Phytoplankton Sampling Program: December 2018 to December 2019

Technical Report A - Annexure B

AGL Gas Import Jetty Project

CEE Technical Report
Phytoplankton Sampling Program
Lower North Arm, Western Port
December 2018 to December 2019

February 2020

CEE Technical Report
Phytoplankton Sampling Program
December 2018 to December 2019

Contents

Figures

Tables

Report to:
[redacted]

Principal
AECOM

Report prepared by:
Scott Chidgey, Peter Crockett, Amelie Mendrinna, Joana Costa
CEE Pty Ltd
Unit 4, 150 Chesterville Rd
Cheltenham, VIC 3192
cee.com.au

Cover photo:
Small chain forming diatom from Lower North Arm Western Port 2019.
Photo by Microalgal Services

CEE Technical Report
Phytoplankton Sampling Program
December 2018 to December 2019

1 Background

AGL Wholesale Gas Limited (AGL) and APA jointly propose to develop a Liquid Natural Gas (LNG) import terminal at Crib Point in the Lower North Arm of Western Port in Victoria, Australia. They propose to moor a floating storage and regasification unit (FSRU) at Crib Point Jetty Berth 2, install gas offloading facilities on the jetty and construct a transfer pipeline between Crib Pt and Pakenham. AGL proposes to engage a contractor to supply and operate the FSRU facility, while APA will develop and operate the gas transfer pipeline.

The Victorian Minister for Planning (the Minister) decided that based on the referral documentation an Environment Effects Statement under the *Environment Effects Act 1978* was required. The Victorian EES process is an accredited assessment process under the EPBC Act. The Minister issued scoping requirements (Ministerial Guidelines) for the EES in February 2018. The Ministerial Guidelines along with the EES referral documents were used to design technical studies that fulfil the requirements for the Environment Effects Statement.

EES referral documents (CEE, 2018a-e) identified the pathways by which the proposal may impact upon the Western Port marine ecosystem. The documents identified that the passage of relatively large volumes of seawater through the heat exchanger of the FSRU would entrain quantities of plankton. The seawater used in FSRU processes would be chlorinated to prevent marine growth developing within the pipework and heat exchangers, and therefore had potential to impact marine communities in Western Port.

One of the recommendations included in the EES referral was that a plankton sampling program be developed to provide information on spatial and temporal variations in plankton populations in Lower North Arm focussing on the proposed location and position of the FSRU intake. This information would be used to inform the evaluation of *"potential for significant short and long-term impacts on marine biota due to entrainment of organisms in seawater for regasification or due to discharge of cooled seawater after use for regasification, including impacts resulting from reduced availability of food for other species, resultant hydrodynamic changes and other impacts such as long-term changes to populations and distribution".*

This technical report describes the plankton community of Western Port from existing information and presents the rationale and methodology for the plankton sampling program, specifically the phytoplankton sampling program. Summary results are also presented. It is one of three reports on the plankton, the others being the zooplankton (planktonic macroinvertebrates) and ichthyoplankton (fish larvae).

Further discussion of the results in the context of hydrodynamic, entrainment and discharge modelling, zooplankton ecology, environmental guideline values and impact assessment are provided in separate EES related documents.

2 Introduction to phytoplankton

Plankton are the microscopic plants and animals that live in the water column. Plankton communities, including the community in the North Arm of Western Port, comprise:

- Phytoplankton, which are the microscopic plants that photosynthesise and one of the key sources of primary production and food for small animals in Western Port and
- Zooplankton, which are the small animals of various feeding groups that provide a source of food for other filter feeding animals including other plankton, invertebrates on the seabed, jellyfish, larval fish and small fish.

Plankton comprise holoplankton that are the plants and animals that spend their entire life cycle drifting in the water columns, while meroplankton are the propagule or larval stages of larger plants or animals that are attached to the seabed or are free swimming.

Phytoplankton obtain their energy through photosynthesis (they are autotrophs). There are also a number of heterotrophic species of phytoplankton that may obtain some or most of their energy by consuming other phytoplankton or bacteria (heterotrophs). Phytoplankton primary productivity supplies a large proportion of the food for marine ecosystems, as they are eaten by a range of zooplankton (micro and macroinvertebrate plankton), larval fish and benthic filter feeding organisms. The primary productivity provided by the phytoplankton in Western Port is supplemented by the primary productivity of benthic micro and macroalgae and seagrasses.

Phytoplankton have some ability to swim in the water column. However, due to their very small size their ability to maintain position in the strong tidal currents and vertical mixing in Western Port is very limited. They drift with prevailing currents and mixing processes. Phytoplankton typically have rapid life-cycles (hours to days) and can respond quickly to changes in the availability of light and nutrients.

Characteristics of the phytoplankton of Western Port were understood from a small number of studies prior to commencement of this program.

The Westernport Bay Environmental Study (Shapiro *et al*. 1975) documented the species of diatoms and some dinoflagellates present in surface waters at four sites distributed from the confluence zone to upper north arm. Samples were collected on 23 occasions from June 1973 to September 1974. Sampling was by horizontal tows at the surface with a fine mesh net. Qualitative abundance of one of the dominant diatom species, *Ditylum brightwellii,* was reported for each survey and site. The abundance of all other species was reported qualitatively for the whole study. Reference to these data are made where appropriate in this report.

Data from subsequent studies are not publicly available but include information on potentially harmful phytoplankton species present in the Flinders aquaculture zone (recent regular monitoring includes more comprehensive data). Data has also been collected in University studies.

Phytoplankton abundance has been monitored by EPA Victoria at three sites in Western Port using chlorophyll-a as a proxy for phytoplankton abundance. EPA monitoring has not assessed phytoplankton composition. Some of the EPA data on chlorophyll-a is incorporated here.

Microalgal Services provide a specialist report discussing phytoplankton ecological characteristics of Lower North Arm Western Port based on the CEE 2018-2019 sampling program. The report is appended to this report (Brett *et al*. 2020).

3 Sampling for EES

The studies for the EES were designed to optimise integration of the hydrodynamic modelling, particle entrainment modelling and understanding of plankton community spatial and temporal variability and dynamics to assess the effects of entrainment on plankton populations from Berth 2 at Crib Point Jetty situated in the main channel in Lower North Arm, and implications for the Western Port ecosystem.

The key interest of the studies was to:

1. Characterise the key components of the North Arm Plankton community
2. Identify spatial patterns along or across the main channel of North Arm where the FSRU will be located that may indicate sensitivity of local populations to entrainment or cold-water discharge
3. Identify seasonal patterns that may inform assessment of impacts of the project under differing seasonal intake and discharge scenarios

The EES plankton studies recognised that water depth, water exchange, current speed, turbulence and proximity of seabed habitats are strong influences on the composition of plankton likely to pass the FSRU intake. The FSRU berthed at Crib Point Berth 2 would be located approximately 600 m offshore from the low tide mark, more than 500 m from the 2 m depth contour and approximately 450 m and 330 m offshore from the 5 m and 10 m depth contours (Chart Datum), respectively. The lower boundary of the nearest saltmarsh-mangrove community southeast and northeast of the Jetty would be more than 1,100 m from of the FSRU intake. The intake on an FSRU at Crib Point jetty would be located approximately 450 m offshore from the likely lower limit of subtidal seagrasses at Woolies Beach at Crib Point.

The studies aimed to document the planktonic character of the water mass of the main North Arm Channel that would be entrained by the FSRU. The methods used were those typically used to quantitatively sample phytoplankton, mesozooplankton (including some meroplankton) and ichthyoplankton (including larger invertebrate meroplankton). The equipment, methods and timing did not target particular species. All plankton sampling used depth integrated samples to sample the same depth band of the water column that would be drawn into the FSRU heat exchange intakes.

The study design was based on the 1982 to 1984 zooplankton investigations of Western Port and Port Phillip Bay (Kimmerer and McKinnon 1985, 1987a, b, c), which provide spatial and temporal information on zooplankton in East Arm and the Western Entrance.

In the EES study, plankton sampling was designed to provide spatial and temporal information comparable between different trophic levels. Samples of phytoplankton, zooplankton and ichthyoplankton were collected monthly for a 13-month period (December 2018 to December 2019) along approximately 19 km of the Lower North Arm Channel.

In addition to the monthly sampling, zooplankton and phytoplankton samples were collected at the Berth 2 site during downloading of the water temperature logger at Crib Point jetty, which occurred at intervals approximately half-way between monthly plankton surveys.

Samples were collected at approximately monthly intervals using standard methods (see Section 0) from December 2018 to December 2019. A total of 4 surveys were conducted in summer and 3 surveys in spring, autumn and winter, to provide seasonal replicates at sampling sites. These seasonal replicates allowed for a seasonal analysis of the plankton community in the Lower North Arm.

3.1 Sites

The location of plankton monthly sampling sites is shown in Figure 3-1. Phytoplankton samples were collected at along the north-south axis (CPN2, CPN1, CPC1, CPS1 and CPS2), and at Crib Point Jetty Berth 2. Sites W10 and E10 were sampled for zooplankton and ichthyoplankton to determine potential influence of seagrass habitat on the characteristics of the plankton along the 10 m depth contour.

The north-south axis sites were positioned to document spatial variability along the tidal gradient in Lower North Arm: waters in the south of Lower North Arm are exchanged with Bass Strait water on a shorter time-scale than those in the north of Lower North Arm. These sites documented the plankton community at sites from near the extensive intertidal and shallow subtidal seagrass beds in Upper North Arm, to sites near the wave and current exposed deep channels in the Confluence Zone.

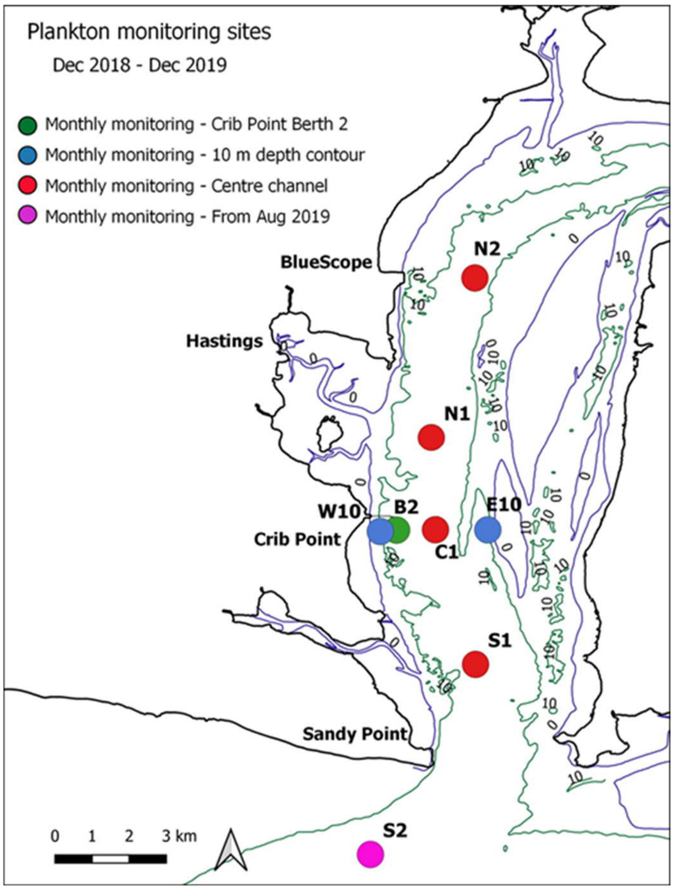

Figure 3-1. Plankton sampling sites in North Arm, Western Port.

Sites along the north-south axis were CPN2, CPN1, CPC1, CPS1 and CPS2. Site CPN1 is near a long-term water quality monitoring site used by EPA Victoria (Hastings) while site CPC1 is in the middle of the channel directly east of Crib Point Jetty Berth 2.

Site CPS1 was expected to show Bass Strait influence, particularly at high tide, due to its proximity to the confluence zone and western entrance segments. The first six months of the sampling program showed conditions at CPS1 were quite similar to other Lower North Arm sites regardless of tide. Therefore, site CPS2 in the confluence zone was added from August 2019 to assess plankton with greater Bass Strait influence.

As discussed below, the timing of surveys was not synchronised with tides, so that the water body sampled at any of the sites on any sampling occasion could have originated from up to 7 km kilometres over the six hours prior to sampling. This essentially randomised the spatial horizontal position of the samples and was consistent with the approach of Kimmerer and McKinnon (1985, 1987a)

3.1.1 Broad Scale Survey

An additional broad-scale, 'snapshot' survey in October 2019 was used to characterise the phytoplankton, zooplankton and water quality along two long transects from the Western Entrance up into East Arm (as far as Corinella) and North Arm (as far as the BlueScope wharves). Sites used in the broadscale study are shown in Figure 3-2. Sites CPP1 to CPP5 are in the same locations as those used by Kimmerer and McKinnon (1985) for which there is existing data on zooplankton communities. Sites CPS2 to CPN2 were the same sites as those used in regular sampling.

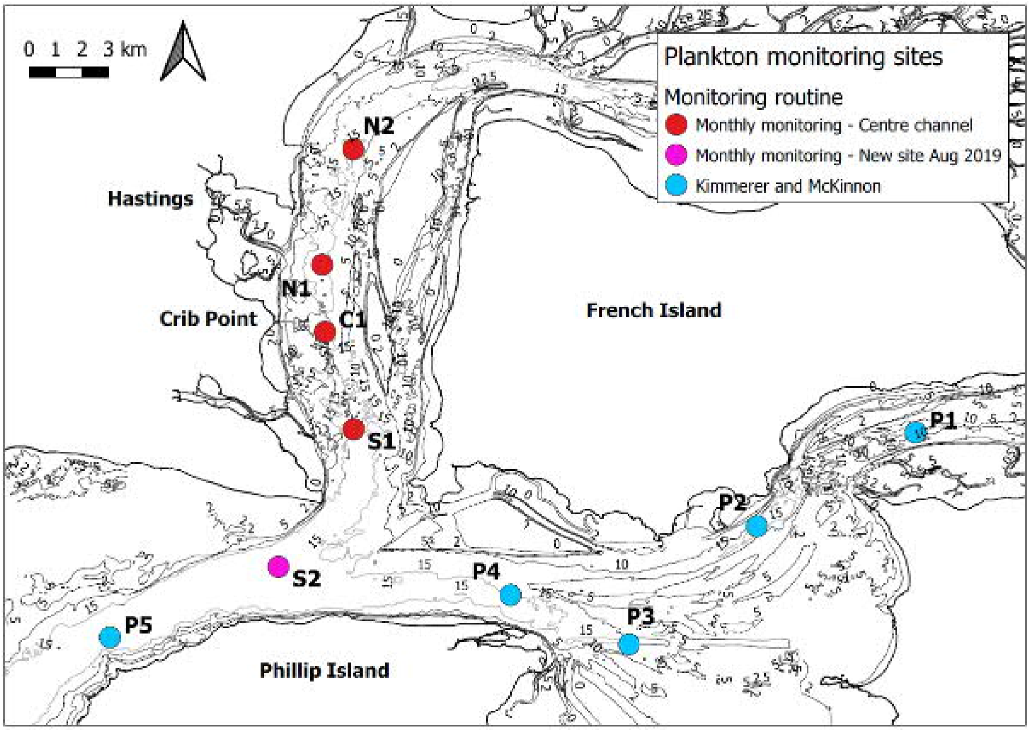

Figure 3-2. Broadscale plankton study sites

including 5 locations from Kimmerer and McKinnon (1985)

l

3.2 Timing

Phytoplankton, zooplankton and ichthyoplankton were sampled at each monthly site visit. Plankton at all sampling sites were sampled at approximately monthly intervals from December 2018 and December 2019 inclusive (13 surveys).

Monthly samples were collected during daytime only, when weather was suitable within approximately one week of the change in month. Mobilising to site, launching, sampling all sites, preserving samples and demobilising took a whole day per survey. Surveys or sampling were not synchronised with tides in any way.

Individual samples (phytoplankton, zooplankton, ichthyoplankton were collected during the same site visit. Phytoplankton and zooplankton samples were usually collected simultaneously. The vessel was usually relocated back to the approximate sample site position between the ichthyoplankton sample collection and the phytoplankton and zooplankton samples due to drift during sampling.

Each monthly survey was separated by a minimum of 3 and a maximum of 6 weeks. Crib Point Jetty Berth 2 site (CPB2) was sampled every other fortnight (total of 25 surveys at this site). The sampling schedule is shown in Table 3-1

Table 3-1. Plankton sampling summary, December 2018 to December 2019

Monthly Survey	Date	S2	S1	B2	W10	E10	C1	N1	N2
Survey 1	11/12/2018		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 2	10/01/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 3	4,11/02/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 4	07/03/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 5	04/04/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 6	03/05/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 7	07/06/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 8	03/07/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 9	02/08/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 10	03/09/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 11	1/10/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 12	30/10/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 13	11/12/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Total number of surveys		5	13	13	13	13	13	13	13

z = zooplankton, i = ichthyoplankton, p = phytoplankton

3.3 Sampling Method

Phytoplankton depth-integrated sampling methods appropriate for the Western Port environment were used and were similar to those used in the Victorian Marine Biotoxin Management Plan (e.g. Fisheries Victoria 2017; EPA Victoria marine monitoring program).

A single depth-integrated water sample was collected for phytoplankton identification and counts, and chlorophyll-a analysis at each site in each survey. Samples were collected using a 15 m long, 19 mm diameter clear PVC hose with valves on both ends and a 1.5 kg weight on one end (Figure 3-3). Phytoplankton samples were preserved with Lugol's potassium iodide solution at a concentration of approximately 0.5 % v/v. Samples for chlorophyll-a analysis were kept cool and in the dark prior to delivery to the laboratory within 24 hours of collection.

Figure 3-3. Phytoplankton sampling with hose

3.4 Subconsultant laboratory processing of samples and report

Phytoplankton in samples were identified and enumerated by Microalgal Services, a NATA accredited specialist phytoplankton laboratory. Phytoplankton were concentrated using membrane filtration, and then samples were placed into Sedgewick-Rafter counting chambers and examined using light microscopy. Microalgal Services planktologists advised on collection and preservation procedures. Microalgal Services provided a report on the ecology and temporal and spatial patterns of the phytoplankton of the samples collected during the program. Microalgal Services report is appended to this report.

Phytoplankton species were identified to the lowest possible taxonomic level (usually genus or species) using morphological features and counted. Phytoplankton abundances were reported as cells per litre.

