and species numbers remained low from October 2016 to January 2019 (Surveys 6-12), but have now returned to and remain well within the range reported before the major decline in spring 2016 – early 2017 (Ross et al. (2021); Figure 62, Figure 63). Abundance and the number of species had increased at some of the mid-harbour sites and decreased at others, but overall, the patterns remained similar and within the range recorded in previous surveys. Site 49 near the mouth of the harbour also had a reduction in macrofaunal abundance and number of species but the recovery began sooner, presumably due to its proximity to the mouth and oxygen recharge events reaching this site earlier (Figure 62, Figure 63).

Patterns in the key sediment and bottom water environmental variables were clear in the PCO analysis (Figure 64), where there was a distinct separation of the external sites into two main groups, based on depth, salinity, redox and oxygen on the first principal component (x-axis) and temperature on the second principal component (y-axis). Along the x-axis, sites with the greatest depth, higher salinities and reduced redox and oxygen concentrations are to the right of the plot (the central basin), and the shallower sites (2, 3, 14 and 45) with lower salinity (influenced by freshwater), higher redox and oxygen concentrations are positioned on the left. These shallower sites are located near the mouth of the harbour and in the World Heritage Area.

The bottom waters in the deeper central basin of Macquarie Harbour are for the most part a marine environment (salinity ~28-30); however, the shallow site 45 in the World Heritage Area experiences lower salinity (averages ~22) particularly during winter and spring when rainfall is the highest (Figure 65; Table 9). Salinity ranged from ~1-20 at the shallow sites 3, 14, and 2 near the mouth during most seasons indicating that freshwater from the catchment is readily mixed with oceanic waters at these sites (Figure 65). These distinct groups also share similar patterns in dissolved oxygen and redox. At sites located in the central basin dissolved oxygen averages <35% at most of these sites however there is substantial variation (<10-50% oxygen) between seasons as noted in this report and visualised in Figure 65. Redox was also greatly reduced at the sites in the central basin in comparison to the shallow sites at either end of the Harbour (Figure 65). Oxygen and redox were positively correlated ($r = 0.7$, $n = 210$, $P<0.001$).

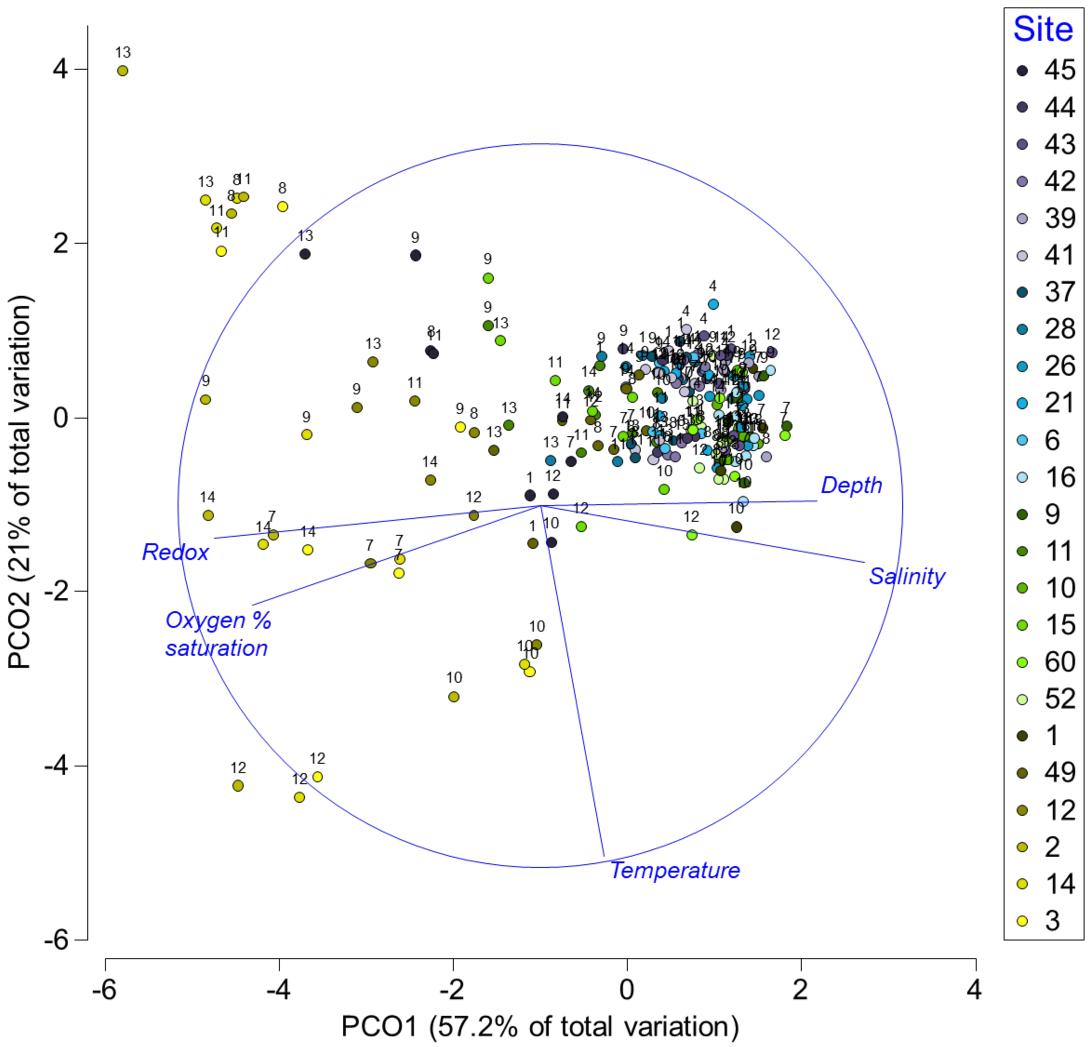


Figure 64 A PCO analysis of sediment and bottom water quality variables collected during surveys 1, 4 and 7-14 at external sites. Not all sites were visited during surveys 2,3 and 5,6 and were excluded from the analysis. Coloured circles indicate the site and the numbers above the circles are the surveys from which these data were collected. Sites listed in the key are ordered by distance from the mouth with site 45 in the world heritage area (the farthest) and site 3 in the mouth of the harbour (the closest).

The differences seen along the y-axis in the PCO plot (Figure 64) reflect the seasonal patterns in temperature exhibited at the shallowest sites nearest the mouth of the Harbour. Generally, temperature averages around 14.5-15.2 ^{0}C at most sites in the Harbour and is far more stable than salinity (Figure 65; Table 9). There is little variation in temperature in the deeper sites located in the central basin. However, the shallow sites 3, 14, and 2 near the mouth can experience seasonal temperature fluctuations of about ± 5°C from the mean of 14.7°C (Figure 65; Table 9). Sites 2, 3 and 14 had the highest temperatures recorded during surveys 10 (Jan 2018) and 12 (Jan 2019) and the lowest temperatures during surveys 8 (May 2017), 11 (June 2018) and 13 (June 2019) (Figure 65).

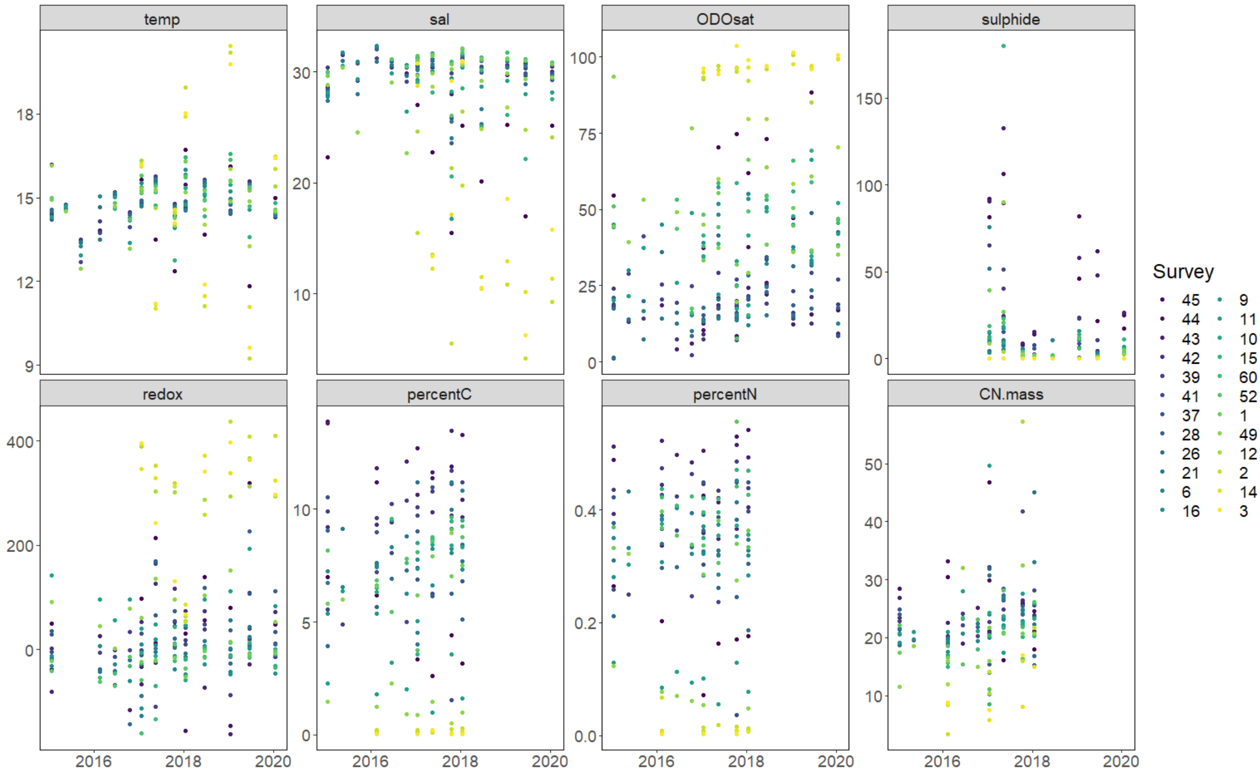


Figure 65 Values for sediment and bottom water quality variables collected at each external site across all surveys. Temp = Temperature, Sal = Salinity, ODOsat = Dissolved oxygen (% saturation), percent C = percentage organic carbon, percent N = percentage nitrogen, CN mass = percentage carbon : percentage nitrogen ratio.

Missing from the PCO analysis are sulphides, percentage organic carbon (% C) and nitrogen (% N), and C to N ratio. Sulphides were measured consistently from surveys 7 onwards. A separate PCO analysis was conducted on all environmental variables (including sulphides) collected during surveys 7-14 (not shown here), but it was found that sulphides explained little of the variation between sites or the patterns of distribution among the macrofaunal community. Sulphide levels were also not correlated to any other variable and were highly variable. Thus, it was decided to remove sulphides from the analysis, which allowed us to include data from surveys 1 and 4 for a more thorough investigation into the remaining environmental variables. However, it is worth noting that low sulphide concentrations were typically found at sites nearest the mouth of the estuary and higher sulphides occurred at the deeper sites in the central basin. Higher sulphide concentrations were most evident at these sites in Surveys 7 (January 2017) and 8 (May 2017) following the low oxygen event in October 2016 (Figure 65).

The % C, % N and C:N ratio of the sediments were measured in surveys 1-10. Values of % N and % C were strongly positively correlated ($r = 0.9$, $n = 464$, $P<0.001$). There is a strong downstream gradient with elevated % C and % N at sites nearest the Gordon River that gradually decrease towards the mouth of the estuary (Figure 65).

An exception to this is site 45, which had lower % C and % N in comparison to other sites in the world heritage area. Carbon and nitrogen isotopes were not measured in all the surveys, but these do provide useful insights into the origins of carbon and nitrogen in the Harbour (Ross et al., 2016b).