Chlorophyll-a was analysed by the Water Studies Centre at Monash University. The chlorophyll-a method involves filtering the 1L sample, mechanical rupturing of cells, extraction of the chlorophyll (and other photosynthetic pigments) using acetone and analysis of the extract by spectrophotometry. The method is accurate over the range 0.2 to 100 µg/L chlorophyll-a.

3.5 Data analysis

As discussed in Section 3, the key interest of the studies was to:

1. Characterise the key components of the North Arm Plankton community
2. Identify spatial patterns along or across the main channel of North Arm where the FSRU will be located that may indicate sensitivity of local populations to entrainment or cold-water discharge
3. Identify seasonal patterns that may inform assessment of impacts of the project under differing seasonal intake and discharge scenarios.

3.5.1 Monthly community composition

The monthly monitoring program at the array of sample sites is shown in Table 3-1. Mean monthly abundance and standard error of the mean (mean± SEM) of phyto-, zoo-, and ichthyo- plankton taxa in Lower North Arm was estimated for thirteen months using the number of samples shown in Table 3-2. The table shows that the number of samples (n) for calculation of mean and standard error ranges from 5 for the first eight months of monitoring phytoplankton to 8 for the last five months of monitoring zooplankton and ichthyoplankton.

Table 3-2. Number of samples (n) used for monthly mean and SEM calculations Lower North Arm Dec 2018 to Dec 2019

Plankton	Dec 2018 to Jul 2019	Aug 2018 to Dec 2019
Phytoplankton	5	6
Zooplankton	7	8
Ichthyoplankton	7	8

3.5.2 Spatial patterns in Lower North Arm

Annual spatial pattern at monitoring sites

Spatial patterns between monitoring sites in Lower North Arm were demonstrated for key biota from annual mean and standard errors calculated from monthly data (Table 3-1). Table 3-3 shows that the number of samples (n) for calculation of mean annual abundance and standard error of the mean (mean± SEM) of phyto-, zoo-, and ichthyo- plankton taxa at monitoring sites in Lower North Arm range 5 at site S2 to 13 at the major monitoring sites.

Table 3-3. Number of samples (n) used for annual mean and SEM calculations Lower North Arm monitoring sites Dec 2018 to Dec 2019

Plankton	S2	S1	B2	W10	E10	C1	N1	N2
Phytoplankton	5	13	13			13	13	13
Zooplankton	5	13	13	13	13	13	13	13
Ichthyoplankton	5	13	13	13	13	13	13	13

Seasonal spatial pattern at monitoring sites

Seasonal spatial patterns between monitoring sites in Lower North Arm were demonstrated for key biota from seasonal mean and standard errors calculated from monthly data (Table 3-1). The table shows that the number of samples (n) for calculation of mean annual abundance and standard error of the mean (mean± SEM) of phyto-, zoo-, and ichthyo- plankton taxa at monitoring sites Lower North Arm was n=3 for autumn, winter and spring and n=4 for summer.

3.5.3 Further data presentation and use

Environment Effects Statement

Further discussion of the results in the context of hydrodynamic, entrainment and discharge modelling, zooplankton ecology, environmental guideline values and impact assessment are provided in separate EES related documents. The assessment of impacts on marine ecosystem components including plankton communities, is a risk-based, 'likelihood X consequence' process informed by statistically determined environmental guidance values (for temperature, chlorine concentration and entrainment proportion with respect to natural flushing), hydrodynamic, entrainment and exposure modelling and understanding of ecological characteristics documented in the technical studies.

Baseline documentation and impact assessment monitoring

The environmental data collected during EES studies, such as those presented in this report, will inform selection of environmental indicators, and the design and statistical power of baseline and operational compliance monitoring programs if the project proceeds.

3.6 Quality

Quality assurance processes samples collected during this task included:

- Sampling equipment was rinsed with fresh seawater prior to sampling, and sampling equipment was rinsed with fresh water following each survey to prevent cross-contamination of samples
- Only new sample containers were used
- The time, date, GPS position and sampling depth was recorded for each sample, along with details of tide state, weather and sea-state
- Samples were preserved in Lugol's solution. Extra preservative was added after several hours to achieve effective preservation if preserved sample colour became light
- Samples were delivered to the analysing laboratory along with chain of custody documentation detailing the sampling dates, sites and sampling depths for each sample.
- Field data were compiled into a database within a few days of sampling
- Laboratory procedures were NATA accredited in-house methods used by Microalgal Services

Quality control for data collected during this task included:

- Checks that the field recorded GPS positions for each sample matched the planned (regular) site location
- Data returned by the laboratory were cross checked against field records and compiled into a database
    - Checks to ensure that the same number of data provided by the laboratory were entered into the database
    - Checks for consistency of data coding (site and sample identifications, taxonomic nomenclature, units)

3.6.1 Data use post EES

The environmental data collected during EES studies, such as those presented in this report, will inform selection of environmental indicators, and the design and statistical power of baseline and operational compliance monitoring programs if the project proceeds.

4 Results

The phytoplankton in Western Port can be broadly divided into diatoms (Bacillariophyceae), dinoflagellates (Dinophyceae) and other flagellates. 'Other flagellates' includes the phytoplankton classes Chrysophyceae, Prymnesiophyceae, Raphidophyceae, Dictyochophyceae, Cryptophyceae, Prasinophyceae and Euglenoidea. The most diverse group were the diatoms with 76 species, followed by the dinoflagellates with 38 taxa. The remaining groups were each represented by between 1 and 7 taxa.

The phytoplankton of Western Port includes species that are autotrophic (obtain all their energy from photosynthesis), heterotrophic (obtain all their energy from ingesting organic matter, bacteria or other small phytoplankton) and mixotrophic (obtain energy from both photosynthesis and heterotrophy). All phytoplankton species are an important part of the food web. Autotrophic phytoplankton absorb nutrients and provide a food source for both zooplankton and heterotrophic and mixotrophic phytoplankton. The mixotrophic and heterotrophic phytoplankton are a very important part of the trophic web, having a key role in the 'microbial loop' that recycles nutrients within the water column, as well as being a major food source for zooplankton (Suthers *et al*. 2019).

Flagellates can move through the water (at a very small scale) using their flagella for propulsion, planktonic diatoms tend to be non-motile, while benthic diatoms may be able to move across surfaces and through sediment.

A summary of the phytoplankton comprising 1 per cent or more of the total number collected over the sampling program is shown in Table 4-1. These 19 phytoplankton taxa accounted for 85 per cent of the total phytoplankton numbers. The remaining 15 percent of phytoplankton is distributed between the remaining 131 identified taxa. The top four taxa were all planktonic diatoms, and all of these are small (5-20 µm) species that form chains (often 200-300 µm long, Figure 4-1). There were two other common planktonic diatoms and one benthic diatom. The remainder of the list are flagellates: two dinoflagellate species, three cryptophytes, two prymnesiophytes, two prasinophytes, and one euglenoid. The diatoms are autotrophs, while flagellates included auto, hetero and mixotrophic species.

Most of the species observed in this study are considered cosmopolitan and are widespread in coastal waters of south east Australia (Hallegraeff *et al* 2009). Any potentially toxic species collected were at densities below aquaculture alert levels.

The phytoplankton community was dominated by small cell-size diatoms and flagellates, with larger celled diatoms occurring sporadically. This is typical of a low-nutrient ecosystem where internal generation and recycling of nutrients is important and population turnover is rapid. This is consistent with the relatively low nutrient input to Western Port from the catchment or exchange with the waters of Bass Strait, which are classified as oligotrophic (low nutrient content).

Table 4-1. Phytoplankton species comprising 1 per cent or more of the population

Class	Species	% total
Diatom (planktonic)	Asterionellopsis glacialis	9.9
Diatom (planktonic)	Skeletonema costatum	9.6
Diatom (planktonic)	Thalassiosira cf. mala	8.1
Diatom (planktonic)	Chaetoceros spp.	6.7
Cryptophyte	Plagioselmis prolonga	6.1
Cryptophyte	Hemiselmis spp.	6.0
Dinoflagellate	Gymnodinium spp.	6.0
Diatom (benthic/planktonic)	Cylindrotheca closterium	5.2
Prymnesiophyte	Chrysochromulina spp.	4.6
Chrysophytes	Ochromonas spp.	4.4
Prasinophyte	Pyramimonas spp.	4.2
Prymnesiophyte	Emiliania huxleyi	3.8
Euglenophyta	Eutreptiella spp.	1.8
Prasinophyte	Nephroselmis pyriformis	1.7
Diatom (benthic)	Nitzschia spp.	1.7
Cryptophyte	Teleaulax acuta	1.5
Dinoflagellate	Heterocapsa rotundata	1.4
Diatom (planktonic)	Dactyliosolen fragilissimus	1.2
Diatom (planktonic)	Bacteriastrum spp.	1.1

Table 4-2 summarises monthly results of the sampling program and shows phytoplankton abundance (cells/litre) by taxonomic class and month. Table 4-2 is colour coded to show the abundance of different classes relative to the range of abundances over the program period. The maximum abundance for any class of phytoplankton was 420,000 cells/litre, while the minimum was 42 cells/litre. The median was 24,000 cells/litre.

The table shows that the dominant group of phytoplankton in each survey were the Diatoms (Baccillariophyceae). Diatoms comprised 54 per cent of the phytoplankton on average and 76 taxa have been identified. Diatoms are autotrophic (photosynthetic) and have golden-brown photosynthetic pigments. The diatoms comprised both planktonic (48 per cent of the total) and benthic species (6 per cent).

The next most abundant group of phytoplankton were Cryptophytes (Cryptophyceae). Cryptophytes comprised 14 per cent of the phytoplankton over the course of the program and 7 taxa have been identified. They are predominantly autotrophic and may be red, brown, blue or green in colour depending on the accessory pigments in their chloroplasts.

Prymnesiophytes (Prymnesiophyceae) and Dinoflagellates (Dinophyceae) each comprised 9 per cent of the phytoplankton. Seven prymnesiophyte taxa and 38 dinoflagellate taxa have been identified. The dinoflagellates included both autotrophic and heterotrophic species. Most of the prymnesiophytes were *Chrysocromulina* sp. which is a mixotrophic species.

Table 4-2. Summary of phytoplankton concentrations (cells/Litre) per month

	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	Total	
n (samples)	6	6	5	6	6	6	6	6	6	6	6	6	6	77	
Total Phytoplankton	4.2E+05	3.4E+05	3.2E+05	3.6E+05	3.3E+05	3.6E+05	4.7E+05	4.9E+05	5.8E+05	6.1E+05	3.1E+05	3.3E+05	2.6E+05	5.2E+06	
Taxa (per survey)	78	75	69	76	79	83	76	67	66	67	85	82	61	150	
Phytoplankton Classes (number of species)														Total	%
Diatoms (76)	2.3E+05	1.6E+05	1.6E+05	2.1E+05	1.4E+05	2.1E+05	2.4E+05	2.6E+05	3.3E+05	4.2E+05	1.7E+05	1.5E+05	1.4E+05	2.8E+06	54
Cryptophytes (7)	4.7E+04	4.6E+04	4.7E+04	4.4E+04	6.1E+04	4.8E+04	9.2E+04	7.1E+04	9.3E+04	7.2E+04	4.3E+04	5.5E+04	2.4E+04	7.4E+05	14
Prymnesiophytes (7)	4.6E+04	4.0E+04	3.7E+04	3.2E+04	3.3E+04	2.8E+04	3.5E+04	4.4E+04	4.4E+04	3.8E+04	3.0E+04	3.4E+04	2.3E+04	4.6E+05	9
Dinoflagellates (38)	4.2E+04	4.2E+04	4.1E+04	2.9E+04	3.9E+04	2.4E+04	3.9E+04	4.6E+04	4.8E+04	2.7E+04	1.8E+04	3.4E+04	3.5E+04	4.6E+05	9
Prasinophytes (marine) (6)	3.3E+04	2.2E+04	2.0E+04	2.4E+04	2.9E+04	3.0E+04	4.1E+04	2.6E+04	3.6E+04	1.8E+04	2.3E+04	2.5E+04	1.3E+04	3.4E+05	7
Chrysophytes (4)	1.8E+04	1.6E+04	1.2E+04	1.3E+04	1.4E+04	1.4E+04	1.7E+04	3.2E+04	2.3E+04	2.7E+04	1.1E+04	1.6E+04	2.5E+04	2.4E+05	5
Euglenophyta (1)	9.4E+03	5.5E+03	4.2E+03	7.7E+03	6.5E+03	9.3E+03	8.0E+03	8.1E+03	6.0E+03	7.7E+03	9.0E+03	5.7E+03	4.0E+03	9.1E+04	2
Dictyochophytes (3)	5.8E+02	6.7E+02	1.2E+03	1.0E+03	2.1E+03	4.2E+02	5.3E+03	7.5E+02	4.8E+03		3.8E+03	3.0E+03	8.3E+01	2.4E+04	0.5
Raphidophytes (2)				9.2E+02	8.3E+02	3.3E+02		2.5E+02			8.3E+01	8.3E+01		2.5E+03	0.05
Ciliate (1)	4.2E+01			8.3E+01	8.3E+01	8.3E+01	8.3E+01			2.5E+02	4.2E+02	3.3E+02		1.4E+03	0.03
Chlorophytes (1)					1.3E+03									1.3E+03	0.03
Cyanoprokaryota (5)										1.3E+03				1.3E+03	0.02
Other (1)					8.3E+01	3.3E+02	4.2E+02							8.3E+02	0.02

<25th percentile (< 4700 cells/litre)	25th-50th percentile (4,700 to 23,800 cells/litre)
50th-75th percentile (23,800 to 42,000 cells litre)	>75th percentile (> 42,000 cells/litre)

Prasinophytes (Prasinophyceae) comprised 7 per cent of the phytoplankton with 6 taxa identified. Prasinophytes are mostly autotrophic and are green algae (having green pigments). Chrysophytes (Chrysophyceae) comprised 5 per cent of the phytoplankton with 4 taxa identified. The Chrysophytes include both autotrophic and mixotrophic species. Euglenophytes (Euglenophyceae) comprised 2 percent of the phytoplankton with just 1 taxon identified (*Eutreptiella*), a green-pigmented mixotrophic species. The remaining phytoplankton classes each comprised less than 0.5 per cent of the phytoplankton by number in each month since December 2018.

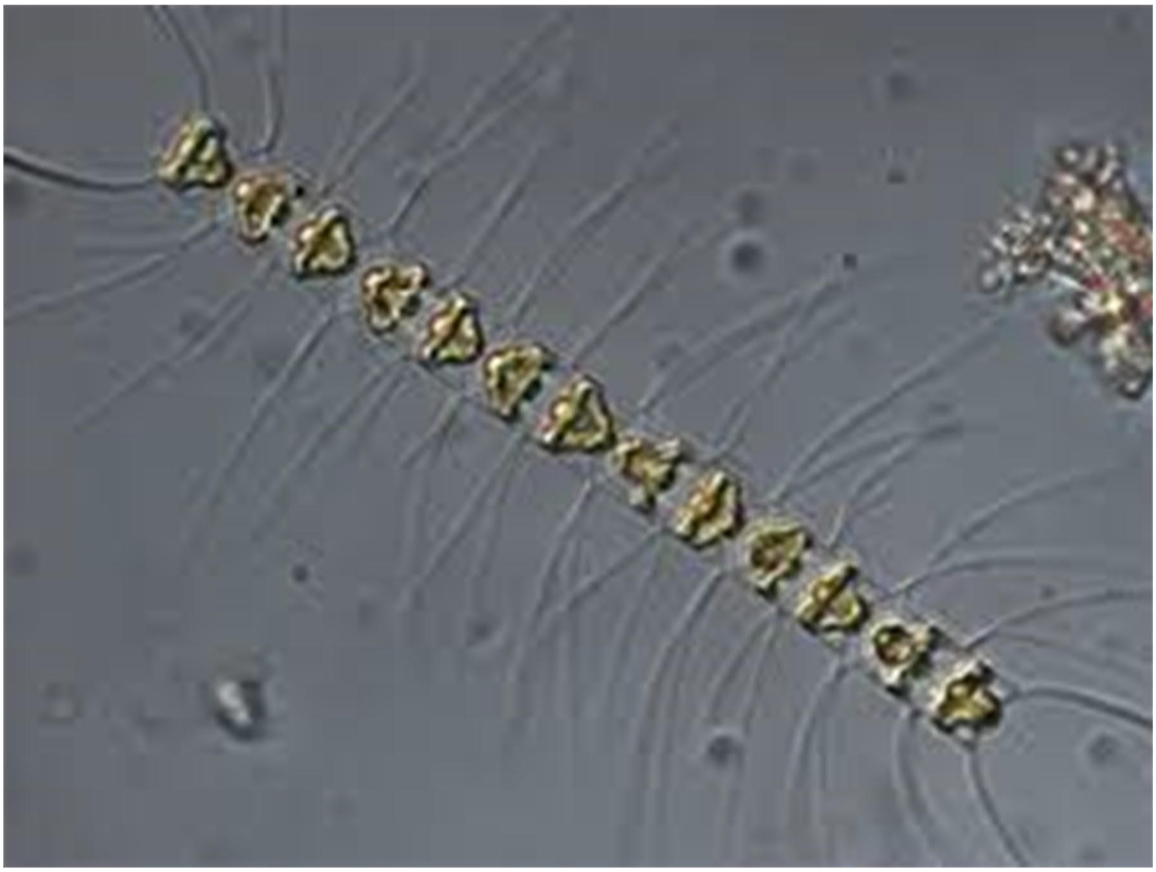

Figure 4-1. Chain forming diatom

Figure 4-2 shows that most of the month to month variability is due to *changes* in the abundance of the top four planktonic diatom species: *Asterionellopsis*, *Skeletonema*, *Thalassiosira* and *Chaetoceros*. These four species comprised 35% of the total phytoplankton on average. All are small, chain-forming species. Their small cell size allows for efficient nutrient uptake and makes them resistant to mechanical damage in the strong mixing conditions. Chain formation and spines on *Chaetoceros* cells control sinking rates, thereby helping these phytoplankton to remain suspended in the water column. Changes in the abundance of the other species are small relative to the diatoms.

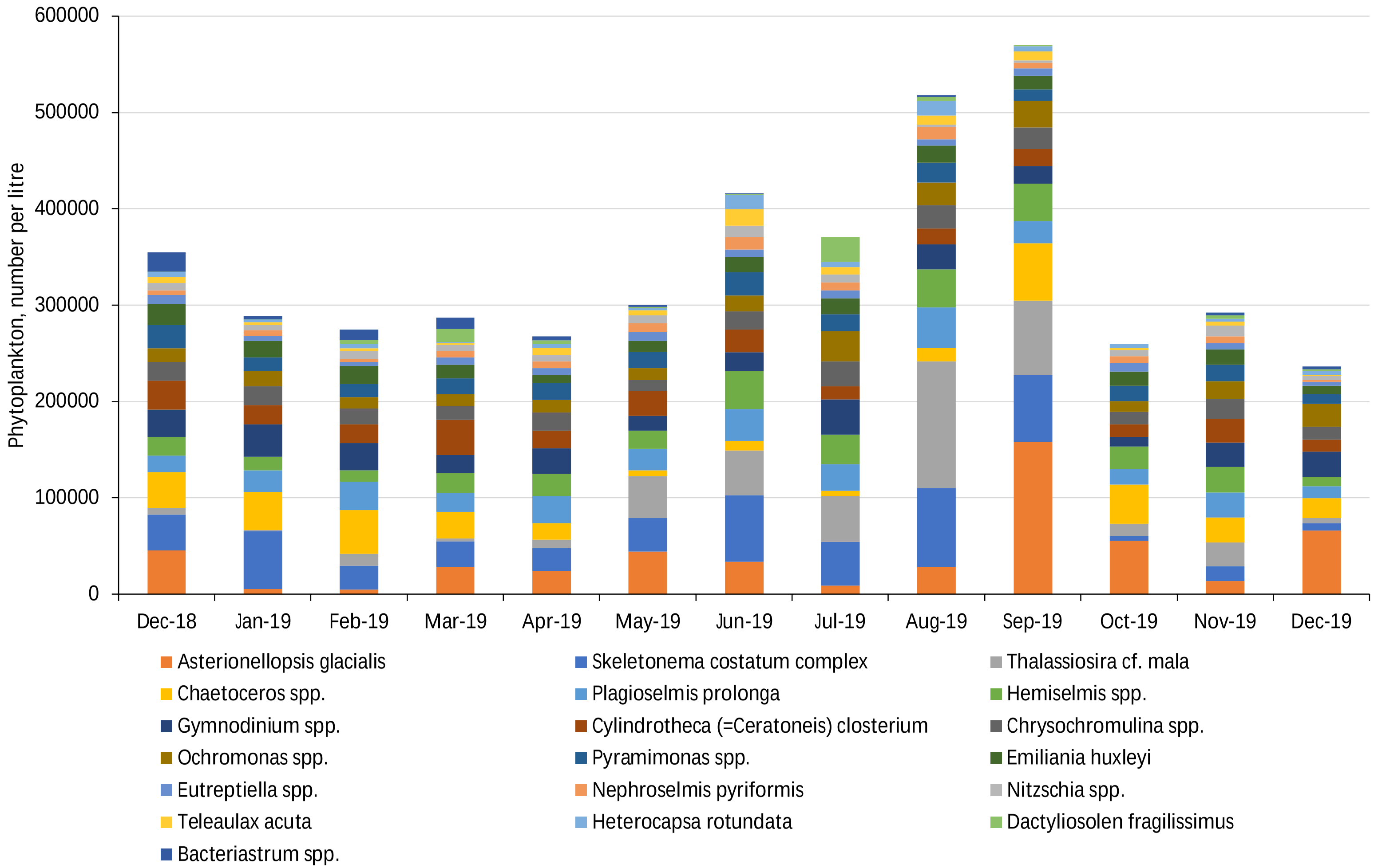

Figure 4-2. Average abundance of species > 1 % of total phytoplankton

4.1 Phytoplankton biomass (Chlorophyll-a)

Figure 4-3 compares monthly phytoplankton numbers to measured chlorophyll-a concentration (a measure of phytoplankton biomass) as well as solar exposure and rainfall for the preceding month. Average chlorophyll-a concentrations in Lower North Arm show minor correlation with observed patterns in average phytoplankton numbers over the period. Chlorophyll-a concentrations and phytoplankton numbers were stable from December 2018 to May 2019. Chlorophyll-a concentrations fell to their lowest in June 2019, despite there being relatively high phytoplankton numbers. This is consistent with phytoplankton down-regulating chlorophyll-a levels during the low-light availability in June. Chlorophyll-a concentrations increased steadily after the winter solstice to September 2019, consistent with a ‘spring bloom’ in phytoplankton biomass. The September peak and October trough are consistent between phytoplankton abundance and biomass – attributed to increased grazing and lower rainfall (lower nutrient input) in September.

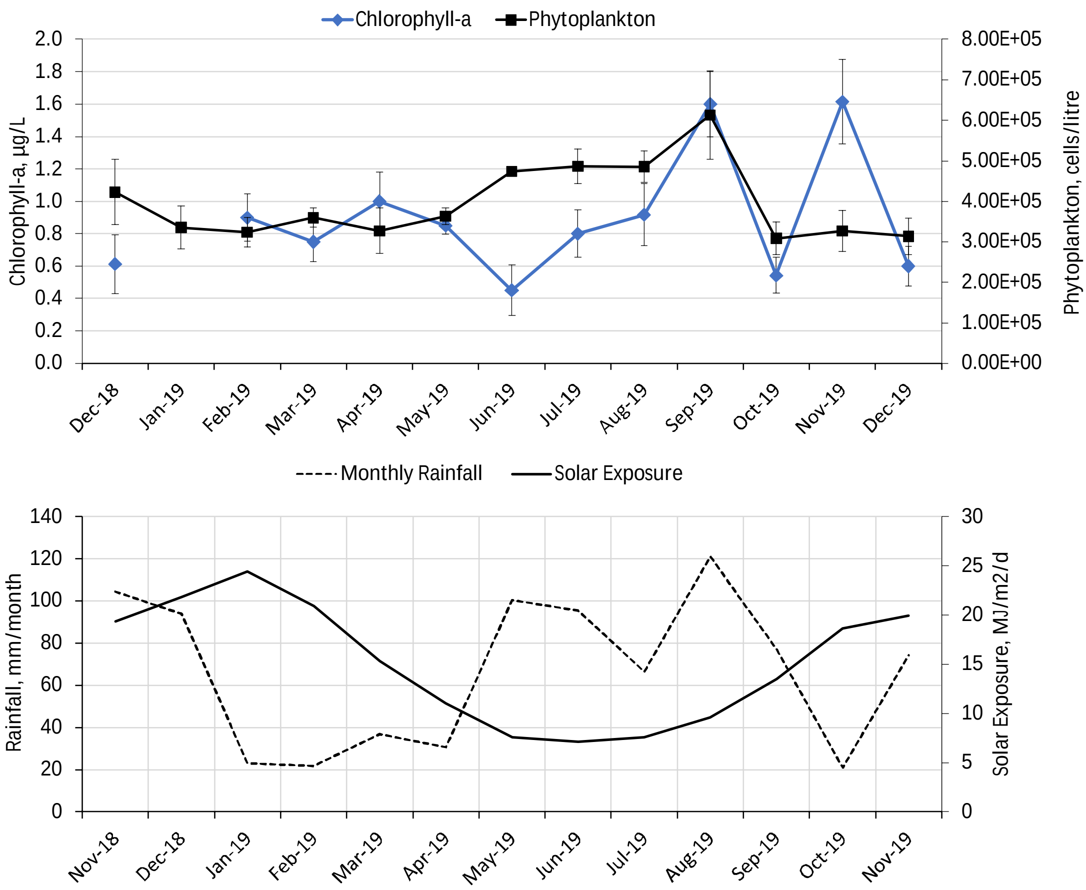

Figure 4-3. Monthly average chlorophyll-a and phytoplankton, Dec-2018 to Dec-2019

4.2 Phytoplankton species richness

Figure 4-4 shows the average number of phytoplankton species per sample in each month, and average phytoplankton abundance. Figure 4-5 shows data for individual sites along with average total phytoplankton abundance. The average number of taxa per sample has ranged from 35 to 45. The highest number of taxa was recorded in May 2019, and the lowest in October and December 2019. There is no clear seasonal trend in the average number of taxa. The maximum change in the number of species between surveys is 19 per cent, and overall variation is only 22 per cent. Increases and decreases in the number of phytoplankton species generally matched increases and decreases in phytoplankton abundance from December 2018 to May 2019, but there is poor correlation from June to December 2019.

Figure 4-5 also shows that there were no consistent differences in the number of species per site and Crib Point Berth 2 had a similar number of phytoplankton species to all other sites.

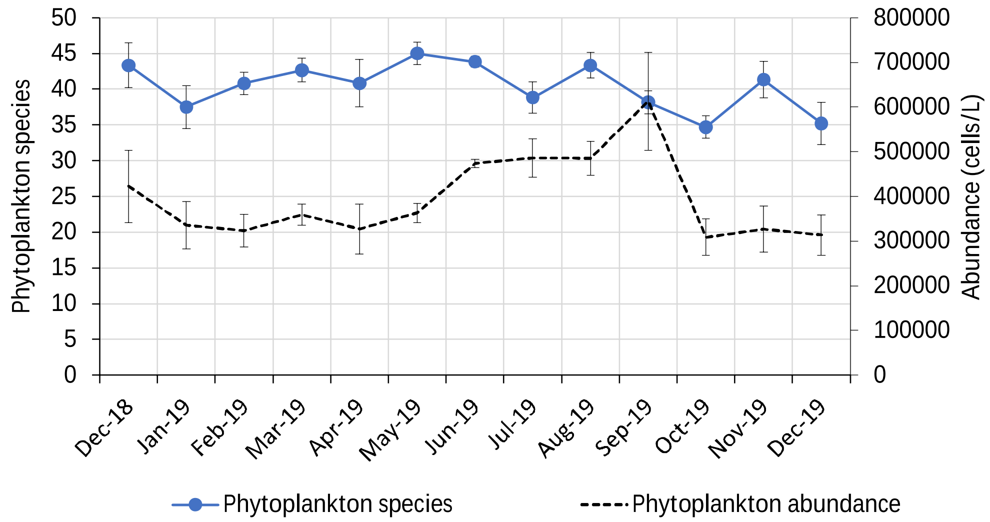

Figure 4-4. Monthly average phytoplankton species per sample, Dec-2018 to Dec-2019
(*Mean ± SEM*)

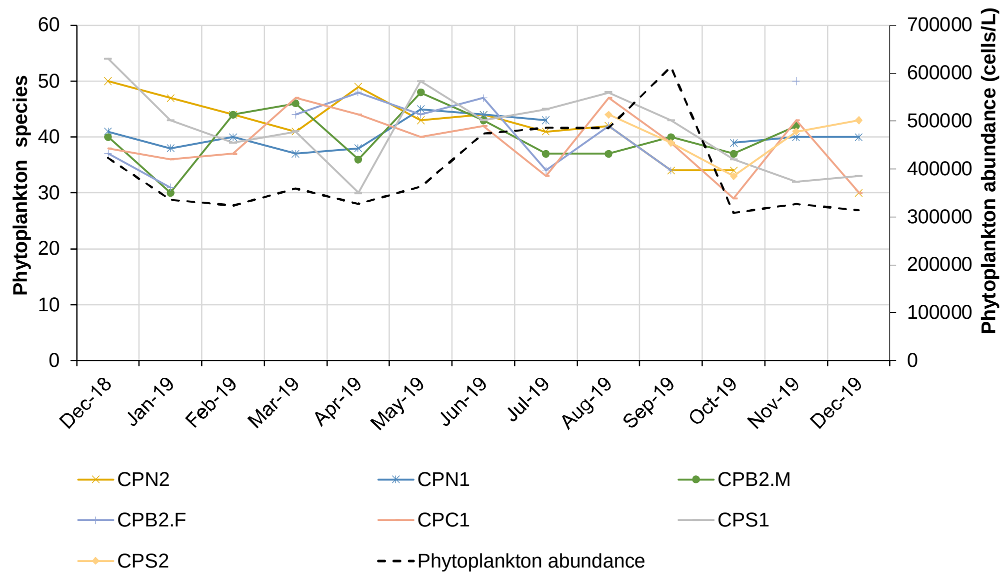

Figure 4-5. Phytoplankton species per site and month

4.3 Planktonic Diatoms

Planktonic diatoms have comprised 49 per cent of the phytoplankton in North Arm. Figure 4-6 shows the average abundance of planktonic diatoms in each survey since December 2018. Patterns match those of the total phytoplankton – variation in planktonic diatoms appears to account for most of the variation in the phytoplankton community. The abundance of planktonic diatoms was relatively stable from December 2018 to June 2019, ranging between 115,000 cells/litre and 200,000 cells/litre. As daylength began to increase in July the abundance of planktonic diatoms showed a steady increase until September 2019 when there were around 400,000 cells/litre. Numbers fell by more than 60 per cent in October however, likely due to predation by copepods and decreased rainfall.

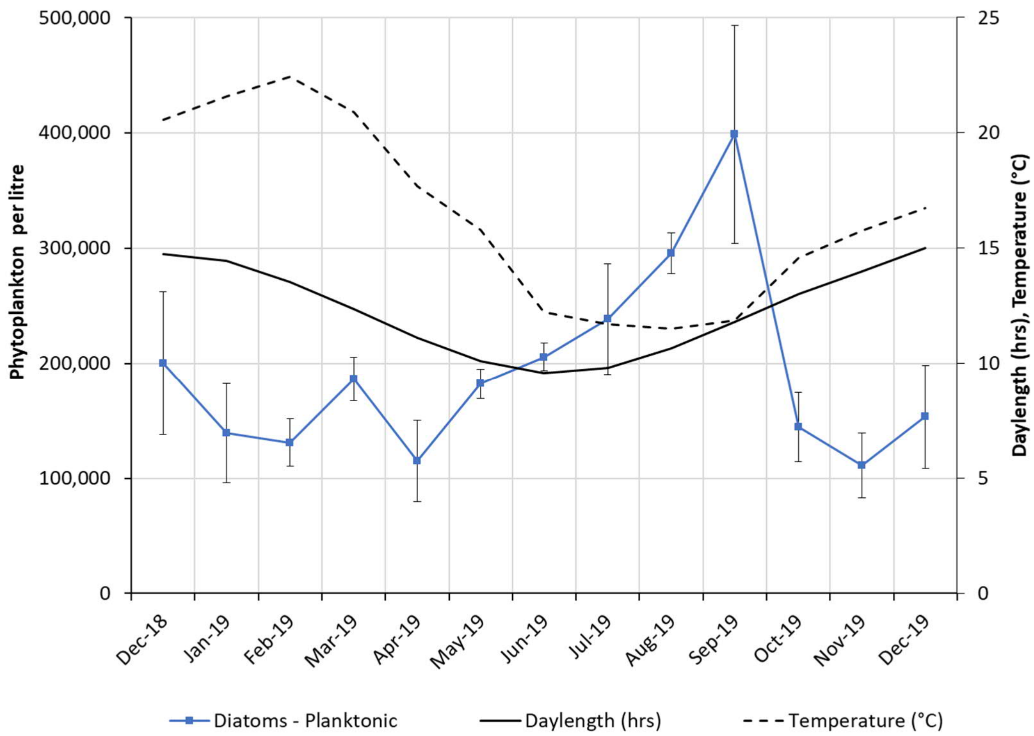

Figure 4-6. Average planktonic diatoms per sample, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.4 Other phytoplankton classes

Figure 4-7 shows abundance of the other major classes of phytoplankton. These were all around half as abundant as the planktonic diatoms. Several classes show a peak in abundance in winter, after the winter solstice in June when solar exposure begins to increase (Figure 4-8).

Cryptophyte abundance (14.5 per cent of total phytoplankton) was relatively stable from December 2018 to May 2019 between 45,000 cells/litre and 60,000 cells/litre, before increasing to over 90,000 cells/litre in June 2019. Cryptophyte numbers remained high until September 2019 when they decreased to a little over 40,000 cells/litre in October 2019.

Numbers of Chrysophytes were around 65,000 cells/litre in December 2018 but steadily decreased to around 40,000 cells/litre in May 2019. Their numbers increased to a peak in July 2019 of 75,000 cells/litre.

Numbers of Dinoflagellates and Prymnesiophytes showed a minor peak in late winter 2019, as well as a marked decrease in abundance in October 2019.

Abundances of benthic diatoms (around 10 per cent of the phytoplankton), Prasinophytes and Euglenophytes have shown little variability and no seasonal trend.