Macrofaunal communities and their relationship with the environment

Based on species composition, the MDS ordination showed a strong separation between external sites, but little difference between surveys (Figure 66). This means that the differences in macrofaunal assemblages between sites was greater than the differences between surveys within sites. The macrofaunal communities separated into three distinct groups. Sites 45 and 2 form the first group (top left), 14 and 3 the second (bottom left), and the remainder of the sites (to the right) form the third (Figure 66a). The separation of these groups correlates with changes in oxygen, redox, salinity and depth along the x-axis (Figure 66c). The groups to the left consistently had higher oxygen concentrations and redox, reduced salinities (<20 on average) and were all shallow sites (<10 m, Table 9). Sites 2, 3, and 14 are situated near the entrance to the ocean and site 45 is near the mouth of Gordon River. These sites also had higher abundances of macrofauna. In contrast the rest of the sites generally had lower oxygen concentrations and redox, high salinities (~28-30) and were > 10m depth with lower abundances of macrofauna (Table 9). Along the y-axis of the MDS plot, sites with a greater number of species occur in the top half of the plot and those with lower species number are found in the bottom half (Figure 66a,c). The macrofauna species common to sites 45 and 2 in the MDS plot were the bivalve *Arthritica semen* and the gastropods *Ascorhis tasmanica* and *Tatea rufilabris* (Figure 66b).

The macrofauna species that were common within the second group of sites (3 and 14) were the bivalve *Atactodea erycinaea* the crustacean *Exoediceros fossor*. The crustacean *Haplostylus* sp. were also found at sites 2, 3, and 14 but not 45 (Figure 66b). All these species are estuarine that can tolerate a wide range of salinities but prefer sandy sediments (Edgar et al., 1999b).

The species correlating with the deep-water sites included the polychaete *Euchone varibilis* and the crustacean Philomedid sp. which were found at marine sites 12 and 49 located in the first third of the estuary near the mouth (Figure 66b) and the polychaete *Pista australis* and the bivalve *Parathyasira resupina* which were more common at the deeper central basin and World Heritage Area sites These species are known to inhabit silty marine environments.

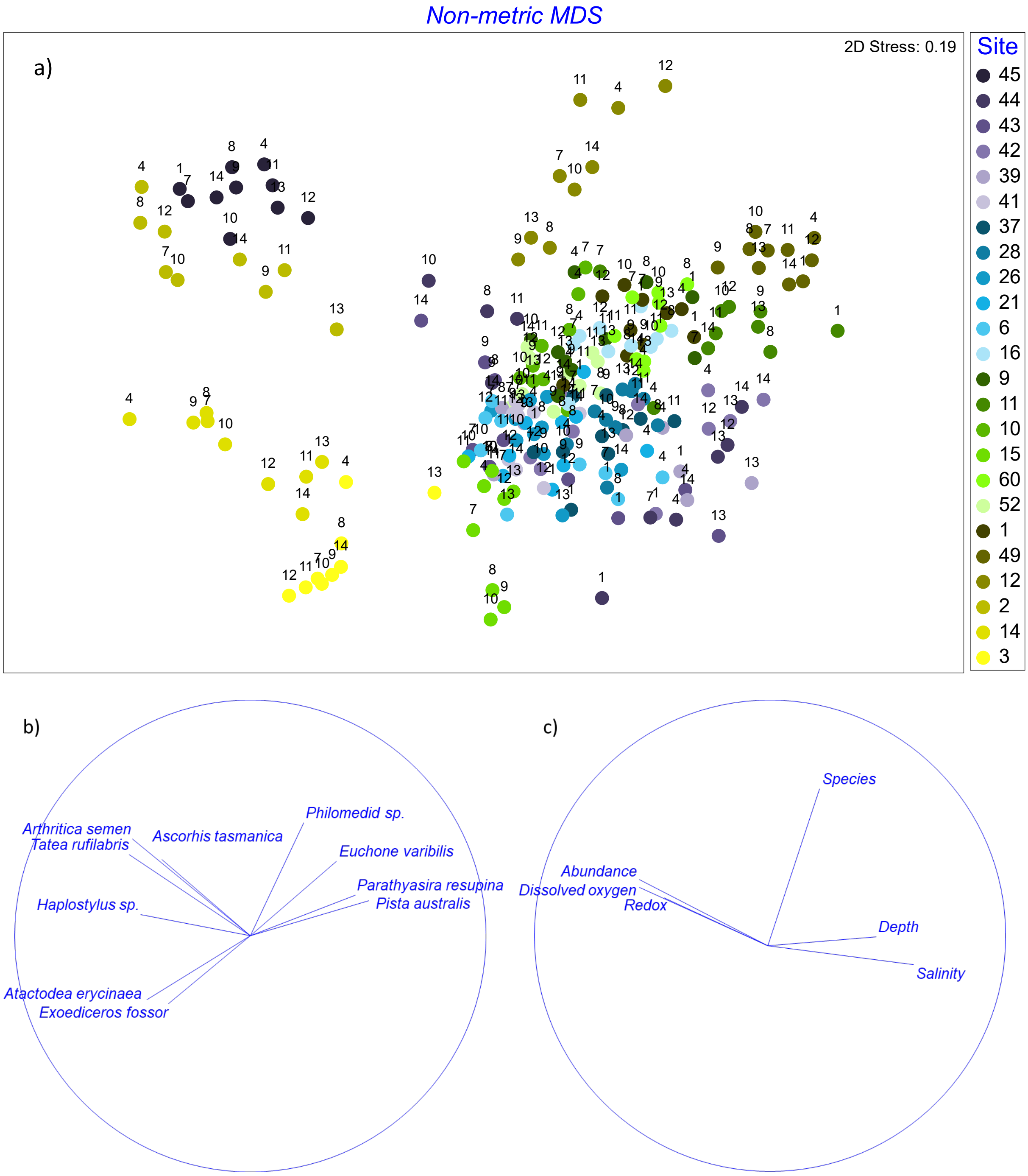


Figure 66 a) An MDS plot showing the patterns in macrofaunal distribution between external sites and surveys. Points closer to each other indicate greater similarities between macrofaunal assemblages. Coloured circles indicate the site and the numbers above the circles are the surveys from which these data were collected. b) Correlations between the MDS plot and macrofaunal species (r ≥ 0.4). c) Correlations between the environmental variables and the MDS plot (r ≥ 0.4). The length of the lines in b) and c) indicate the strength of the correlation, with the circle having a radius of 1.0.

Harbour wide change in macrofauna communities and recommendations for future monitoring

Two other key studies assist in the understanding of harbour wide change of macrofauna communities in Macquarie Harbour. Ross et al. (2016b) compared the results of benthic surveys of harbour and lease sites undertaken in early 2015 against baseline surveys of farm and external sites conducted between 1999-2003 and in 2012. The comparison with baseline surveys highlighted a change in the broader benthic ecology over the past 15 years, with arguably the greatest change occurring in the last 2 years (relative to the 2015 survey), as demonstrated by a measurable increase in total abundance, species richness and species diversity. These observed changes have had an influence at a functional level, with a decrease in burrowing taxa and an increase in the more static suspension and deposit feeding tube builders. Whilst there could be a range of explanations for this change, such as a recovery from the effects of mining or changes in the regulation of catchment inflows influx of organic matter associated, the authors concluded that it is highly likely that the addition of nutrients and organic matter from fish farming has played a role in stimulating benthic productivity.

Ross et al. (2016b) report also noted that there was limited capacity to identify changes beyond the deeper central harbour region where most of the farming now takes place, which raised several questions about the extent of any impacts, and in particular the potential effects on the faunal communities in the extensive shallow regions of the harbour. For example, has the benthic ecology of the shallower regions of the harbour changed, and do the shallower communities possibly provide an important reservoir for recruitment and recovery of benthic communities in the deeper regions? Thus, an additional survey of sites in these shallower regions in 2018 was conducted (see Ross et al., 2021). The same sites were visited as those surveyed in the 1996 benthic survey sites from (Talman et al., 1996) and a more recent survey of the WHA sites conducted in 2015 (Barrett et al., 2016). In the 2018 survey it was found that there was some consistency in the faunal composition and distribution patterns between the 1996 and 2018 surveys but there were also some differences. For example, in the World Heritage Area, there was an increase of the small bivalve *Arthritica semen*, the gastropod *Tatea rufilabris* and the amphipod *Paracorophium* sp 1 and a decrease in the abundance of the amphipod *Limnoporeia yarrague*, mysid *Haplostylus* sp. and orbiniid polychaete *Leitoscoloplos bifurcatus* in 2018 compared with 1996 (Figure 24 & 25). The authors noted that all these species can be highly variable in numbers in estuarine systems, both spatially and temporally, and therefore these differences are not necessarily unusual or significant. Also, with only two sampling points it is not possible to definitively determine whether the broader changes between surveys observed in the faunal communities from the shallow sites in the central and northern parts of the harbour reflect a longer-term shift in the benthic ecology or are natural temporal fluctuations between surveys; additional surveys in future years would help establish this more clearly.

In this study we were able to compare communities, the number of species and total abundances of macrofauna from 24 external sites across 14 surveys conducted from 2015 - 2020. No historical data for the external sites visited was available for comparison in this study. In the multivariate analysis, there was little evidence to suggest that macrofaunal communities had changed over time. However, any changes were masked by the differences in communities between sites being greater than those recorded between surveys within sites. What the results did show was that during the low oxygen events of 2016 and 2017 there was a reduction in the number of species and faunal abundance. This change was largely restricted to deeper sites in the central basin and the World Heritage Area. Populations of macrofauna have recovered but the length of time varied and appeared to directly relate to the magnitude and spatial extent of oxygen recharge. Sites closest to the mouth of the Harbour where summer/autumn oxygen recharge events happen first recovered the fastest, whereas the deeper southernmost sites were the slowest to recover.

Given that macrofauna are proving to be useful indicators of the environmental conditions of Macquarie Harbour it is recommended that benthic surveys are incorporated in the MHEMP monitoring program, initially biennially, but more or less frequently depending on environmental conditions (e.g., oxygen dynamics and recovery). We also recommend that the sites include some of the original shallow sites monitored by (Talman et al., 1996) given that the vast shallow areas in the harbour appear to be acting as a “reservoir for recruitment” for the deeper sites following low oxygen events.

Several sediment variables measured during this study were also valuable indicators of change in the environment and we recommend these to be collected alongside macrofauna. These include sediment redox, C and N content and isotopic signatures. Sulphides have not proven as valuable in Macquarie Harbour compared to elsewhere and were typically higher and more variable.

Performance against indicator limits

As a requirement of Schedule 3 BEMP Macquarie Harbour - Marine Farming licence conditions relating to environmental management of a finfish farm, marine farming operations must comply with environmental standards. For water quality, the indicators and limits were established following initial MHBEMP monitoring in which the 20th or 80th percentile values at 2 or 20 m were adopted as limits. More specifically, the rolling annual median indicator values for the combined compliance region (see Figure 1) sites, where directly attributable to marine farming operations, must not exceed the indicator limits specified in Table 10.

Table 10 Indicators and Limits for water quality compliance in Macquarie Harbour

Indicator	Limit
Ammonia (at 2 metres)	0.033 mg-N/L (max.)
Ammonia (at 20 metres)	0.024 mg-N/L (max.)
Nitrate (at 2 metres)	0.053 mg-N/L (max.)
Oxygen (at 2 metres)	6.82 mg/L (min.)

At both 2 and 20 m, 12-month rolling median ammonia concentration has remained below the trigger limits (Figure 67, Figure 68). Similarly, the 12-month rolling median for oxygen concentration at 2 m has remained above the trigger limit (Figure 69), though there has been a slight downward trend since winter 2012 and throughout 2016 dissolved oxygen concentration observations were at times below the trigger limit (as indicated by the bottom whisker of the boxplots).