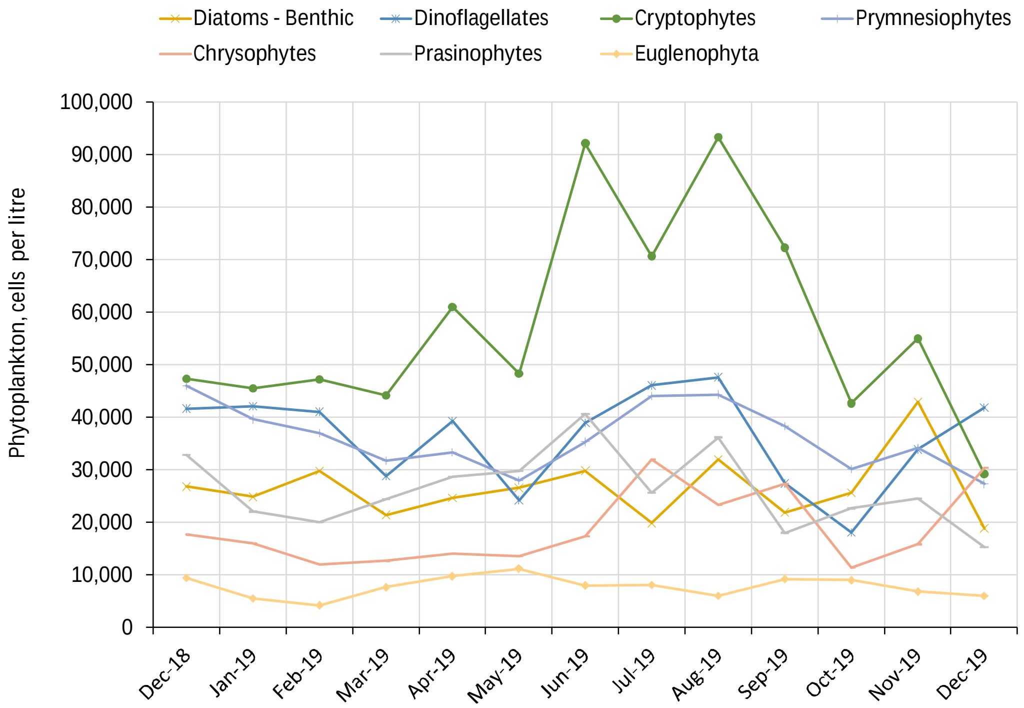

Figure 4-7. Average abundance of major classes, Dec-2018 to Dec-2019

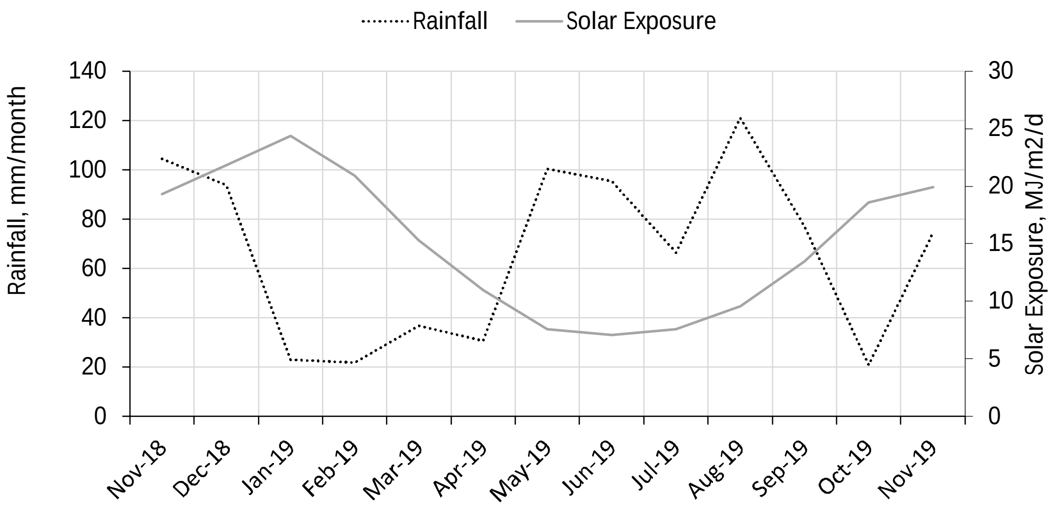

Figure 4-8. Rainfall and solar exposure Dec-2018 to Dec-2019

4.5 General Spatial patterns – Lower North Arm

The figures that follow illustrate spatial patterns in the phytoplankton in Lower North Arm. The distances shown are the distance from Bass Strait measured from a line between West Head and Point Grant along the centre of the channel. As just three surveys during the 'spring bloom'

from August to October have been completed at site CPS2 in the confluence zone, data for site CPS2 is left out of some charts. Data for all sites during August to October are plotted separately where appropriate. Data for the monthly and fortnightly at Crib Point Berth 2 are plotted separately.

Figure 4-9 shows that phytoplankton concentrations are typically higher in the north of Lower North Arm (CPN2 averages 465,000 cells/litre) than the south (CPS1 averages 350,000 cells/litre). Crib Point Berth 2 shows intermediate phytoplankton abundance.

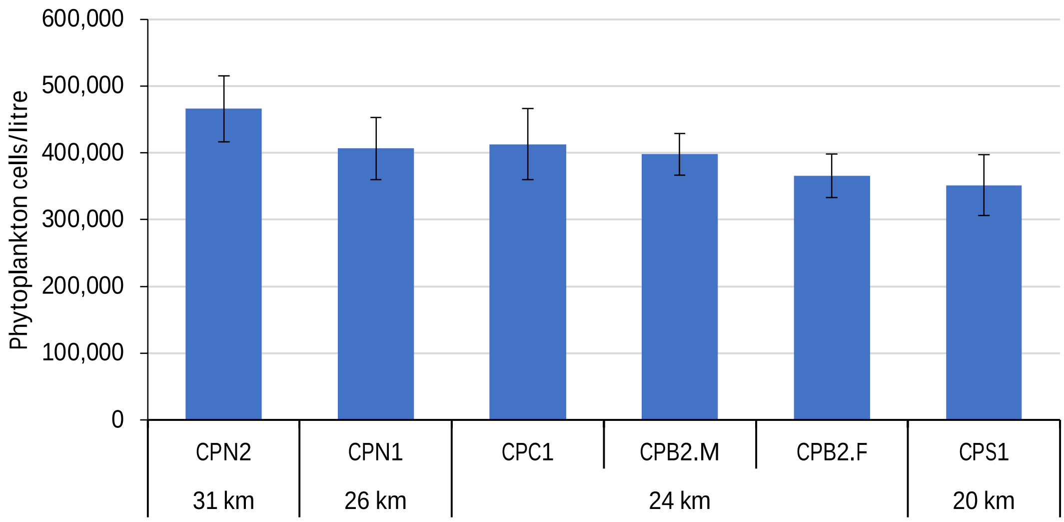

Figure 4-9. Average total phytoplankton abundance per site, Dec-2018 to Dec-2019
(*Mean* ± *SEM*)

Figure 4-10 shows average total phytoplankton abundance for the five months when site CPS2 in the confluence zone has been sampled (CPN1 was only sampled three times in this period). There is a large amount of spatial variability (between sites) but temporal variability (within sites) is also high. Overall there is no clear spatial pattern. Phytoplankton abundance at CPS2 was similar to other sites.

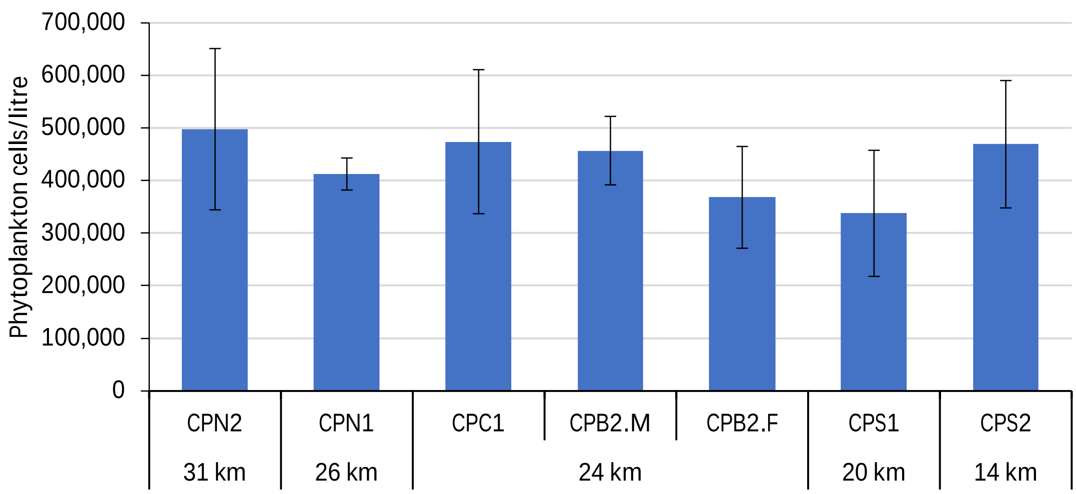

Figure 4-10. Average total phytoplankton per site, Aug-2019 to Dec-2019
(*Mean* ± *SEM*)

Figure 4-11 shows the spatial variation in average phytoplankton biomass estimated by chlorophyll-a concentrations. Average Chlorophyll-a varied from 0.8 µg/L at CPC1 to 1.05 µg/L at CPS1. Based on these data, the middle of Lower North Arm has lower phytoplankton biomass than its extreme north and south, however error bars indicate there are no statistically significant differences between sites.

The annual range in phytoplankton biomass values is similar to that for southern Port Phillip Bay sites reported in the 1990s (Beardall *et al*. 1996).

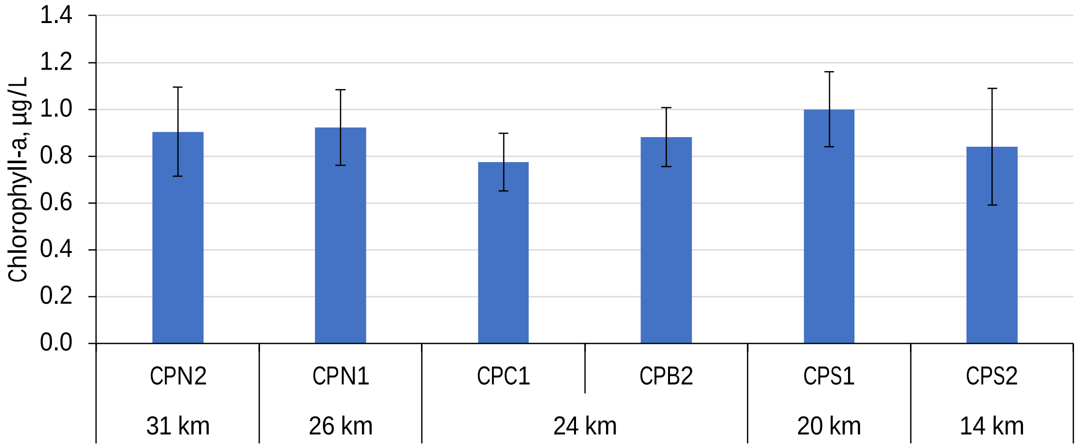

Figure 4-11. Average chlorophyll-a concentrations, Dec-2018 to Dec-2019
(*Mean ± SEM*)

Figure 4-12 shows that there is no consistent difference in the number of species between sites in the north and south of Lower North Arm. There are typically between 38 and 43 species per sample at each site (40 per sample at Crib Point Berth 2). Figure 4-5 showed that species numbers at each site were similar throughout the year.

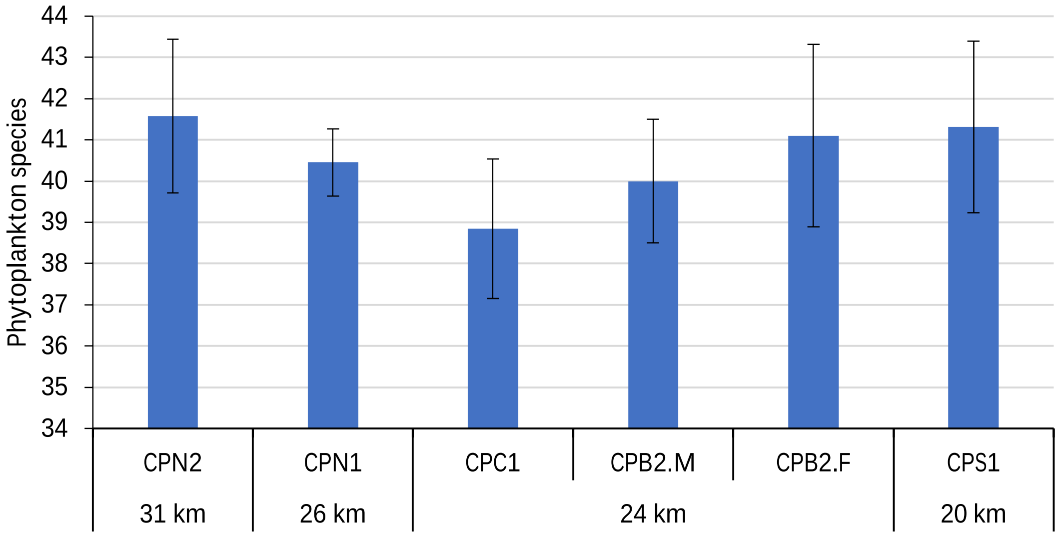

Figure 4-12. Average phytoplankton species per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

Figure 4-13 shows that for surveys including site CPS2 in the confluence zone there is no spatial pattern in the number of species per site. This sampling period during the 'spring bloom' did however have a slightly lower average number of species than for all surveys.

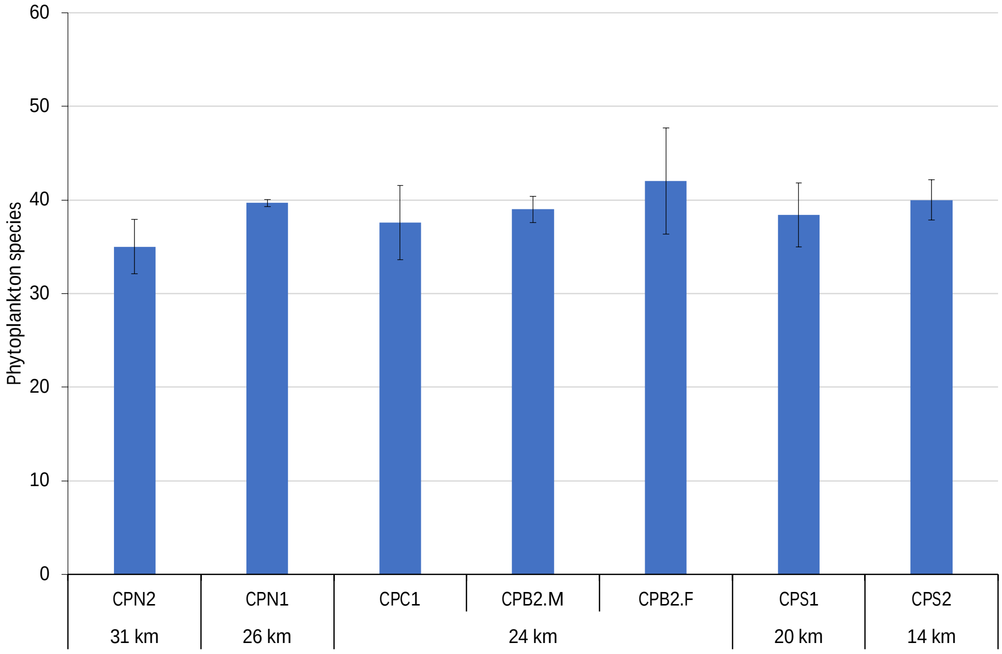

Figure 4-13. Average phytoplankton species per site, Aug-2019 to Dec-2019
(*Mean ± SEM*)

4.5.1 Diatoms

Figure 4-14 shows the abundance of the diatoms, the dominant group of phytoplankton. The figure plots planktonic (49% of the phytoplankton) and benthic diatoms (6% of the phytoplankton) separately. Planktonic diatoms were more abundant at CPN2 than sites near Crib Point Jetty and CPS1 in most surveys. Benthic diatoms were also more abundant in the north of Lower North Arm than the south, consistent with the large areas of mudflats in the north. The longer residence times of water in the north of Lower North Arm may support higher diatom abundance through recycling of nutrients within the plankton.

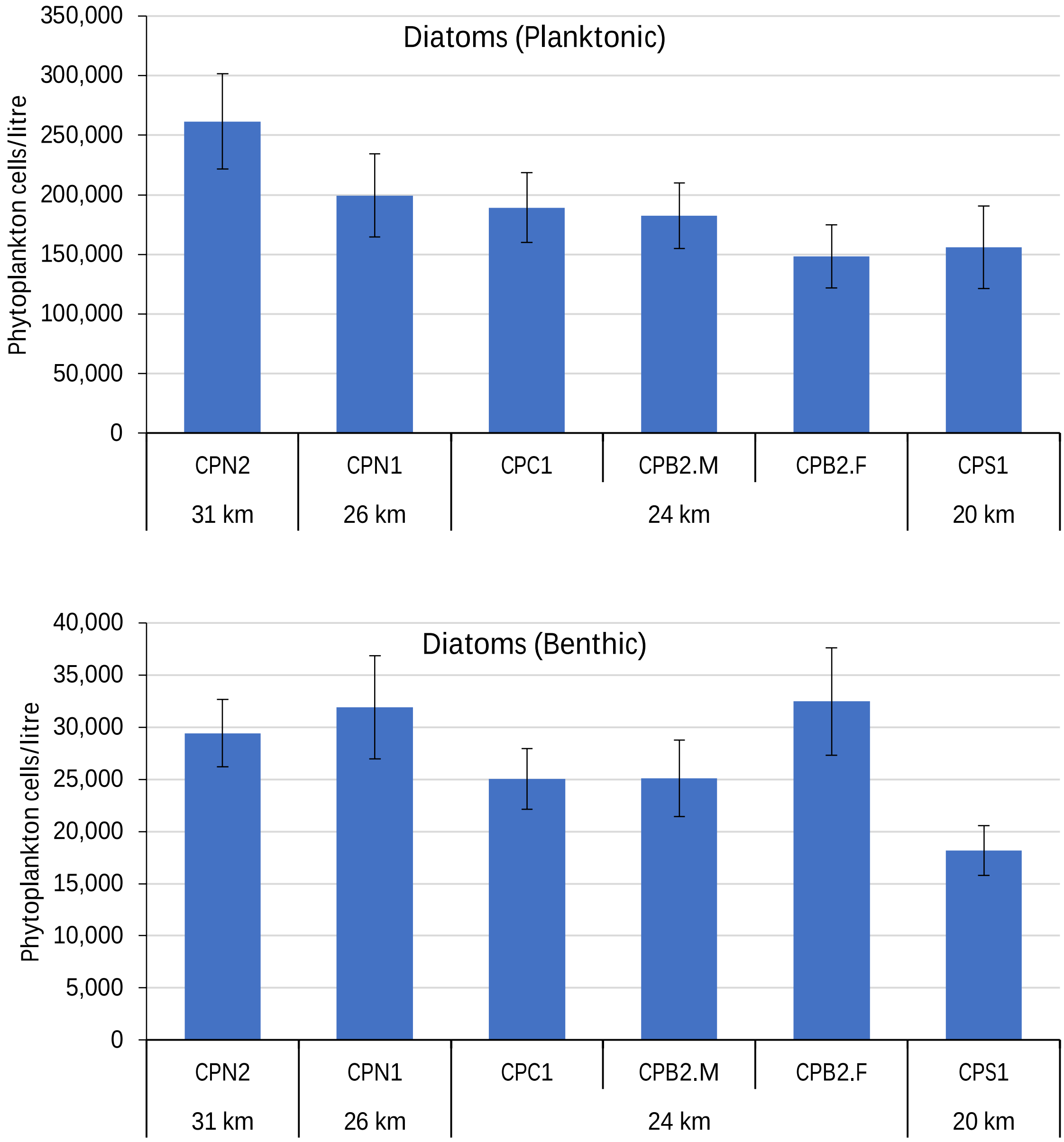

Figure 4-14. Planktonic and benthic diatom abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.2 Cryptophytes

Figure 4-15 shows there were no differences in Cryptophyte abundance from the north to the south of Lower North Arm and that abundances at Crib Point Berth 2 are similar to those elsewhere. Cryptophyte abundance at CPS2 in the confluence zone for the August-October 2019 surveys were similar to those in Lower North Arm.

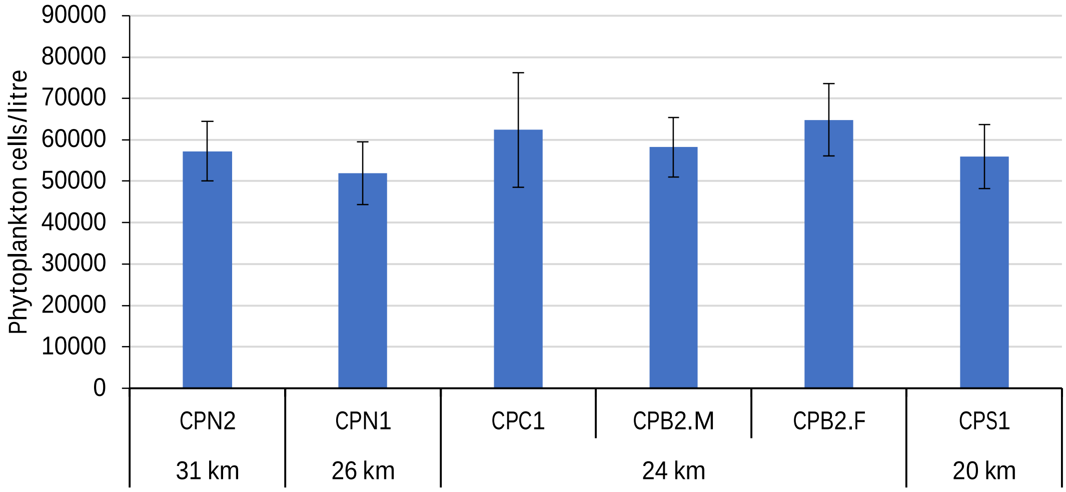

Figure 4-15. Cryptophyte abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.3 Dinoflagellates

Figure 4-16 shows that Dinoflagellates were more abundant in the south of Lower North Arm (around Crib Point and CPS1) than the sites further north (CPN1 and CPN2). For months when CPS2 was included in the sampling, dinoflagellate abundances at CPS2 were similar to the south of Lower North Arm.

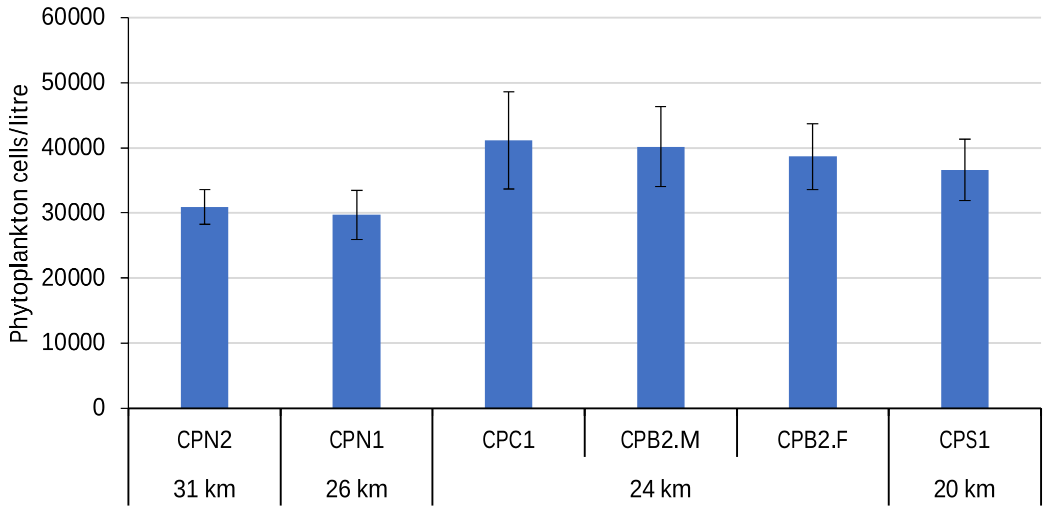

Figure 4-16. Dinoflagellate abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.4 Prymnesiophytes

Figure 4-17 shows no pattern in Prymnesiophyte abundance relating to location in the Lower North Arm. The fortnightly surveys at Crib Point Berth 2 have found lower Prymnesiophyte abundance than monthly surveys at all other sites.

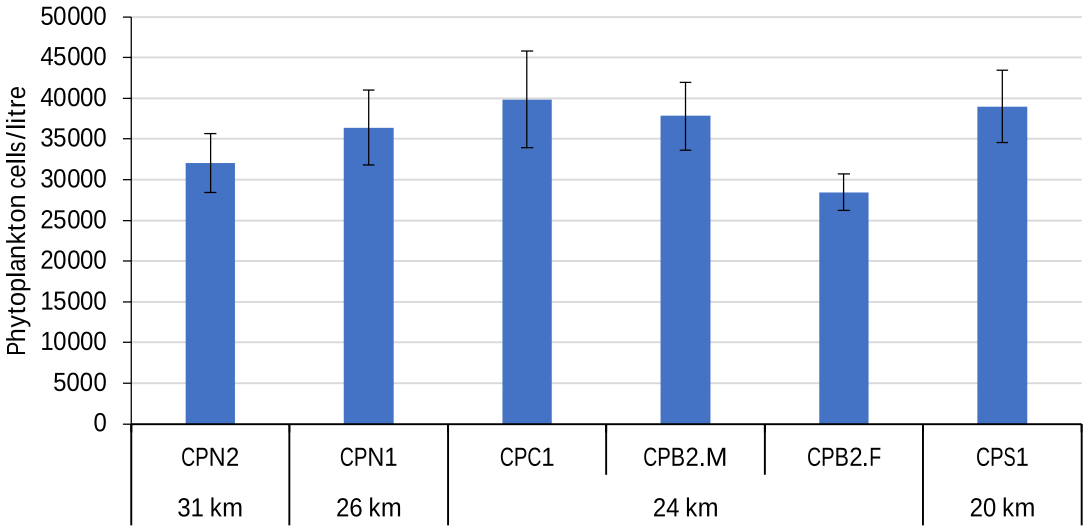

Figure 4-17. Prymnesiophyte abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.5 Prasinophytes

Figure 4-18 shows that Prasinophytes abundance tends to be higher the north of Lower North Arm (29,000 cells/litre) than the south (21,000 cells/litre) and that numbers Crib Pt Berth 2 are consistent with this pattern.

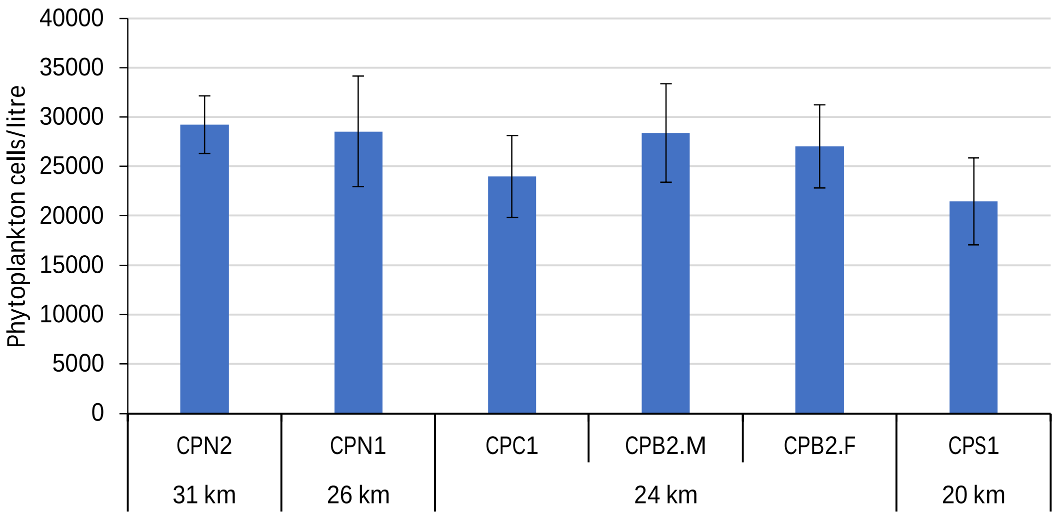

Figure 4-18. Prasinophyte abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.6 Chrysophytes

Figure 4-19 shows that patterns in Chrysophyte abundance are similar to those of dinoflagellates, with lower and less variable numbers in the north than the south of Lower North Arm. Numbers at Crib Point Berth 2 are consistent with this pattern, numbers were higher at CPC1, but with a large error.

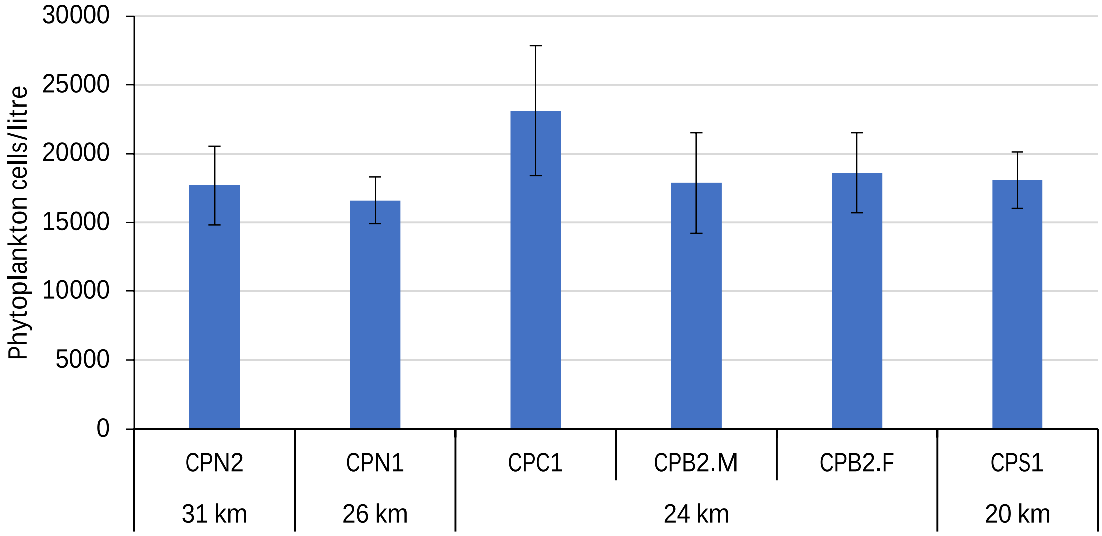

Figure 4-19. Chrysophyte abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.7 Euglenoids

Figure 4-20 shows that Euglenoid abundance is higher at the north sites of Lower North Arm, a pattern also observed in Diatoms and Prasinophytes.

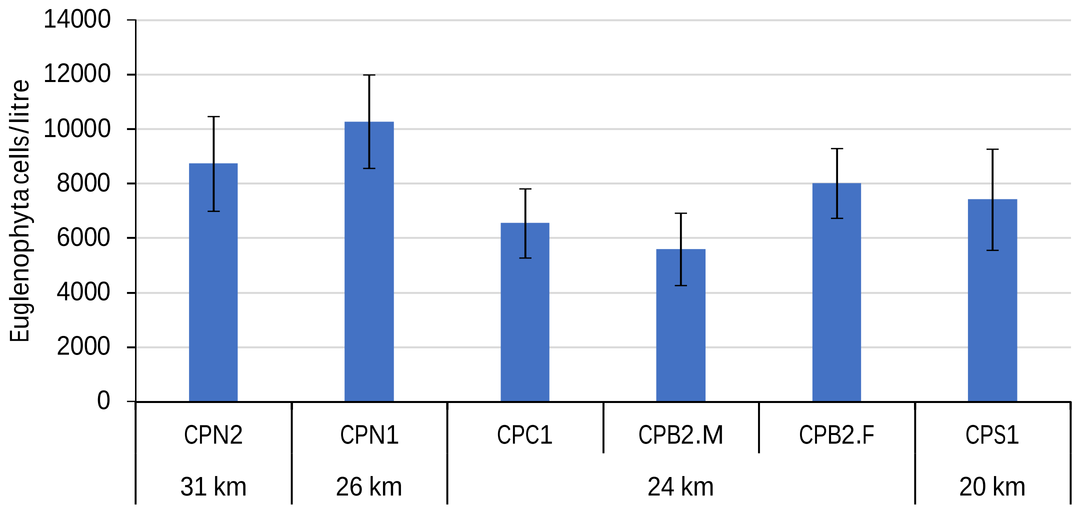

Figure 4-20. Euglenophyte abundance per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

4.5.8 Broadscale Phytoplankton Survey

A broadscale survey in October 2019 documented phytoplankton abundance and diversity at 10 sites in the Western Entrance and Confluence Zone (CPP5 and CPS2) the Lower North Arm (CPS1 to CPN2) and in the Rhyll (P4, P3) and East Arm (P2, P1) segments. A map of these sites is shown in Figure 3-2.

Figure 4-21 shows the abundance of the key phytoplankton classes. Diatoms were the most abundant class (25%), followed by Cryptophytes (21%), Prymnesiophytes (20%), Dinoflagellates (17%) and Chrysophytes (11%). Other classes accounted for 5% of the phytoplankton or less. This shows a quite different community structure to the average for December 2018 to October 2019 when diatoms comprised 55 per cent of the phytoplankton. Even when the sites in the Rhyll and East Arm segments are excluded, there were many more flagellates in the October broad scale study than the average for the year. Furthermore, diatoms accounted for over 55 per cent of the phytoplankton in the October monthly survey two weeks prior. Evidently there was a shift in the phytoplankton community over the September to October period. Grazing pressure from zooplankton was the highest for the sampling period at this time (see below) and there was a corresponding decrease in overall phytoplankton abundance and biomass.

Phytoplankton abundances varied markedly over the study area from around 100,000 cells/L to over 500,000 cells/L. Abundances were lowest in the confluence zone, south of Lower North Arm and at site P2 in east arm. Abundances were very high in the Rhyll segment owing to high numbers of flagellates including Cryptophytes, Prymnesiophtes, Dinoflagelates and Chrysophytes. Higher numbers were also present in the north of Lower North Arm and site P1 in east arm.

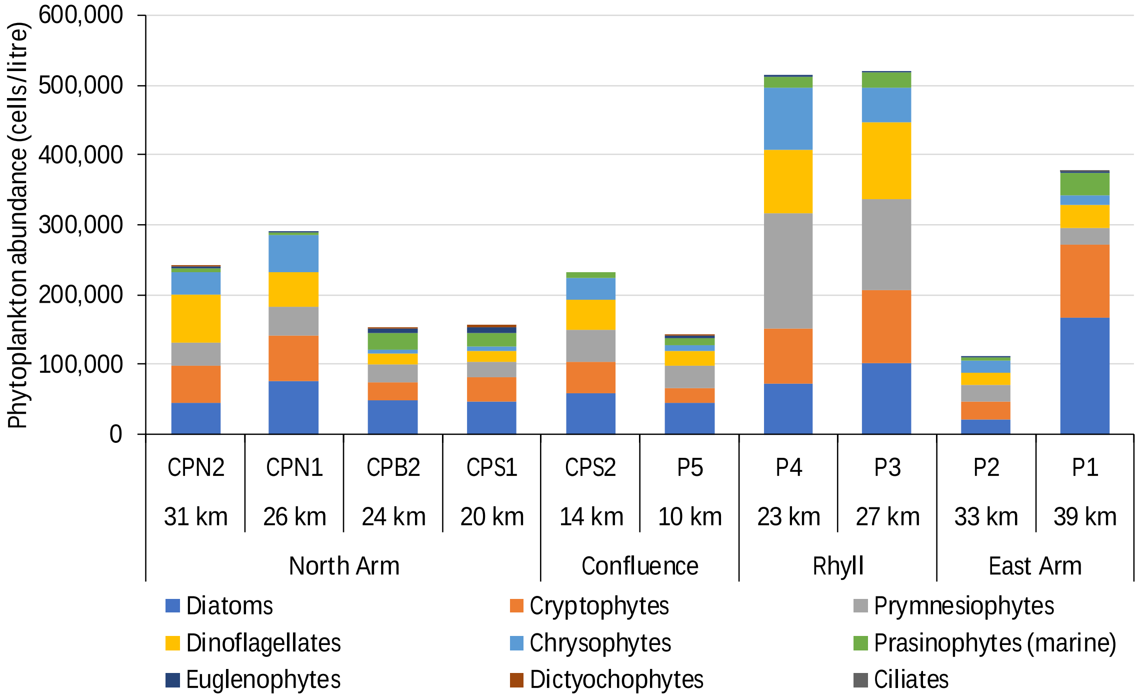

Figure 4-21. Broadscale patterns in of key phytoplankton classes, October 2019

5 References

Beardall J., Roberts S., Royle R. (1996). Phytoplankton in Port Phillip Bay: spatial and seasonal trends in biomass and primary productivity. Yarralumla, ACT: Port Phillip Bay Environmental Study. CSIRO Environmental Projects Office.

CEE (2018a). Plume Modelling of Discharge from LNG Facility at Crib Point, Western Port – AGL Gas Import Jetty Project. Report for AGL.

CEE (2018b). Marine Ecosystem Protected Matters – AGL Gas Import Jetty Project. Report for AGL.

CEE (2018c). Modelling and Assessment of Biological Entrainment – AGL Gas Import Jetty Project. Report for AGL.

CEE (2018d). Assessment of effects of cold-water discharge on marine ecosystem at Crib Point – AGL Gas Import Jetty Project. Report for AGL.

CEE (2018e). Chlorine in FSRU Seawater Processes – AGL Gas Import Jetty Project. Report for AGL.

Hallegraeff G., Beardall J., Brett S., Doblin M., Hosja W., de Salas M. & Thompson P. (2009) Phytoplankton. NCCARF.

Kimmerer, W.J., McKinnon, A.D. (1985). A Comparative Study of the Zooplankton in Two Adjacent Embayments, Port Phillip and Westernport Bays, Australia. Estuarine, Coastal and Shelf Science 21:145-159.

Kimmerer W. and McKinnon A.D. (1987a). Zooplankton in a marine bay.I. Horizontal distributions used to estimate net population growth rates. Mar Ecol Prog Ser 41: 43-52.