Nitrate concentration at 2m has also remained under the trigger limits, although throughout 2015, when aquaculture operations were operating at the historical maximum, nitrate concentrations approached the trigger limit (Figure 70). This may reflect the rapid transformation of ammonia to nitrate via the process of nitrification.

The current parameters and depths limits do not appear to be adequate as environmental standards to monitor and protect the environmental health of Macquarie Harbour. The decline in oxygen concentrations in bottom and mid-waters and associated deterioration in benthic conditions has been well documented (e.g, Ross et al., 2017) and has motivated reductions by the EPA since early 2017 to reduce the pressure on the harbour and allow for environmental recovery (see http://epa.tas.gov.au/regulation/salmon-aquaculture/macquarie-harbour/management-determinations). Of note, there is only a single limit for oxygen at 2 m depth. An oxygen limit for bottom and mid waters is strongly recommended to ensure the adequate protection of the flora and fauna of Macquarie Harbour. This review has also highlighted the importance of total N as a proxy for both organic and inorganic N within the system; we suggest this be included in sampling and future reporting on environmental condition in the harbour. Additionally, given that ammonia appears short lived within the system, consideration should be given to the inclusion of nitrate as an indicator and limit for bottom waters. Finally, the review has shown that the Macquarie Harbour system appears to be relatively uniform in terms of nutrient and oxygen concentration, highlighting that water column conditions within the compliance region are not independent of what is happening within the broader system (or vice versa). This highlights the importance of ongoing monitoring of all system drivers (e.g. river inflows, water elevation, STP inputs) to assist with the interpretation and attribution of change in the indicators and their levels.

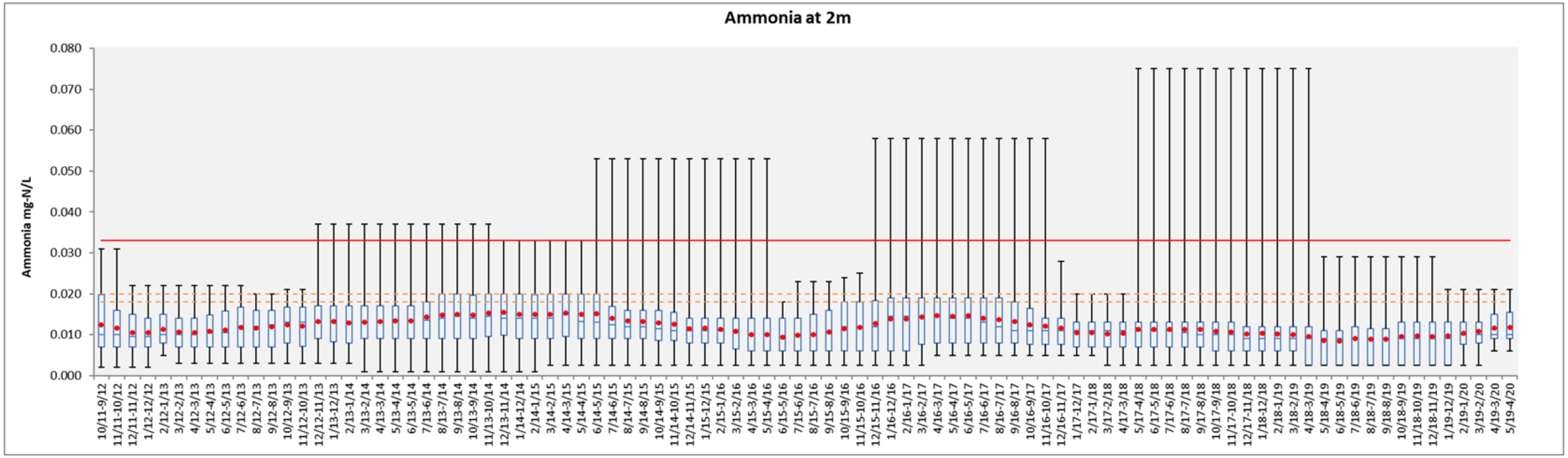


Figure 67 Performance against indicator limit for ammonia at 2 m.. Box and whisker plots show median (centre line), 20th and 80th percentiles (ends of box) and maximum and minimum recorded values (ends of whiskers). Red dot is the mean. Horizontal axis shows month/year-month/year of the 12-month rolling period of assessment. Horizontal red line represents the limit for that parameter as listed in Schedule 3 to Macquarie Harbour marine farming licence conditions. Horizontal orange dashed lines represent the modelled 80th percentile at maximum biomass for the new EIS and Alt Biomass scenarios (combined seasons/stations). Source: DPIPWE.

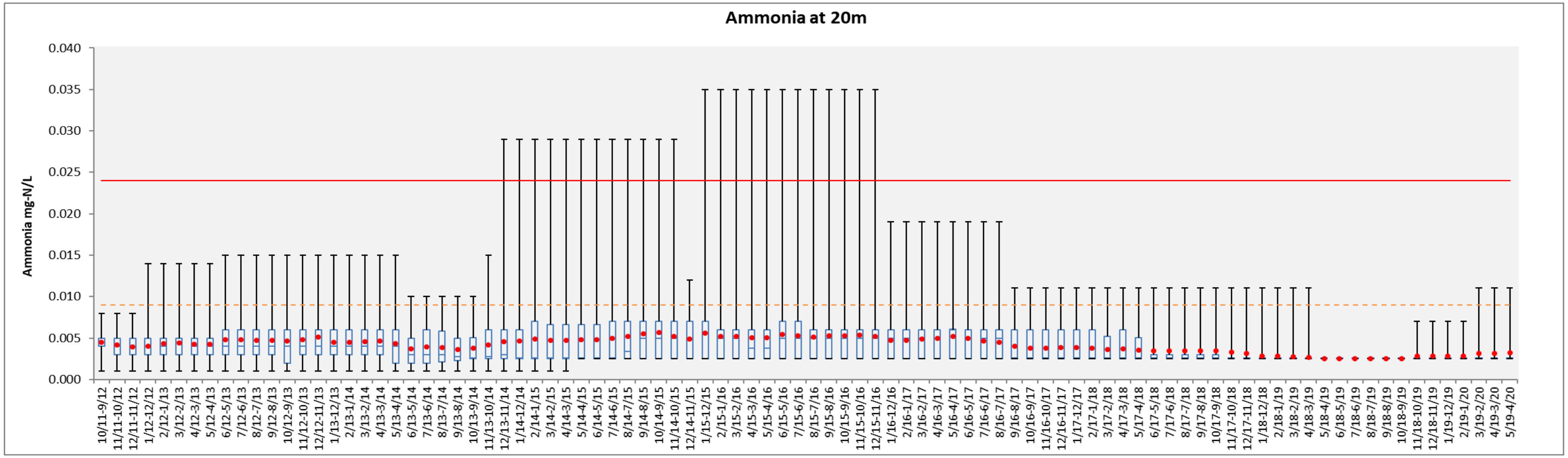


Figure 68 Performance against the indicator limit for ammonia at 20 m. Box and whisker plots show median (centre line), 20th and 80th percentiles (ends of box) and maximum and minimum recorded values (ends of whiskers). Red dot is the mean. Horizontal axis shows month/year-month/year of the 12-month rolling period of assessment. Horizontal red line represents the limit for that parameter as listed in Schedule 3 to Macquarie Harbour marine farming licence conditions. Horizontal orange dashed lines represent the modelled 80th percentile at maximum biomass for the new EIS and Alt Biomass scenarios (combined seasons/stations). Source: DPIPWE.

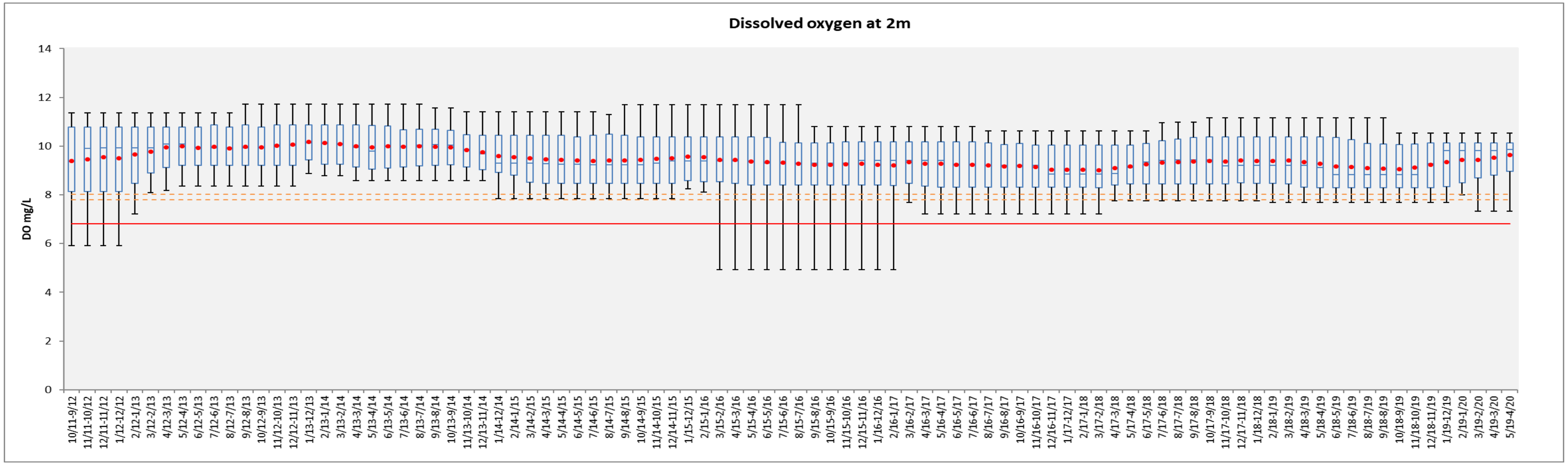


Figure 69 Performance against the indicator limit for dissolved oxygen at 2 m. Box and whisker plots show median (centre line), 20th and 80th percentiles (ends of box) and maximum and minimum recorded values (ends of whiskers). Red dot is the mean. Horizontal axis shows month/year-month/year of the 12-month rolling period of assessment. Horizontal red line represents the limit for that parameter as listed in Schedule 3 to Macquarie Harbour marine farming licence conditions. Horizontal orange dashed lines represent the modelled 20th percentile at maximum biomass for the new EIS and Alt Biomass scenarios (combined seasons/stations). Source: DPIPWE.

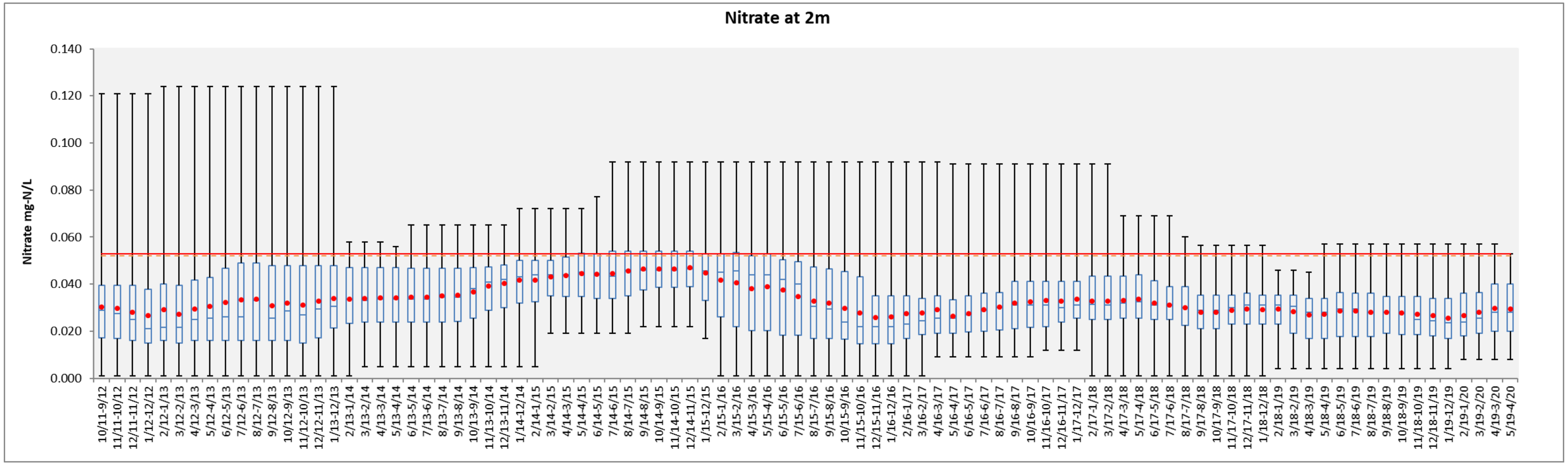


Figure 70 Performance against the indicator limit for nitrate at 2 m. Box and whisker plots show median (centre line), 20th and 80th percentiles (ends of box) and maximum and minimum recorded values (ends of whiskers). Red dot is the mean. Horizontal axis shows month/year-month/year of the 12-month rolling period of assessment. Horizontal red line represents the limit for that parameter as listed in Schedule 3 to Macquarie Harbour marine farming licence conditions. Horizontal orange dashed lines represent the modelled 80th percentile at maximum biomass for the new EIS and Alt Biomass scenarios (combined seasons/stations). Source: DPIPWE

Conclusion

Salmonid aquaculture has a long history in Macquarie Harbour with recent expansion seeing production reach >20,000 tonnes in 2015/16. This is largely due to the favourable conditions provided by permanent stratification and the fresh to brackish surface waters which limit the prevalence of amoebic gill disease. While favourable for growing fish, this stratification minimises vertical mixing, which, when combined with the shallow and narrow entrance to the ocean, results in naturally low dissolved oxygen condition in bottom and mid-waters. However, there has been a steady decline in dissolved oxygen in Macquarie Harbour since 2009, and in recent years, low dissolved oxygen conditions have been associated with a deterioration in sediment condition, including increased *Beggiatoa* bacteria and a decline in benthic infauna (Ross and Macleod, 2017). As such, the maximum permissible biomass has progressively been lowered by the EPA since early 2017 to reduce the pressure on the harbour and allow for environmental recovery; the most recent biomass determination (May 2020) set the limit for salmonids (salmon and trout) at 9,500 tonnes for the next two years. Over the MHEMP monitoring period which began in late 2011, there have been several dissolved oxygen recharge events. The drivers of these events include major storm events that assist large volumes of oceanic waters to enter the system and low flow periods in summer/autumn during which oceanic waters are not impeded by fresh-brackish surface waters near the entrance and more easily enter over the shallow entrance of the harbour.

Ammonia concentrations have not increased in Macquarie Harbour over the MHBEMP period analysed (2012-2020); however, total N has increased indicating that nutrients are being retained within the system. In estuaries with residence times of weeks to months (i.e. as is the case in Macquarie Harbour with a residence times of up ~40 - 110 days), dissolved N forms entering the system from rivers are often predominantly utilised within the estuary whereas in estuaries with brief residence times (<1 week), dissolved nitrogen largely exits the estuary and is utilised in coastal and shelf waters (Seitzinger and Sanders, 1997). Dissolved nutrients entering the surface waters of Macquarie Harbour from aquaculture do so in the central harbour where the residence time is at the lower end of these estimates. Although flushing from the harbour may help explain why there is no clear relationship between phytoplankton biomass, nutrient concentrations or standing aquaculture biomass, the increase in total N in the harbour is consistent with increased microbial production and utilisation (e.g. bacteria and archaea) of dissolved N inputs.

Ammonia oxidising bacteria and archaea fix carbon dioxide and obtain energy from ammonia. As such, they increase the organic matter in Macquarie Harbour and consume oxygen in doing so. This has two important implications: 1) the consumption of oxygen contributes to the deoxygenation of the harbour, particularly in deeper waters where archaea are highly abundant, and 2) it increases the organic matter loading of Macquarie Harbour, which can further contribute to deoxygenation as this matter is decomposed (i.e. whether it be the prokaryotes themselves, mixotrophic or heterotrophic plankton that consume them, or larger fauna). Ammonia oxidising archaea were an order of magnitude more abundant in the deeper waters of Macquarie Harbour than were ammonia oxidising bacteria (Ross et al., 2016a). These lineages have been shown to be predominantly stenohaline marine species and increase in abundance between 13–22 times from freshwater to marine environments (Zhang et al., 2015). Due to their abundance at depth, ammonia oxidising archaea are likely to play a very important role in nitrification in the Macquarie Harbour system (Ross et al., 2016a) as they are able to access nutrients that photosynthetic organisms cannot. Ammonia oxidising bacteria, however, are more abundant in surface waters and may be an important food source for mixotrophs and heterotrophs such as nanoflagellates.

There has been a change in phytoplankton composition with nanoflagellates now representing a major component of the assemblage. It is possible that this change has resulted from the proliferation of prokaryotes, which are reportedly more abundant now in MHBEMP samples than they were previously (Stephanie Fulton AST, personal communication). A study in a North American lake found that 30–100% of heterotrophic nanoflagellates ingested bacteria depending on depth and season (Bennett et al., 1990) and it has been shown that the transfer of nutrients into the classical food web from microbial communities by the ingestion of nanoflagellates by zooplankton is potentially very important (Bennett et al. 1990). Additionally, Cryptophytes now comprise a major component of the phytoplankton community when historical information, though limited, suggests they were a far lesser component historically. Conversely, Euglenoids and Bacillariophytes appear to have decreased in importance, though it is possible their abundance has not changed as there is no quantitative historical data for which to make a reliable comparison.

The present review of the MHEMP data collected from 2011- 2020 highlight changes in the dynamics of some of the key water quality parameters in Macquarie Harbour, notably dissolved oxygen, nitrate and total N concentrations. There is also some evidence that the phytoplankton community has changed with Cryptophytes now being dominant and nanoflagellates increasingly being an important group. The report also describes the pivotal role that microbial production is likely to be playing in the harbour and how it responds to aquaculture. Clearly the monitoring program is providing the data that allows for such a robust assessment of ecological condition, however, there does appear to be some redundancy in the number of sites monitored and a need to revisit some of the parameters measured (and not measured). It is also clear that the current parameters and depths used as indicator limits do not appear to be adequate as environmental standards to monitor and protect the environmental health of Macquarie Harbour. To this end, recommendations with respect to knowledge gaps and future monitoring are detailed below:

Knowledge gaps and recommendations

Monitoring

The monitoring program meets the requirements of the Schedule 3 BEMP Macquarie Harbour - Marine Farming licence schedule relation to water quality monitoring. This includes monthly sampling at 10 sites across a range of parameters and depths (Figure 71). The current program includes significantly more sites (Figure 72) and parameters. Many of these sites and parameters were added based by industry on the recommendations of the MHDOWG working group to better understand the influence of river and ocean inputs on carbon and nitrogen loads in the system, and thus, their influence relative to aquaculture on oxygen levels. There are also EPA water quality sites that are monitored quarterly by the EPA and sensor strings collecting high frequency data on oxygen and salinity (and other parameters) at multiple sites throughout the harbour. This data provides an ideal opportunity to re-assess the efficacy of the current suite of parameters, spatial distribution of sites and sample frequency to detect changes in ecological function. This requires a more detailed assessment, but it is clear from this review that:

- the surface waters of the harbour are relatively uniform in terms of physico-chemistry and nutrient concentrations. Additionally, while there is a longitudinal gradient in the harbour in deeper waters whereby water that has resided in the harbour for longer periods of time (i.e. in the upper reaches) has lower dissolved oxygen and higher nutrients, there is a minor latitudinal variation at depth. Monitoring sites that display similar temporal patterns could be rationalized, provided, however, enough sites remain in place to ensure the longitudinal patterns and the influence of the end members (i.e. rivers and ocean) can be identified.
- the temporal analysis in this report was undertaken at a select few of the deeper MHBEMP sites (HG1, KR1, CHN, CHE, PET3, WH1 and WH2) based on evidence of minimal spatial variation. Given that this was enough to encapsulate both spatial and water column variation, this highlights that number of sites could be rationalised.
- similarly, phytoplankton communities were relatively uniform throughout Macquarie Harbour and the number of sampling sites could be reduced,

provided that the remaining sampling regime incorporates enough sites to identify variation longitudinally within the harbour.

	Parameter	Nutrients		Physico-chem	Phytoplankton	
	Method	(Niskin bottle)		(Water meter)	(Niskin bottle)	
	Analyte	• Nitrate • TAN • TKN-N (sites 4, 7, 9, 10 and 11 only)		• Temp • Salinity • DO • DO saturation • Turbidity	• Spp abundance • Spp diversity • Chla pigment by UV-vis	
Site No	Site depth (m) Approx	Sample depth (m)	Total No samples for site	Full depth profile	sample depth (m)	Total No samples for site
1	10	2/B2	2	yes	IS/2	2
2	8	2/B2	2	yes	IS/2	2
3	30	2/10/20/B2	4	yes	IS/2	2
4	9	1/2/B2	3	yes	IS/2	2
5	9	2/B2	2	yes	IS/2	2
6	35	2/10/20/B2	4	yes	IS/2	2
7	40	1/2/10/20/B2	5	yes	IS/2	2
8	20	2/10/B2	3	yes	IS/2	2
9	20	1/2/10/B2	4	yes	IS/2	2
10	30	1/2/10/20/B2	5	yes	IS/2	2
11	8	1/2/B2	3	yes	IS/2	2
		Samples/survey	37			22

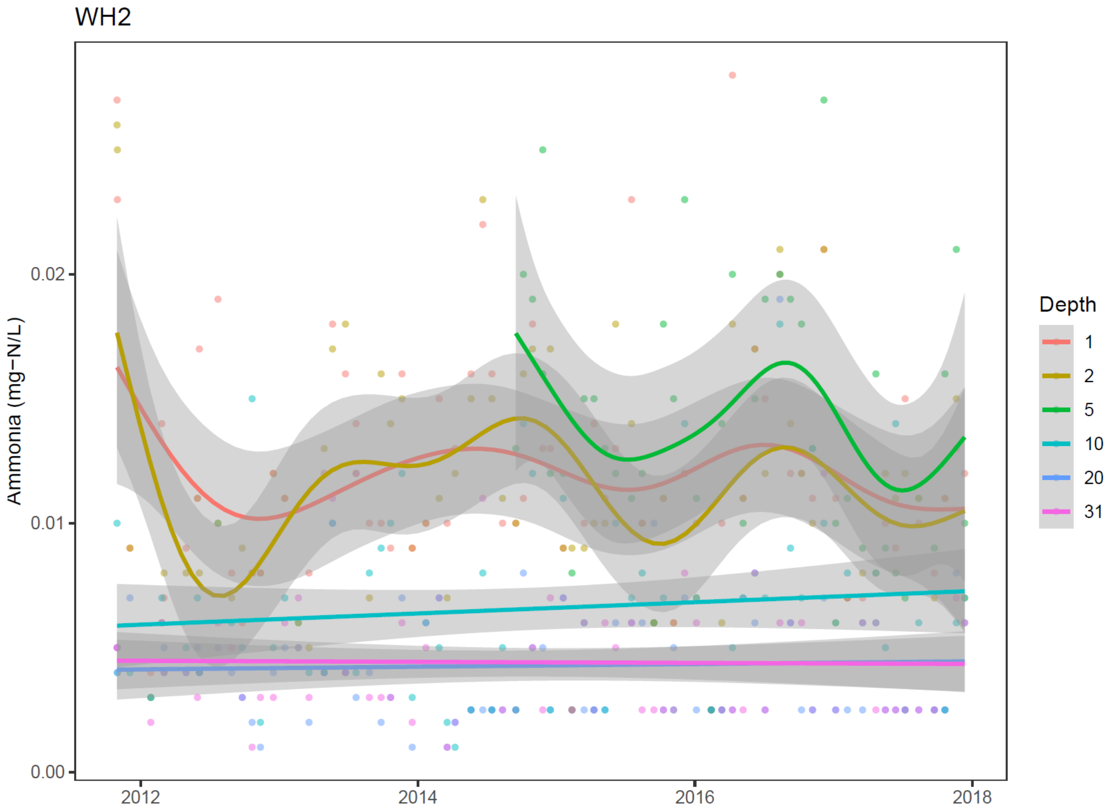
IS = 12m integrated sample, B2 = Bottom minus 2m