Kimmerer W. and McKinnon A.D. (1987b). Zooplankton in a marine bay.II. Vertical migration to maintain horizontal distributions. Mar Ecol Prog Ser 41: 53-60.

Kimmerer W. and McKinnon A.D. (1987c). Growth mortality and secondary production of the copepod Acartia tranteri in Westernport Bay, Australia. Limnol, Oceanogr. 32(1):14-28.

Shapiro M.A. (ed.) (1975) Westernport Bay Environmental Study, 1973 -1974. Environmental Study Series No. 502. Ministry for Conservation, Victoria

Suthers I., Rissik D., Richardson A. (2019). Plankton: A Guide to their Ecology and Monitoring for Water Quality. Second Edition. Published by CSIRO. Australia.

Victorian Fisheries Authority (Accessed 2019). Marine Biotoxin Management Plan. 5th addition. August 2015. Avaliable at: https://vfa.vic.gov.au/aquaculture/publications/shellfish-quality-asurance/marine-biotoxin-management-plan

6 Appendices

6.1 Additional charts

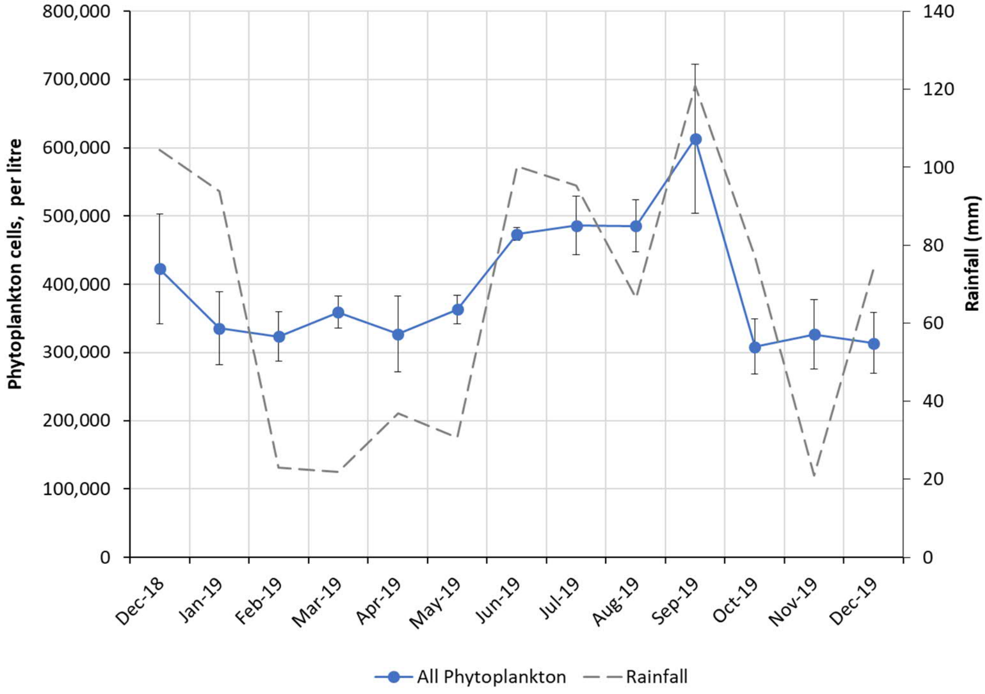

Figure 6-1. Monthly average phytoplankton abundance, Dec-2018 to Dec-2019
(*Mean ± SEM*)

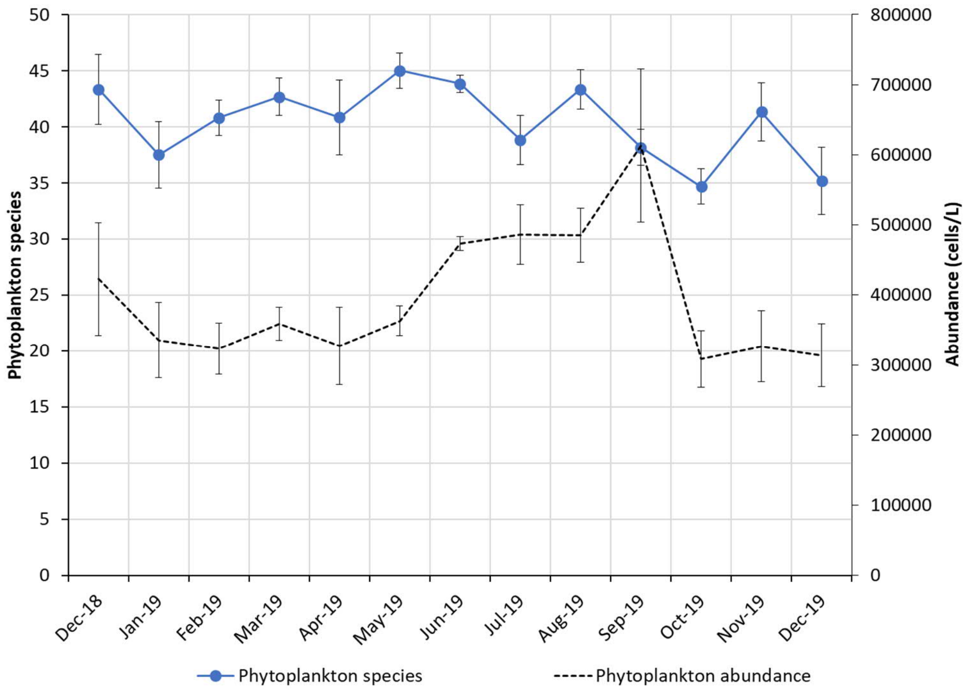

Figure 6-2. Average number of phytoplankton species over time
(*Mean ± SEM*)

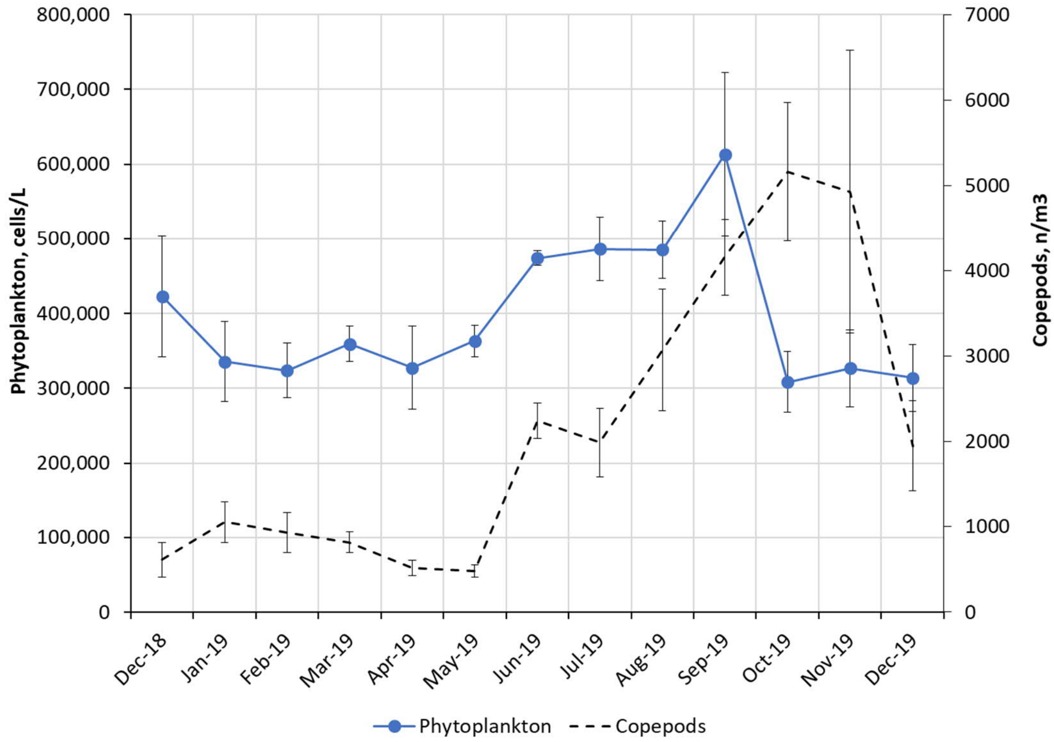

Figure 6-3. Monthly phytoplankton and copepod abundance
(*Mean ± SEM*)

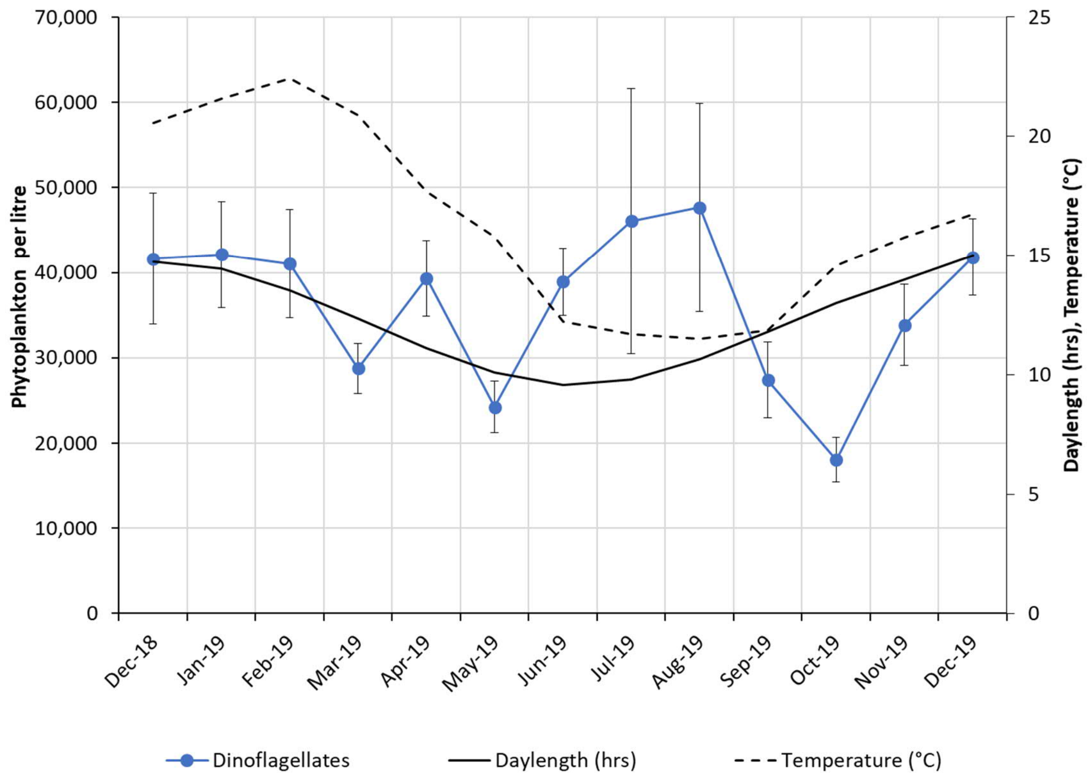

Figure 6-4. Monthly Dinoflagellate abundance
(*Mean ± SEM*)

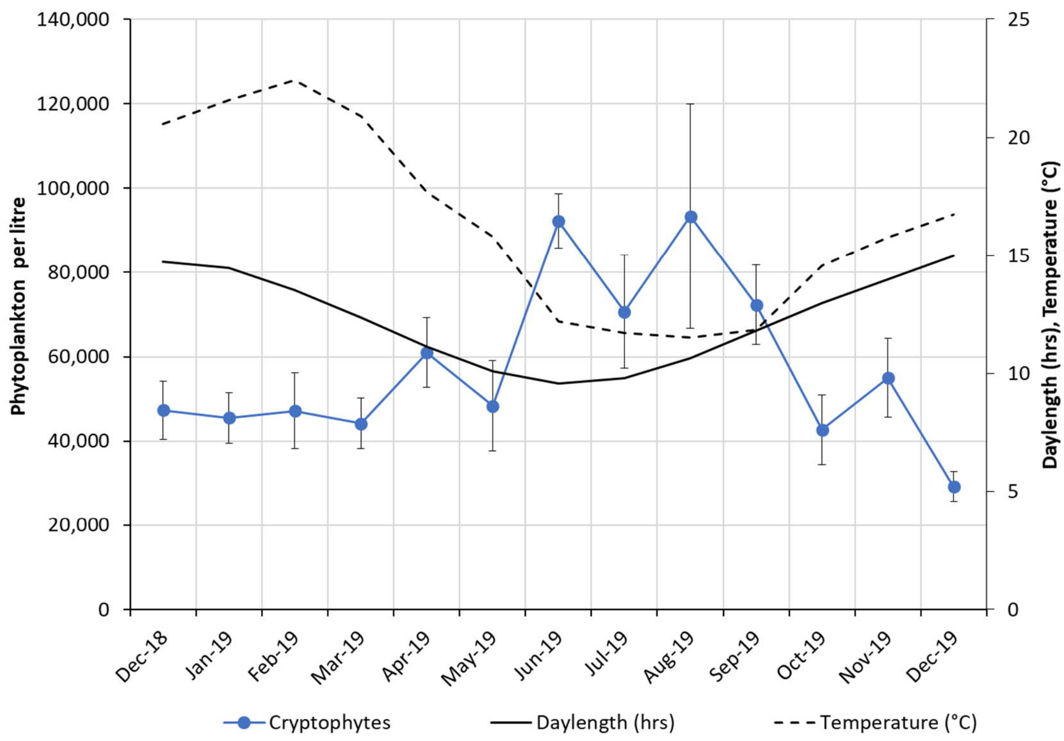

Figure 6-5. Monthly Cryptophyte abundance
(*Mean ± SEM*)

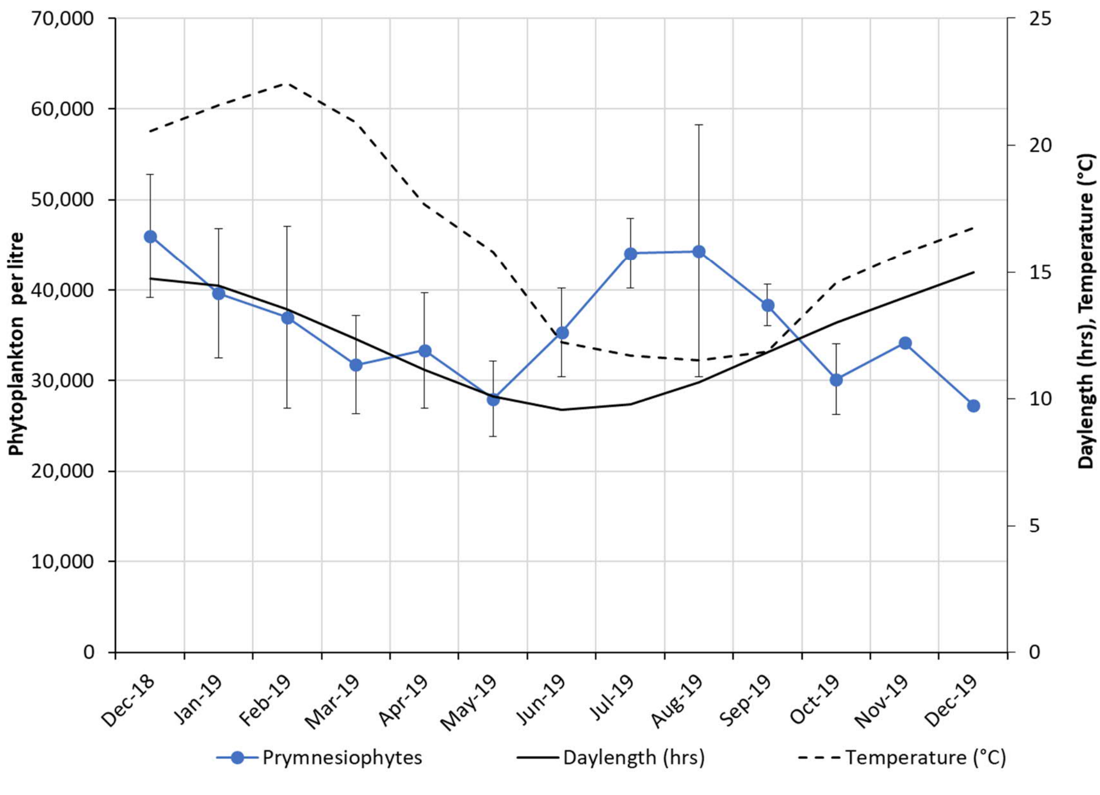

Figure 6-6. Monthly Prymnesiophyte abundance
(*Mean ± SEM*)

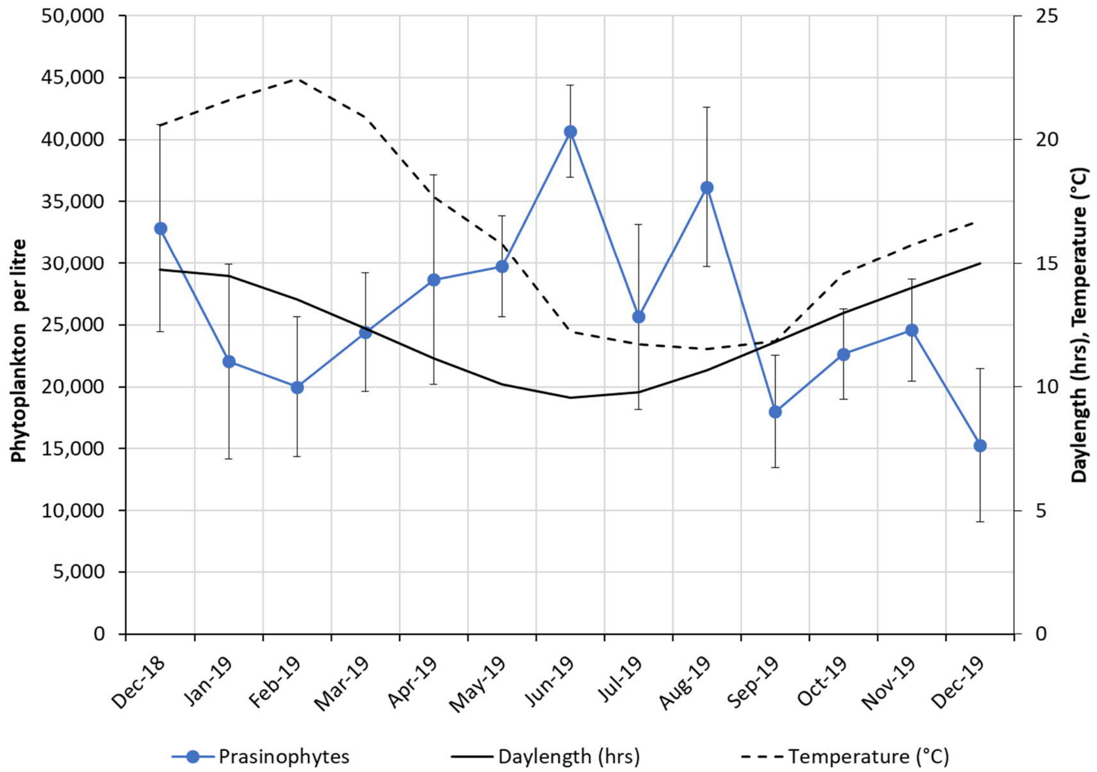

Figure 6-7. Monthly Prasinophyte abundance
(*Mean ± SEM*)