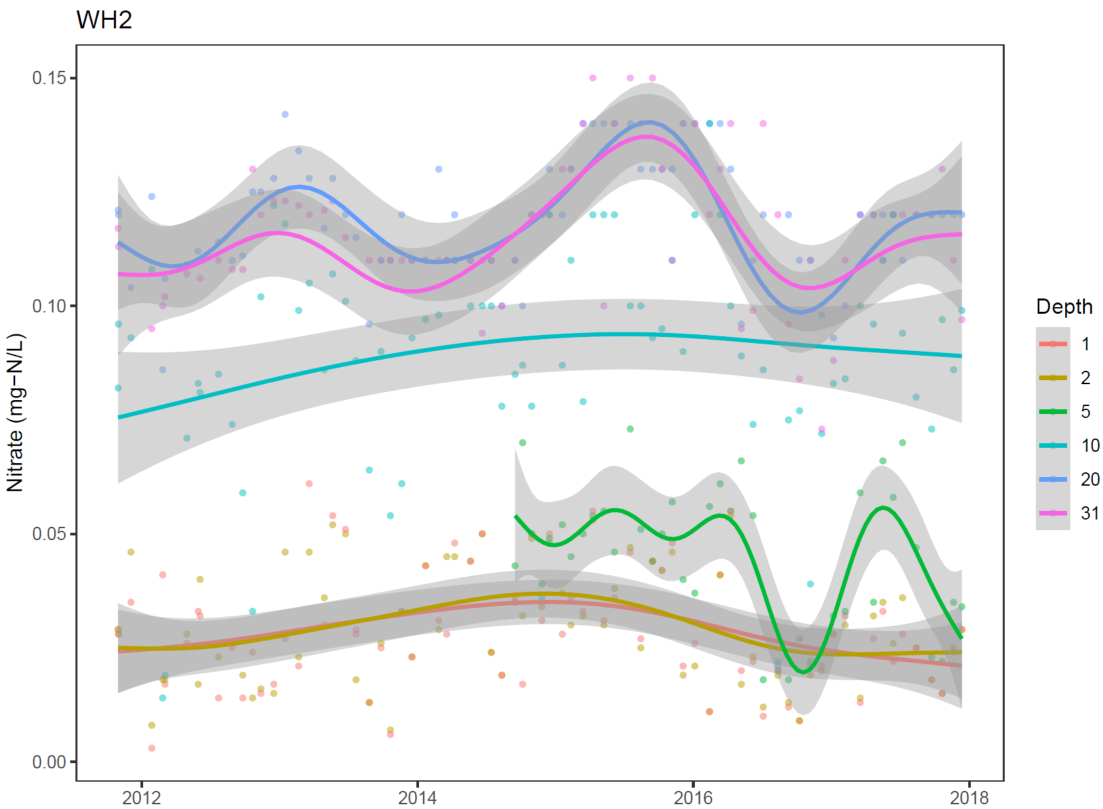
Figure 71 Sampling requirements for each monitoring site

Site No (Licence)	Site Name	Site Code
1	Hells Gates 1	HG1
2	Swan Basin	SB
3	King River 1	KR1
4	Central Harbour North	CHN
5	Cosy Channel	CC
6	Central Harbour East	CHE
7	Central Harbour 1	CH1 (sampling ceased Sept 13)
8	Petuna 3	PET3
9	World Heritage North	WHN
10	World Heritage 2	WH2
11	World Heritage 1	WH1
Additional	Hells Gates 3	HG3
Replaced CH1	Central Harbour 5	CH5 (sampled from Oct 2013)
Additional	C8	C8 (sampled from Dec 2013)
Additional	C10	C10 (sampled from Dec 2013)
Additional	GR1	GR1 (sampled from Dec 2013)
Additional	GR2	GR2 (sampled from Dec 2013)
Additional	KR4	KR4

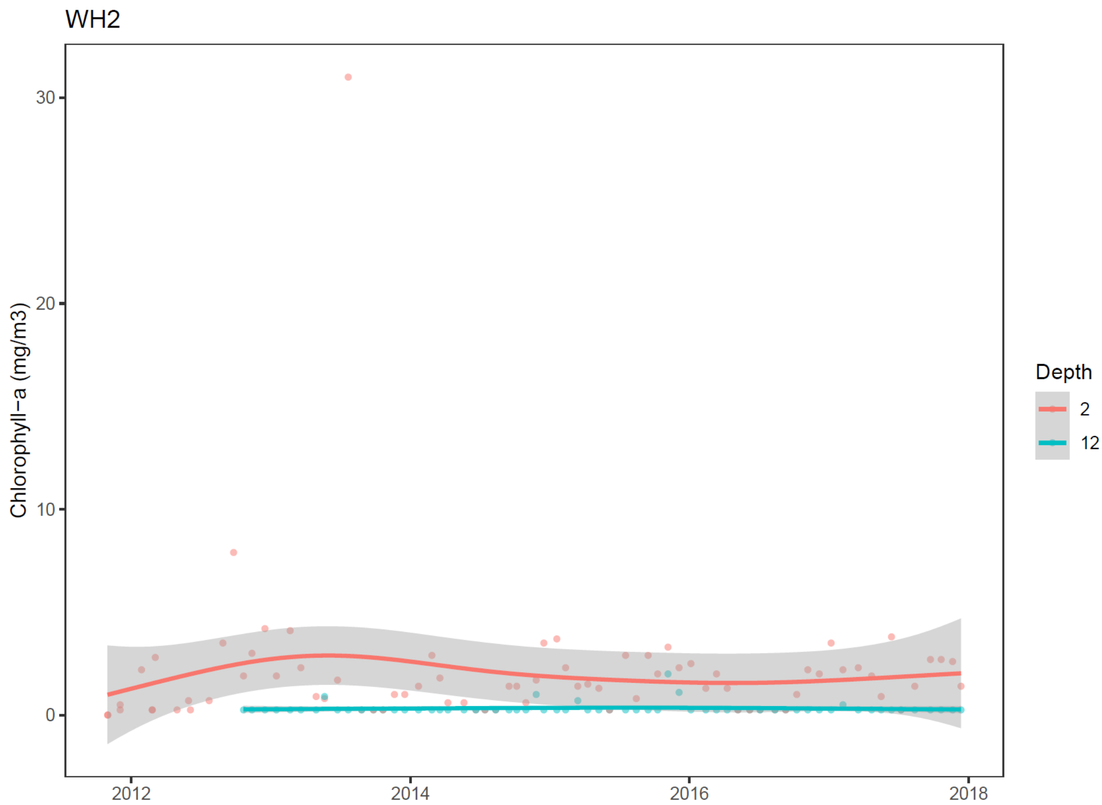
Figure 72 Current monitoring sites

- At several sites nutrient samples are collected and analysed at both 1 and 2m and at 20m and the bottom. The concentrations and temporal trends are extremely similar (see ammonia and nitrate examples at WH2 below).

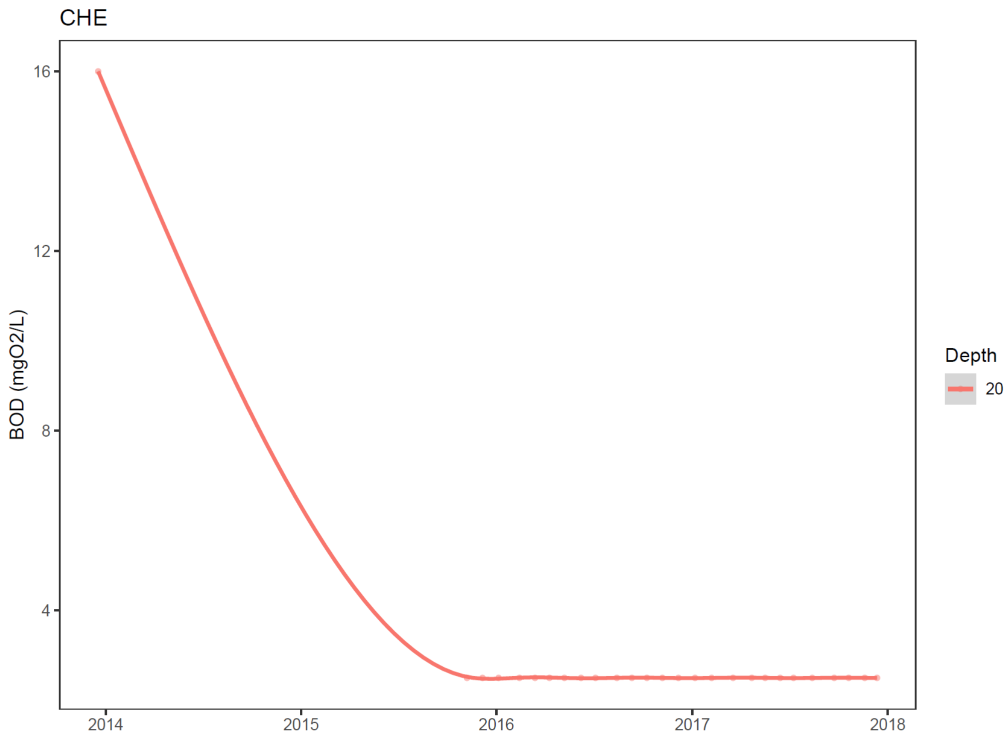




- chlorophyll-*a* concentrations are measured from samples collected at 2m using a Niskin bottle and for 0- 12 m using an integrated sample. Chlorophyll-*a* concentrations are rarely above the minimum detection limit in the integrated sample and therefore provide little useful information to the MHBEMP assessment (see below example from WH2).



- phytoplankton community composition is also assessed from both 2m and the 12m integrated sample at every site. This assessment has shown that phytoplankton are largely restricted to the surface layer of Macquarie Harbour and therefore the 2 m Niskin sample is a good indicator of phytoplankton abundance and composition at any given time. As such, the 12 m integrated sample could also likely be removed from sampling, but first, a pilot assessment is recommended to investigate whether any species are aggregating at the pycnocline.
- The review highlighted that phosphorus availability may limit primary productivity in Macquarie Harbour given the high concentrations of nitrogenous compounds. As such, we recommend that phosphate be included in BEMP sampling in the future at the core longitudinal sites.
- The measurement of NPOC isn't a formal requirement of the BEMP schedule, but its inclusion has provided informative for monitoring the organic carbon pool. Like phosphate we recommend it be included in BEMP sampling in the future at the core longitudinal sites.
- BOD is often below AST detection limits and the data provides little useful information (see below from CHE)



- At some sites nitrate only is measured and at other sites both nitrate + nitrite and nitrate. The latter provides the opportunity to calculate nitrite concentrations. Nitrite concentrations are very low relative to nitrate and there appears little value in measuring both at the respective sites
- At several sites, each of TN, TKN and TKN filtered in addition to the dissolved forms of nitrogen are measured. It seems highly likely that this could be rationalised.

Trigger limits

The current parameters and depths limits do not appear to be adequate as environmental standards to monitor and protect the environmental health of Macquarie Harbour. The decline in oxygen concentrations in bottom and mid-waters and associated deterioration in benthic conditions has been well documented (e.g., Ross et al., 2017) and has motivated reductions by the EPA since early 2017 to reduce the pressure on the harbour and allow for environmental recovery (see http://epa.tas.gov.au/regulation/salmon-aquaculture/macquarie-harbour/management-determinations). Of note:

- the use of a single trigger limit at 2 m depth for dissolved oxygen is insufficient to protect the flora and fauna of Macquarie Harbour, most of which is below 2 m. An oxygen limit for bottom and mid waters is recommended.
- the review highlights the importance of total N is a proxy for both organic and inorganic N within the system; we suggest this be included in sampling and future reporting on environmental condition in the harbour

- ammonia appears short lived within the system; consideration should be given to the inclusion of nitrate as an indicator and limit for bottom waters.
- the review has shown that the Macquarie Harbour system appears to be relatively uniform in terms of nutrient and oxygen concentration, highlighting that water column conditions within the compliance region are not independent of what is happening within the broader system (or vice versa). This highlights the importance of ongoing monitoring of all system drivers (e.g., river inflows, water elevation, STP inputs) to assist with the interpretation and attribution of change in the indicators and their levels.
- Although macrofaunal surveys have been conducted regularly in Macquarie Harbour, particularly since early 2015, they are not a formal requirement of the MHEMP program. Given that macrofauna are an important indicator of environmental conditions of Macquarie Harbour it is recommended that benthic surveys are incorporated in the MHEMP monitoring program, initially biennially, but more or less frequently depending on environmental conditions (e.g., oxygen dynamics and recovery).
- We suggest that future benthic surveys include a subset of the original shallow water sites sampled by Talman et al. (1996) given that the vast shallow areas in the harbour appear to be acting as a “reservoir for recruitment” for the deeper sites following low oxygen events.

Knowledge gaps

Microbial activity

The findings of this review and other recent studies on Macquarie Harbour highlight the key role that microbial activity is playing in the degradation of organic matter and drawdown of oxygen. Targeted observations to understand the specific organisms present and the factors that influence the rates of microbial degradation, oxygen utilization and nutrient remineralization, will reduce uncertainty of our understanding of the harbour biogeochemistry to help better constrain these processes in biogeochemical model efforts. This work has been supported through FRDC 2016 – 067 and some of the initial outputs have already been published (Da Silva et al., 2021).

Nanoflagellates

Nanoflagellates have been shown to be highly effective predators of prokaryotes (i.e. bacteria and archaea) and can therefore play an important role in the ecology of a system. Given this group has appeared to establish itself over recent years, there may have been a shift in the harbour plankton ecology. Identifying the species comprising “unidentified nanoflagellates” would assist in understanding their trophic role. It is difficult, or impossible, to identify nanoflagellates to species level using light microscopy (Davies et al., 2016). It can be achieved with scanning electron microscopy (Kim and Jung, 2005), transmission electron microscopy (Bergesch et al., 2008, Leroi and Hallegraeff, 2004), staining and fluorescence microscopy (Tsai et al., 2005), molecular techniques (Lim et al., 2001) or their trophic level can be established through the presence of chloroplasts or the ingestion of fluorescently marked cells (Christakill et al., 1999). All of these techniques are time consuming, relatively expensive and probably not able to be implemented in ongoing MHBEMP sampling, however, a single directed sampling, if only to establish the trophic level of the nanoflagellates, would greatly assist in future interpretation of the Macquarie Harbour phytoplankton community.

The abundance of unidentified nanoflagellates at greater depths needs quantification. If this newly established species/lineage is predominantly a predator of prokaryotes they may be able to inhabit waters below the photic zone, unlike autotrophic and mixotrophic phytoplankton. While this group was more abundant in the 2m Niskin sample than the 12 m integrated sample, bacteria are most abundant in surface waters, so it is still plausible that they are relatively abundant at greater depths, particularly if they feed on archaea, which are far more abundant than bacteria at depth (Ross et al., 2016a). Ideally, this investigation should investigate archived samples to quantify the abundance of these species in early MHBEMP sampling to determine their abundance when they were not counted.

Chryptophytes

This genus is known to flourish in low light, high nutrient environments making them particularly well suited to Macquarie Harbour. Further, many species are known to be mixotrophic and consume prokaryotes (i.e. bacteria and archaea) and phytoplankton meaning they are likely to be very important within the system. Silicified phytoplankton such as diatoms require silicate for growth and, if it is limiting, it could explain the dominance of other phyla groups such as Chryptophytes. Measurements of silicate in future should be considered to provide further insight into the factors that dictate the phytoplankton composition and abundance in Macquarie Harbour.

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Appendix i

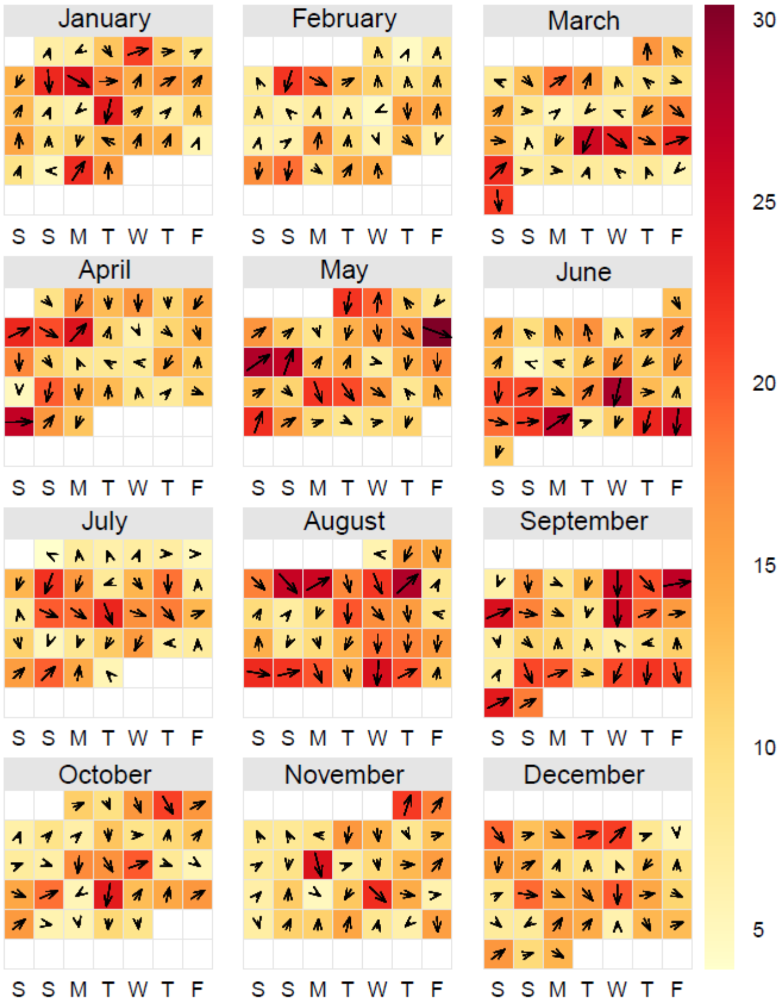

Figure: Daily wind direction (arrows) and speed (knots) at Cape Sorell in 2012.

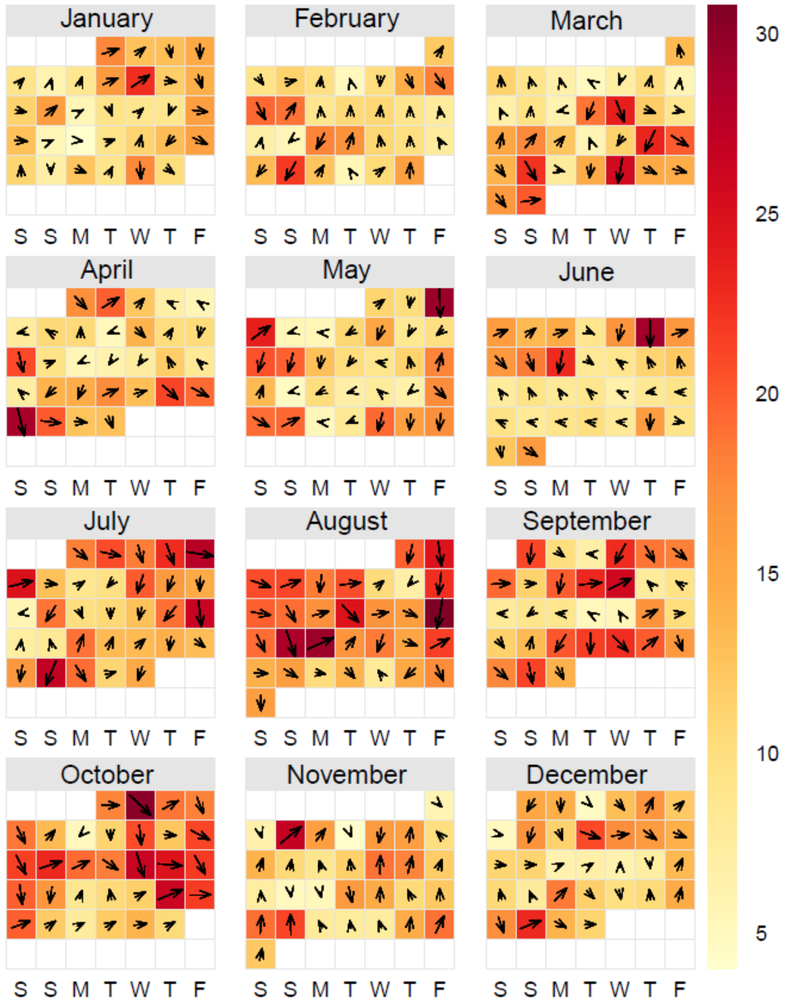

Figure: Daily wind direction (arrows) and speed (knots) at Cape Sorell in 2013.

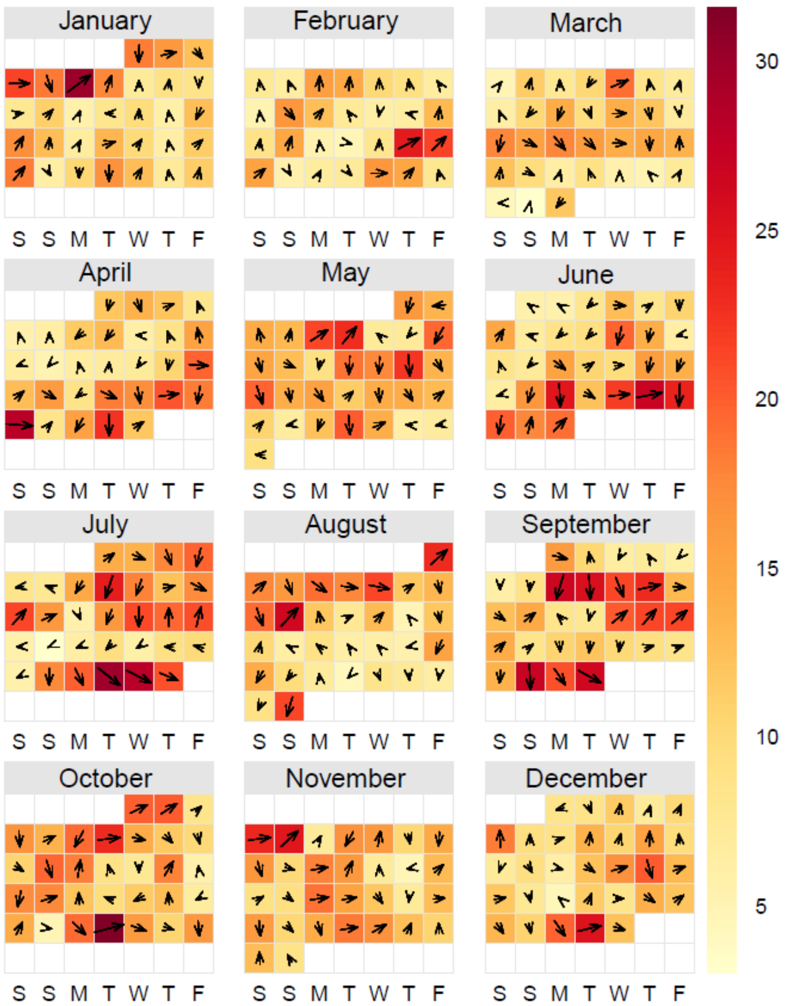

Figure: Daily wind direction (arrows) and speed (knots) at Cape Sorell in 2014.