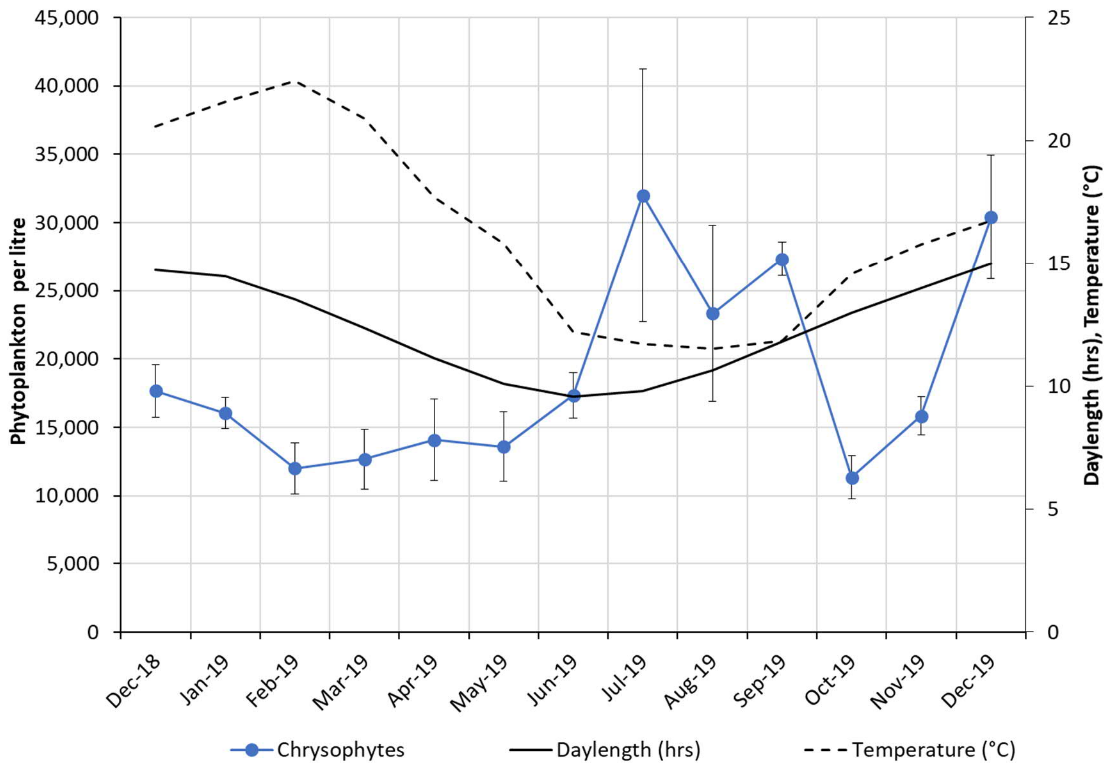

Figure 6-8. Monthly Chrysophyte abundance
(*Mean ± SEM*)

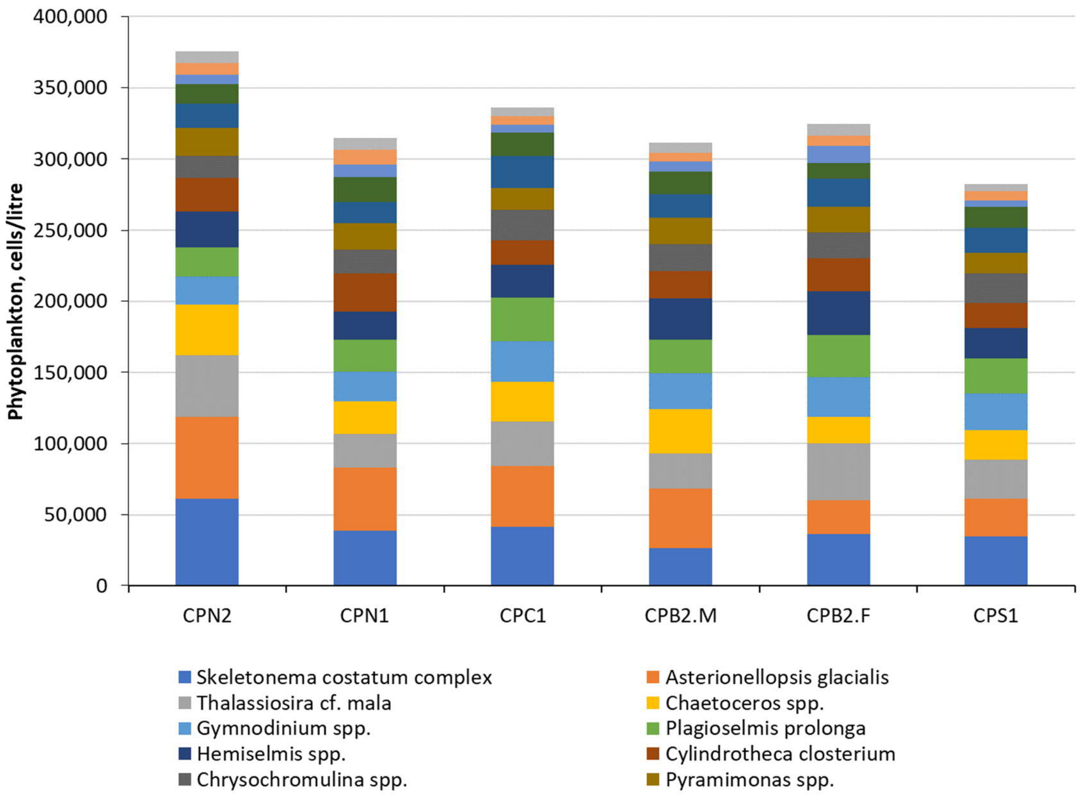

Figure 6-9. Abundance of top 15 species per site, Dec-2018 to Dec-2019

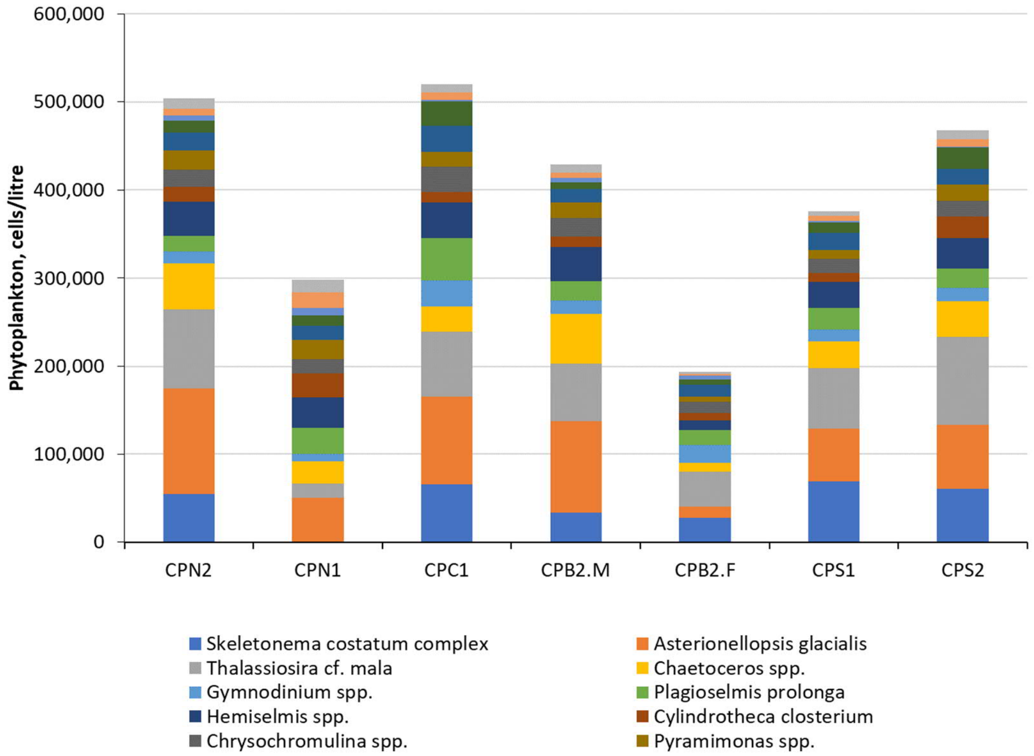

Figure 6-10. Abundance of top 15 species per site, Aug-2019 to Dec-2019

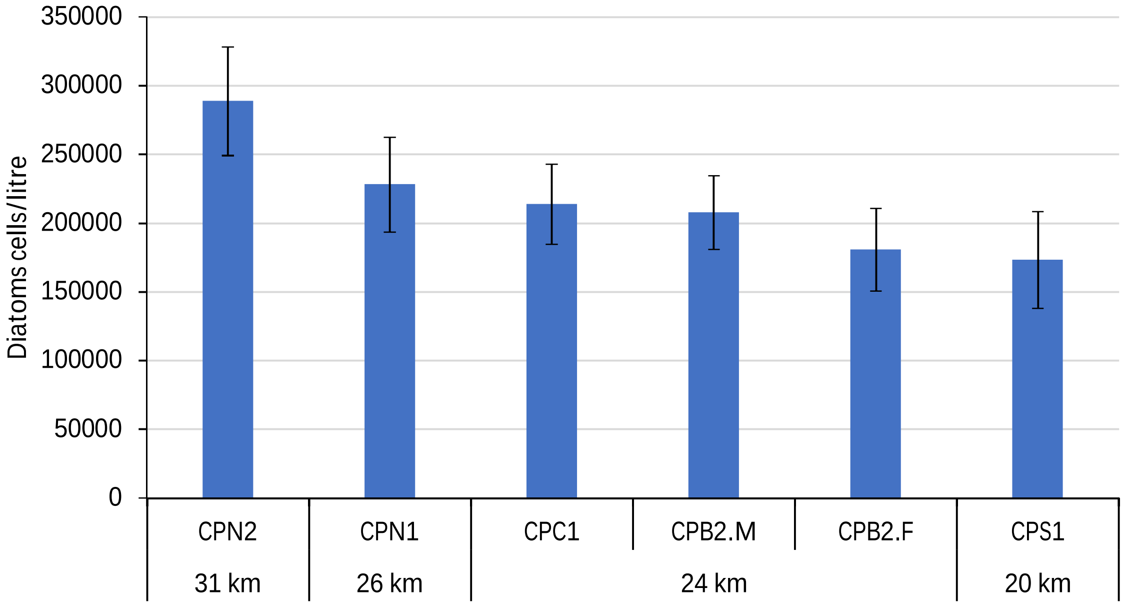

Figure 6-11. Total abundance of diatoms (benthic and planktonic) per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

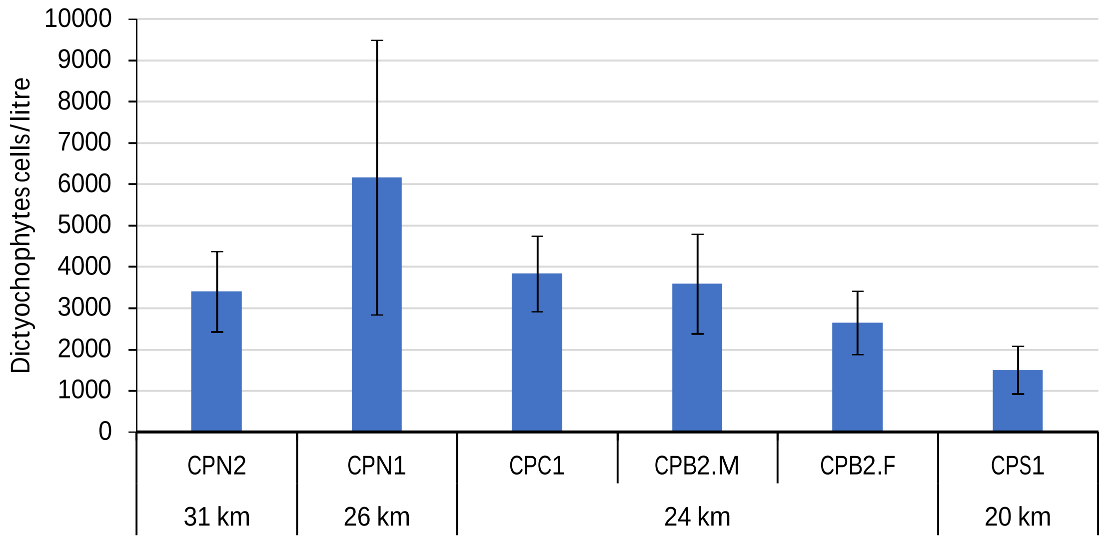

Figure 6-12. Total abundance of Dictyochophytes per site, Dec-2018 to Dec-2019
(*Mean ± SEM*)

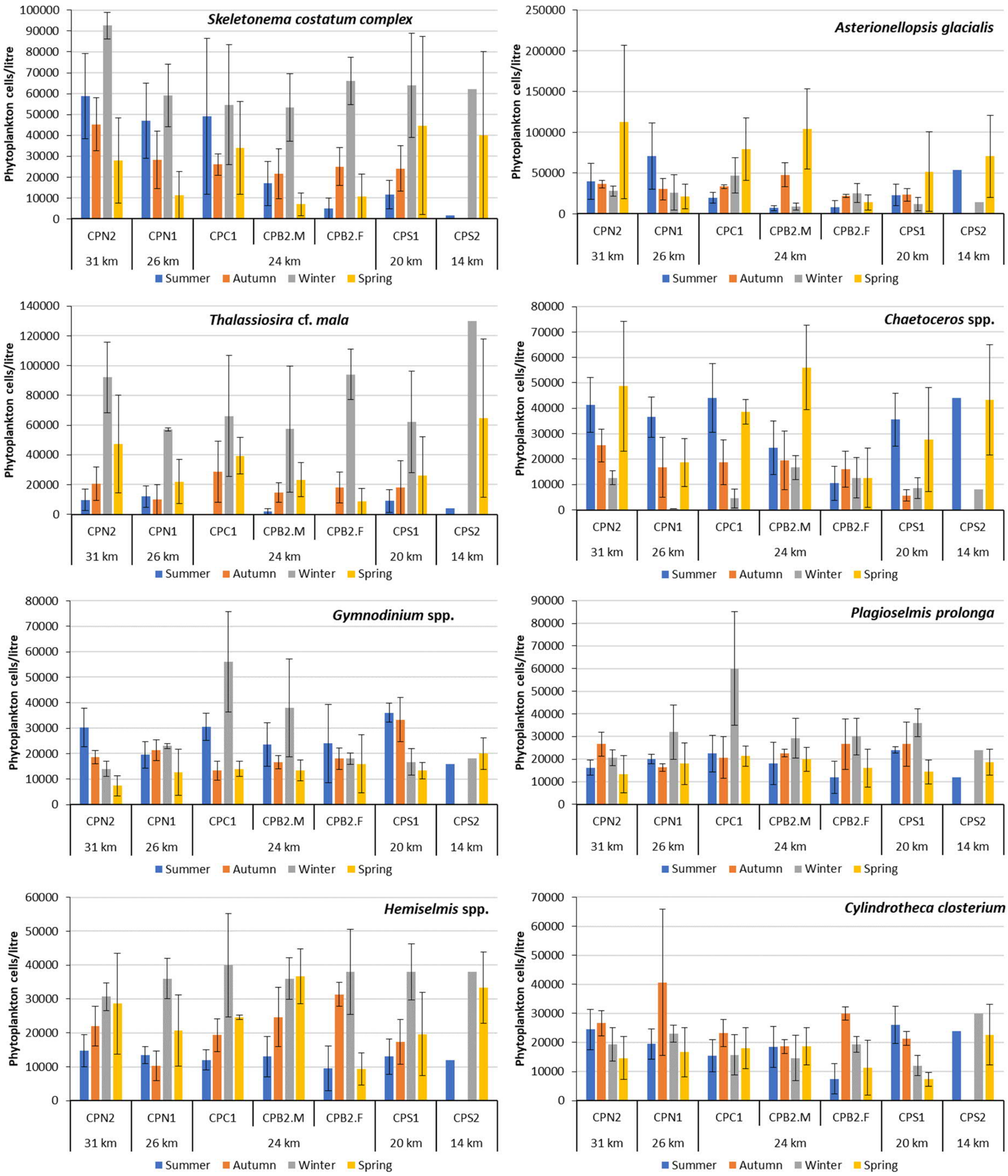

Figure 6-13. Seasonal average abundance of top 15 species

(*Mean ± SEM*)