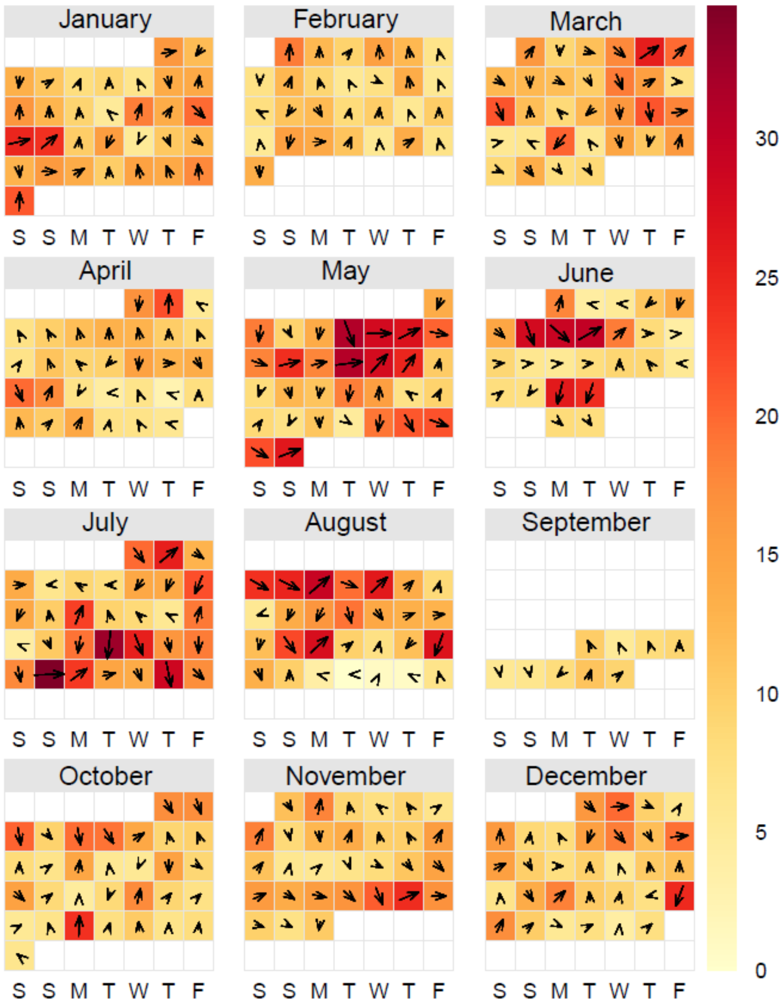

Figure: Daily wind direction (arrows) and speed (knots) at Cape Sorell in 2015.

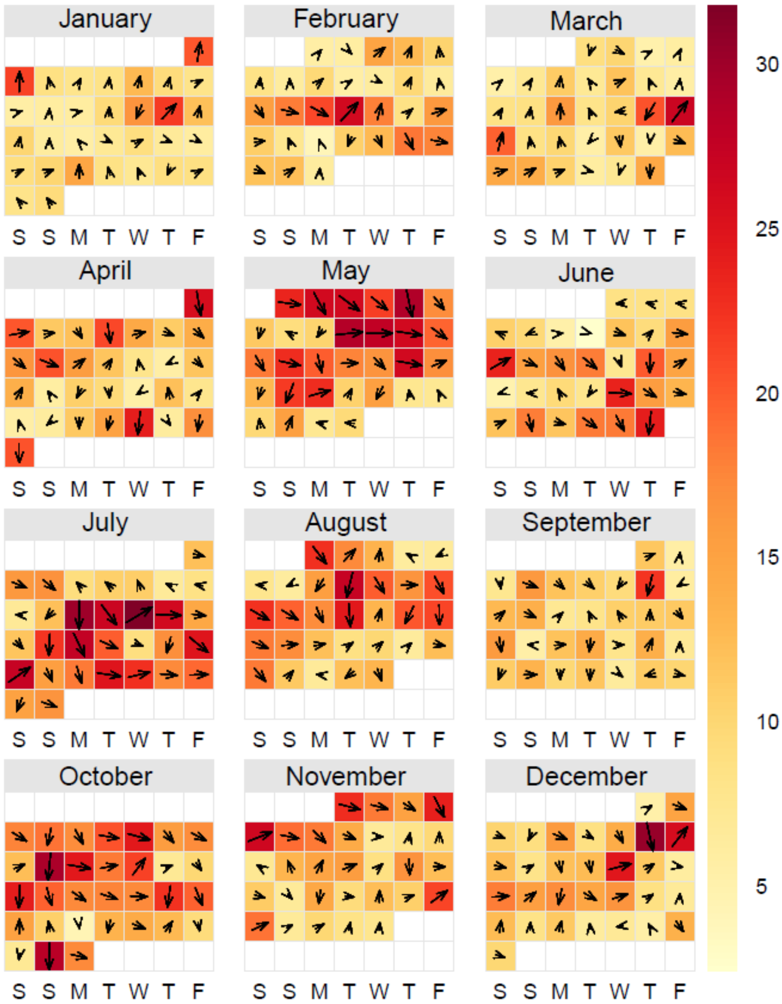

Figure: Daily wind direction (arrows) and speed (knots) at Cape Sorell in 2016.

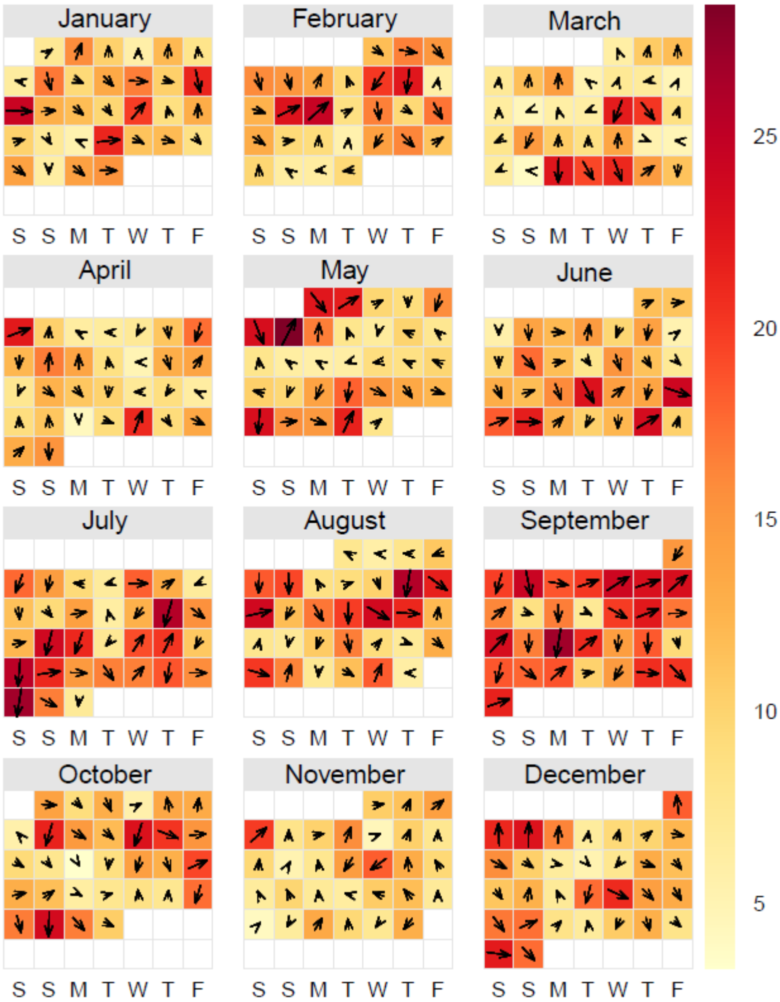

Figure: Daily wind direction (arrows) and speed (knots) at Cape Sorell in 2017.

Appendix ii

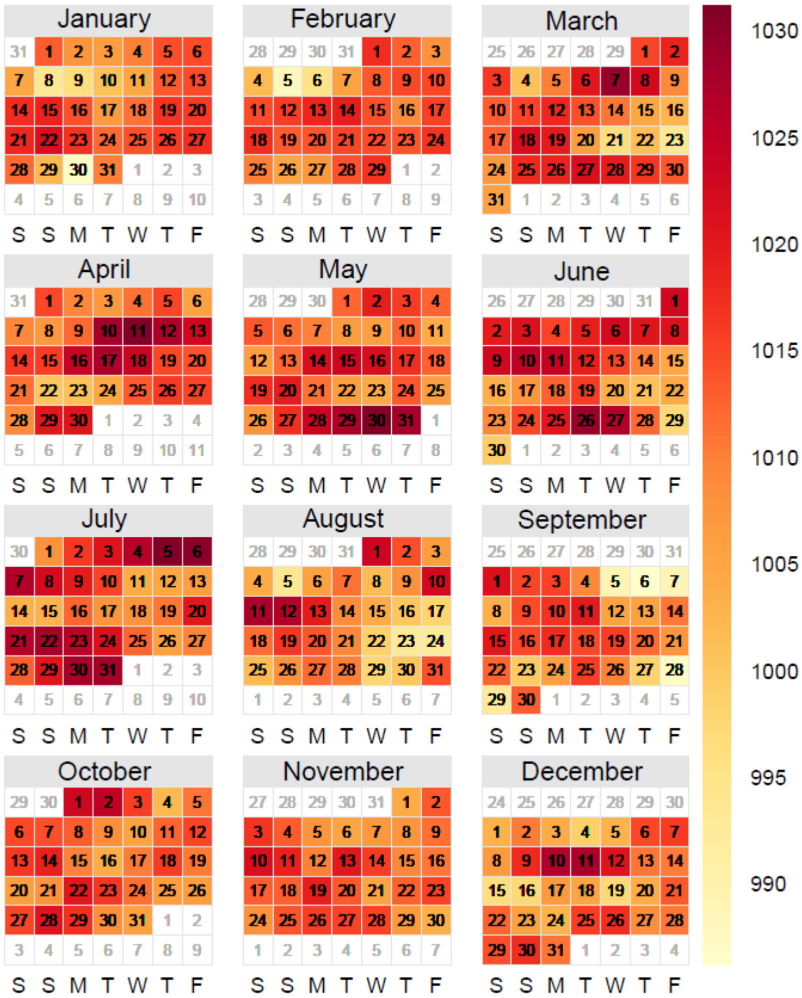

Figure: Daily barometric pressure (kPa) at Cape Sorell in 2012.

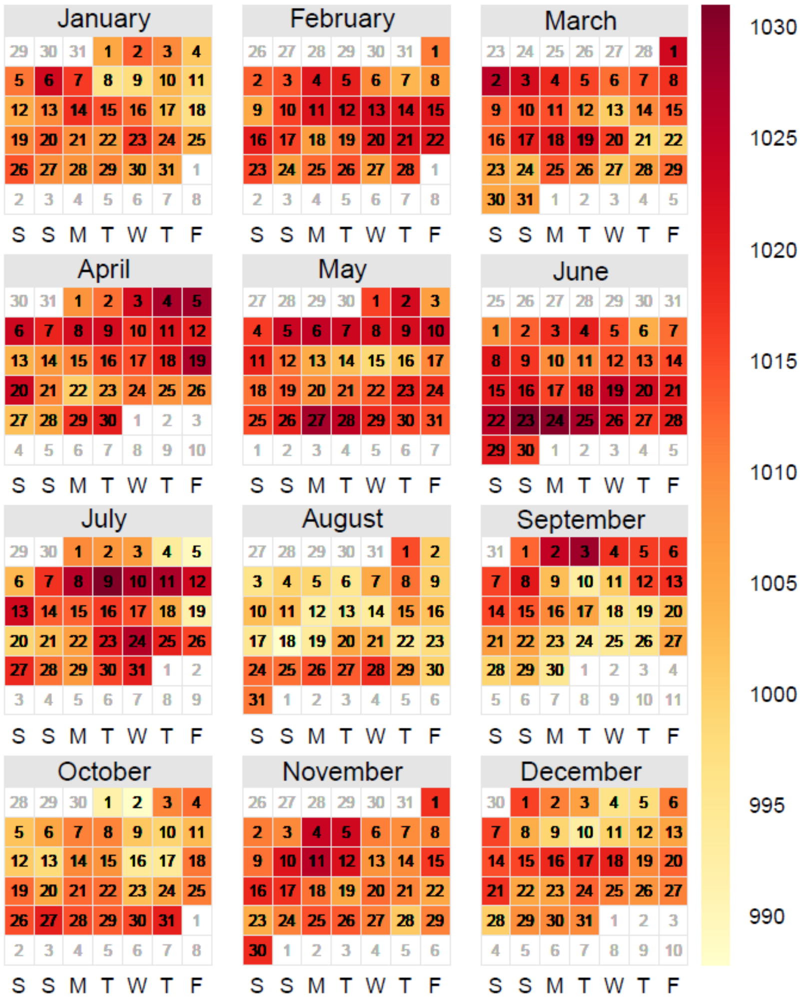

Figure: Daily barometric pressure (kPa) at Cape Sorell in 2013.

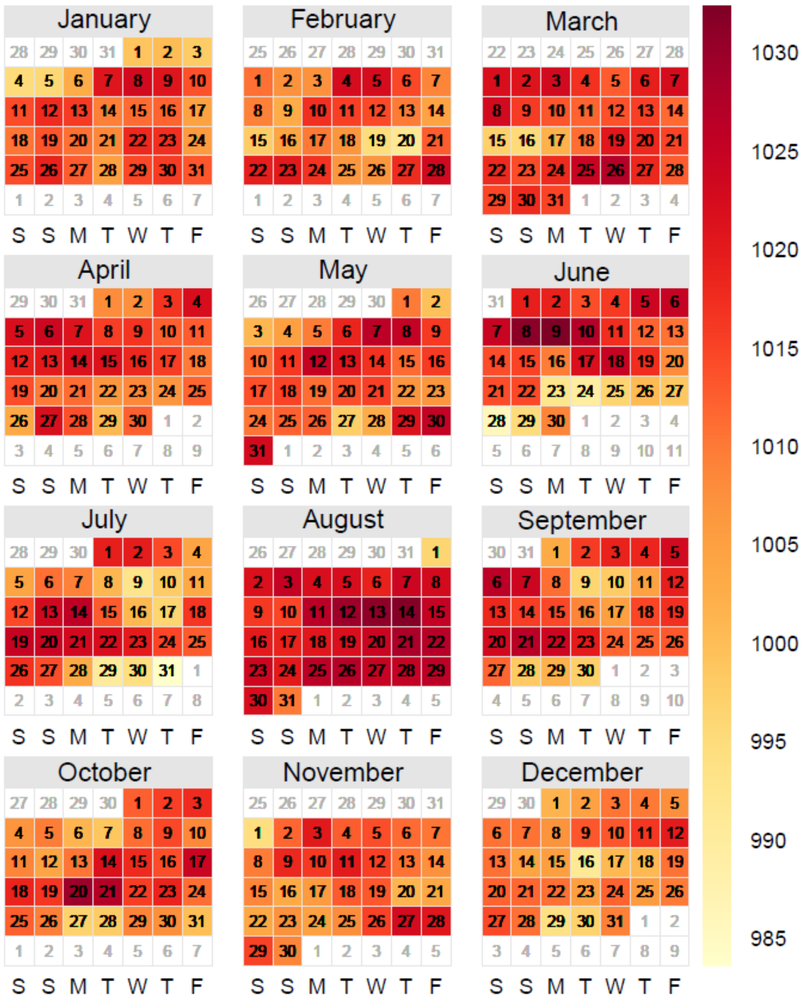

Figure: Daily barometric pressure (kPa) at Cape Sorell in 2014.

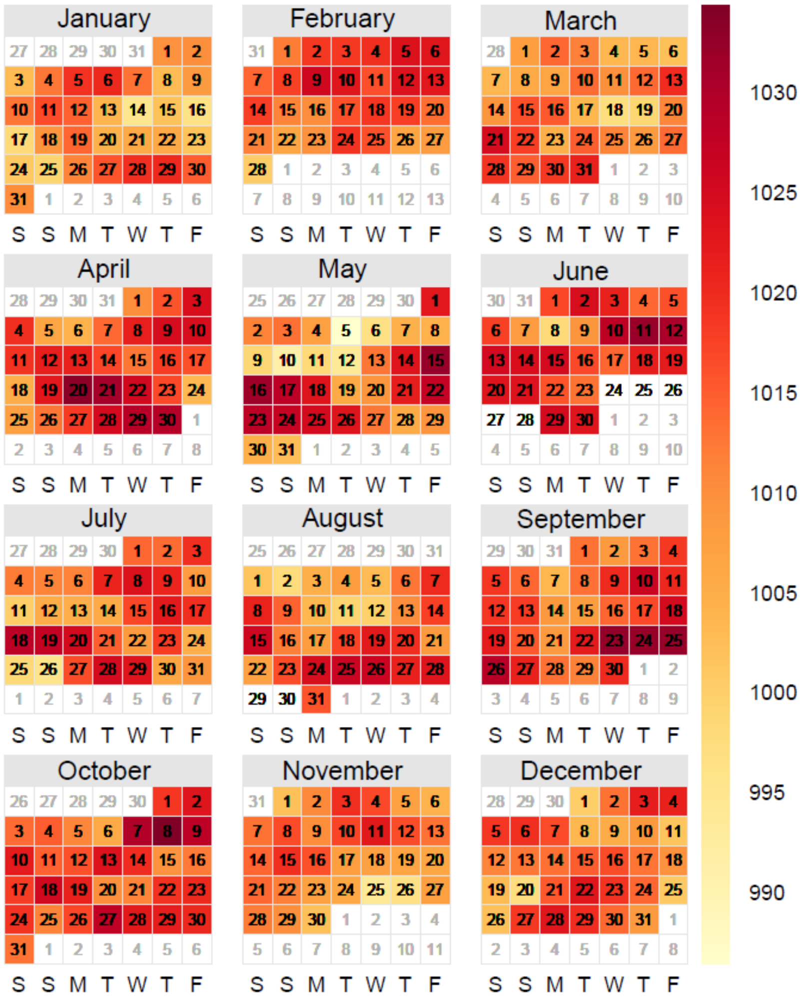

Figure: Daily barometric pressure (kPa) at Cape Sorell in 2015.

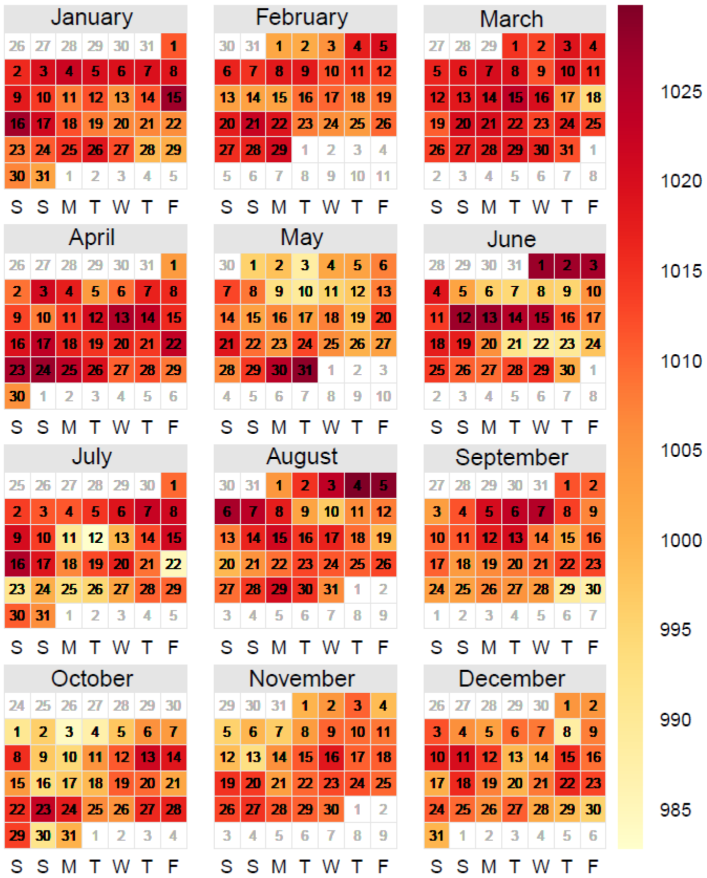

Figure: Daily barometric pressure (kPa) at Cape Sorell in 2016.

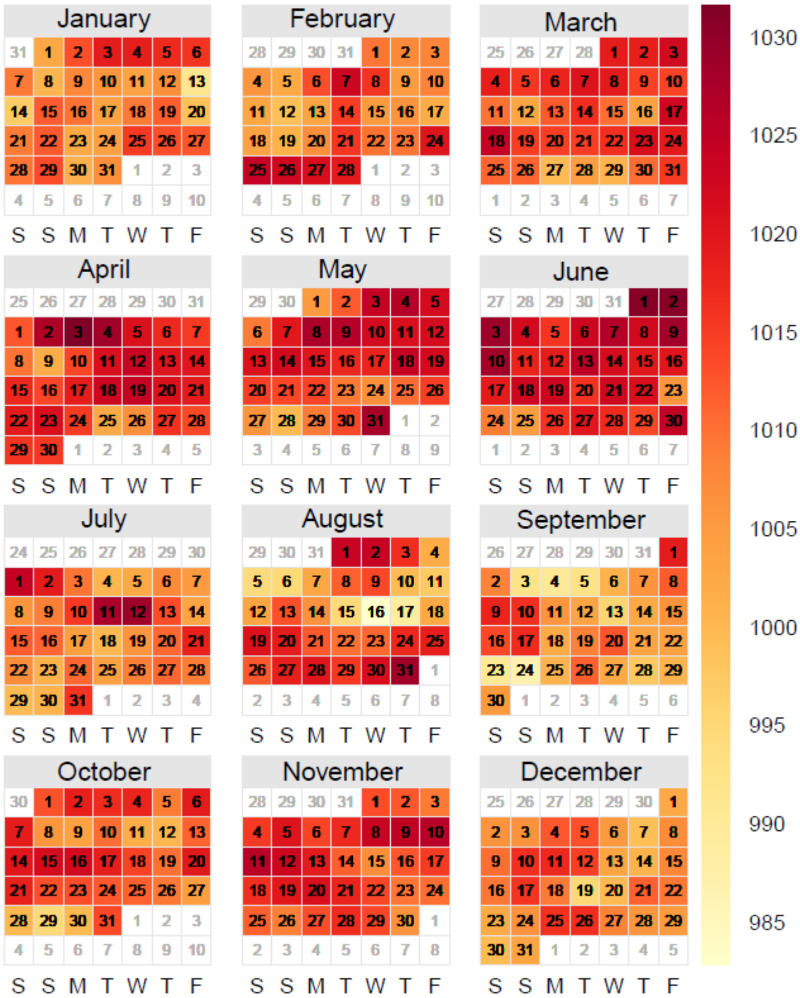

Figure: Daily barometric pressure (kPa) at Cape Sorell in 2017.