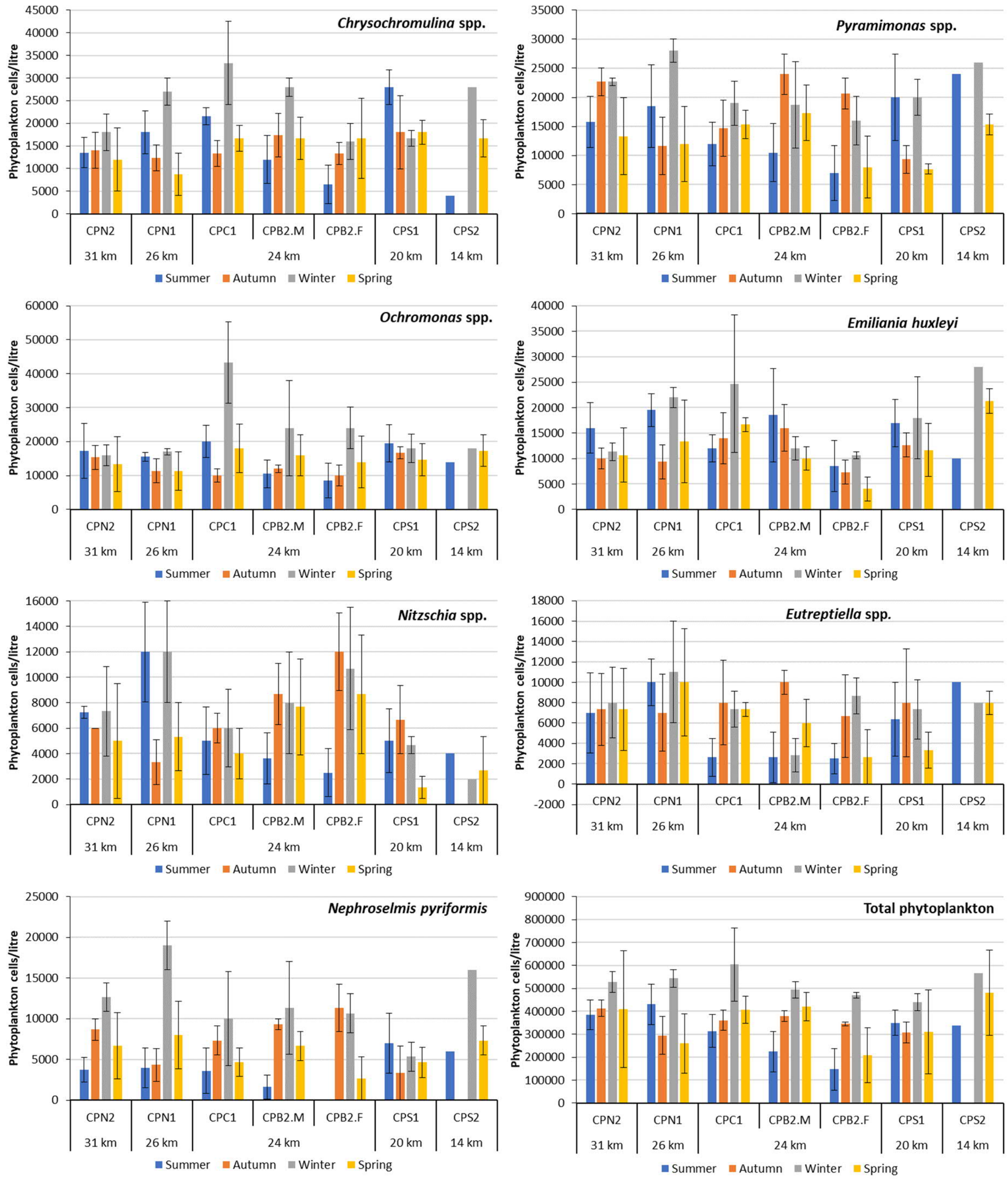

Figure 6.13 (*cont.*) Seasonal average abundance of top 15 species

(*Mean ± SEM*)

6.2 Microalgal Services Subconsultants Report

The phytoplankton of Lower North Arm, Western Port based on a 13-month sampling program." Report to CEE Pty Ltd Environmental Scientists and Engineers. January 2020.

Authors:
Dr Steven Brett, Dr David Hill and Dr Tamsyne Smith-Harding
Microalgal Services, 308 Tucker Road, Ormond VIC 3204

Report follows

"The phytoplankton of Lower North Arm, Western Port based on a 13-month sampling program." Report to CEE Pty Ltd Environmental Scientists and Engineers.

January 2020

Dr Steven Brett, Dr David Hill and Dr Tamsyne Smith-Harding
Microalgal Services, 308 Tucker Road, Ormond VIC 3204

Introduction

Phytoplankton are minute, single celled organisms that live suspended in the water column. They range in size from less than one micron to around 1mm. Many phytoplankton species can photosynthesize, using the energy of sunlight to convert CO_2 into usable forms of chemical energy. Through photosynthesis they are responsible for the majority of primary production in the marine environment and produce around 50% of the world's oxygen. Not all phytoplankton species photosynthesize however; some rely instead on ingestion of organic materials or other organisms to provide the energy for growth and reproduction. The role of phytoplankton as primary producers and/or consumers of lower trophic levels means that they are fundamental in all marine food webs.

Surprisingly, the phytoplankton flora of Western Port is poorly understood. While significant effort has been expended in the study of seagrasses, invertebrates and fish of the region - there have been few studies of phytoplankton (for overviews see Sparkes 2016, Keough 2018). A small study of phytoplankton was carried out as part of the original Westernport Bay Environmental Study (Shapiro et. al. 1975), describing some of the species present, and looking at seasonal changes in a single species. Early monitoring of the Flinders aquaculture zone also provided some very limited information on potentially harmful species, but provides no information on general phytoplankton composition. More recent monitoring of this zone, while more comprehensive, is not publicly available (M. Harris pers. comm). University studies of zooplankton populations in invertebrates in the 1980s incorporated some phytoplankton analysis, and a small study of dinoflagellate cysts was also carried out, although none of this data appears to have been published (D. Hill pers. comm.).

While Western Port has been the subject of long running environmental monitoring programs, the study of phytoplankton has largely been neglected. Most studies have used chlolrophyll-a measurement as a proxy for phytoplankton biomass (eg EPA 2011). The measurements reveal significant variations in biomass in different regions of the bay, and at different times of the year – but composition of the phytoplankton communities has been totally ignored. The current understanding of phytoplankton is elegantly summed up in the following statement:

> 'There is a significant knowledge gap with regard to the species composition, assemblage structure and ecology of phytoplankton in Western Port. Information on species dominance patterns and how they change spatially and temporally (both within and between years) is completely lacking, as is their behaviour with respect to identified nutrient sources and circulation patterns within the bay.' (Keough 2018)

This report presents findings from a one-year study of phytoplankton abundance and community composition in the Lower North Arm of Western Port. While the current study is more detailed (and provides substantially more quantitative data) than previous studies, the scope targets the area for operation of a floating storage and regassification unit (FSRU) proposed to be moored continuously at Crib Point jetty. The intent of the study was to inform an Environmental Effects Statement for the

FSRU project. Hence, the data provide information for one area, over a 13-month period. A much longer, more comprehensive study of all zones of Westernport would be needed to fully address the knowledge gaps identified for broader environmental management of Western Port as discussed by Keough (2018).

Materials and Methods

The Lower North Arm of Western Port is the water body to the west of French Island which includes the Port of Hastings. It connects the southern reaches of Western Port to the Upper North Arm at the top of French Island. The Lower North Arm is relatively narrow and possesses a deep central channel (15m). While net water movement is clockwise around French Island, the area is subject to semi-diurnal tides and wind action, which strongly affect water movement in the area. The water body is well-mixed and turbulent with a persistent sediment load.

Phytoplankton samples were collected from six sites in the Lower North Arm between December 2018 and December 2019. One litre integrated water column samples (10-15m hosepipe sampler) were collected monthly from sites in the centre channel, and fortnightly from the Crib Point Berth. All samples were preserved with Lugol's Iodine at time of collection.

On arrival at the laboratory, samples were concentrated using membrane filtration, then sub-samples were placed into Sedgewick-Rafter counting chambers and examined using light microscopy. Full species counts were undertaken to provide a snapshot of the levels of all phytoplankton species present at the time of sampling. Phytoplankton species were identified using morphological features to the closest taxonomic level possible (generally genus or species).

CEE Pty Ltd was responsible for program design, collection of samples and analysis of data. Microalgal Services carried out phytoplankton sample analysis and was requested to provide qualitative comments on the ecology of the phytoplankton communities. More detailed analysis of the phytoplankton data and assessment of potential effects of the operation of an FSRY was carried out by CEE.

Results and Discussion

The flora observed in Lower North Arm water samples comprised almost exclusively marine species, and was dominated by the small chain-forming planktonic diatoms Skeletonema, Asterionellopsis, Thalassiosira and Chaetoceros. Larger planktonic diatoms and benthic diatoms were present, but less numerous. The samples also contained a wide range of small flagellate species. While cryptomonads were the most abundant flagellates observed, prymnesiophytes, dinoflagellates, prasinophytes, ochrophytes and euglenoids were also well represented. The samples generally had high levels of sediment.

The overall species composition of the samples did not change markedly over time, with the dominant species persisting throughout the thirteen-month period. Total phytoplankton cell numbers, however, showed some variations between sites and with time. Cell numbers ranged from 100,000 to 900,000 cells/L; levels which are quite common in healthy estuarine or coastal marine phytoplankton communities. Table 1 shows the relative abundance of major phytoplankton groups.

Phytoplankton Group	Relative Abundance %	Taxa Observed
Diatoms - planktonic	41.9	54
Diatoms - benthic	10.4	20
Dinoflagellates	9.3	37
Cryptophytes	14.7	7
Haptophytes	9.8	9
Prasinophytes	6.4	6
Ochromonads	5.1	4
Euglenoids	1.7	1

Table 1: Relative abundance (%) of major phytoplankton groups in Lower North Arm water samples December 2018 to December 2019. Relative abundances based on total cell numbers from all sites.

Diatoms

The most abundant group of organisms observed throughout the study was the **diatoms** (Bacillariophyceae), a microalgal group capable of living in a wide range of habitats. Diatom cells are unique in being surrounded by a cell wall (frustule) composed of silica. The diatoms produce lipid as a storage product, and despite having a silica cell wall, they are an important food source for other phytoplankton, zooplankton, larger invertebrates and planktivorous fish.

Small planktonic chain-forming diatoms were the dominant forms in the samples from Western Port. *Skeletonema*, *Asterionellopsis*, *Chaetoceros* and *Thalassiosira* are all common, cosmopolitan diatoms that are widespread in estuaries and inshore coastal areas. Individual cells of all these diatoms are robust and relatively small, and join together to form long chains. Chains of *Skeletonema* are straight, those of *Asterionellopsis* are stellate or spiral, while the discoid cells of *Thalassiosira* are attached to one another via fine threads. *Chaetoceros* chains are distinguished by distinct spines (setae) which emanate from the corners of each cell.

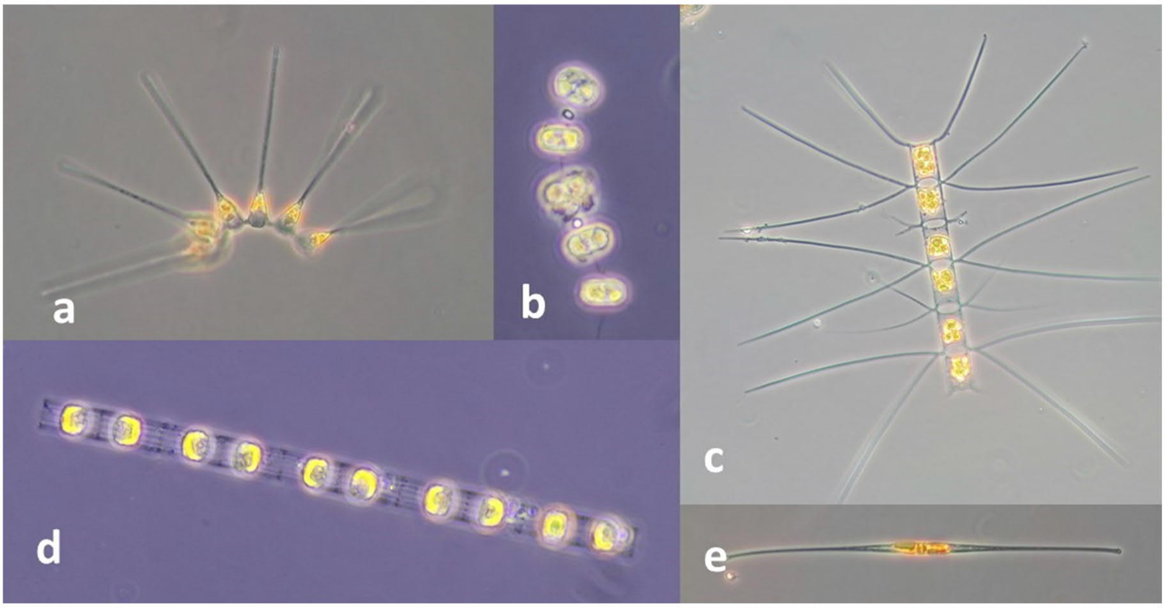

Figure 1. Small chain-forming planktonic diatoms a) *Asterionellopsis* b) *Thalassiosira* c) *Chaetoceros* and d) *Skeletonema*. e) *Cylindrothcea* lives as single cells and is common in both benthic and planktonic environments.

The small cell size of these colonial diatoms allows efficient uptake of nutrients and enables rapid growth. The formation of chains, and features such as spines, are believed to control the rate of sinking in the water column, enabling optimal uptake of light and nutrients. Under favourable conditions these diatoms are capable of doubling their numbers daily, and all are known to be capable of forming dense blooms. Although individual cells of chain-forming diatoms were often very small (5-20um) the chains they formed in the Western Port samples were large - commonly measuring between 200-300um in length.

While small rapidly-growing, chain-forming diatoms dominated samples, larger planktonic species were also present. Larger, cylindrical diatoms like *Dactyliosolen*, *Guinardia* and *Leptocylindrus* were intermittently recorded at low numbers from sites across the study area. These species can exist individually or may be found in short chains. These slower growing diatoms are often more common and abundant in clearer open water, and may become dominant as blooms of smaller diatoms die out. These large diatoms do not appear as robust as smaller diatoms, and it is common to see damaged cells in turbulent environments.

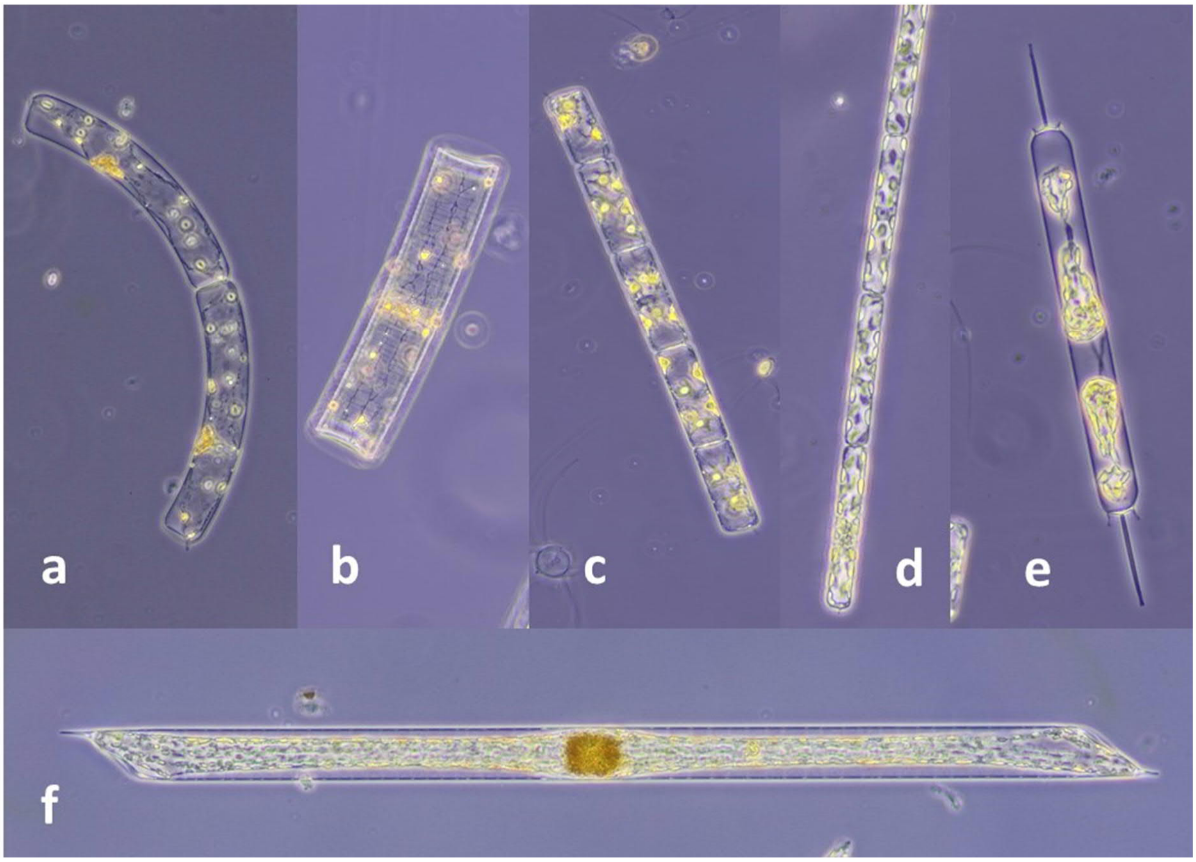

Figure 2. Large planktonic diatoms. a-c) *Guinardia* (*G. striata*, *G. flaccida* and *G. delicatula*) d) *Dactyliosolen* e) *Ditylum* f) *Rhizosolenia*

Benthic diatoms were also an important component of the flora in all samples. *Cylindrotheca* was found in high numbers in all samples and at all sites. This cosmopolitan diatom is a common inhabitant of sediments, but can also often be found free floating in the plankton. The cells are fusiform; needle-like with a slight widening in the middle. *Cylindrothecea*, like many other benthic diatoms, has the ability to move on surfaces and through sediments via secretion of mucilage from pores in its silica frustule. Species of *Nitzschia*, *Navicula*, *Pleuorsigma*, *Entomoneis* and *Licmophora*,

while not as abundant as *Cylindrotheca*, were also present in most samples. Numerous other benthic species were also observed intermittently. The persistent presence of benthic diatoms in samples suggests that there is significant disturbance of bottom sediments in the Lower North Arm, and that waters are turbulent enough to ensure that a population of benthic species remains suspended in the water column at most times. Benthic species are considered to be important primary producers in Western Port (Keough 2018) and, as such, are worthy of separate investigation.

Flagellates

While the diatoms were the dominant components of the phytoplankton, a wide range of flagellates were also present in all samples. Flagellates are single celled organisms that are capable of moving through their environment by beating or rhythmical movement of long, whip-like structures called flagella. The flagellates are a diverse assortment of organisms from numerous divergent groups. They have traditionally been distinguished from one another by differences in their pigmentation, the way that they move, the products that they produce, the structure of their cell walls, and (more recently) molecular techniques.

Total levels of flagellates in the Western Port water samples were roughly equivalent to the total levels of diatoms. This numerical equivalence was not, however, reflected in biomass due to the relatively small sizes of most flagellate species. The small size of flagellates enables them to divide rapidly and under favourable conditions many species can reproduce once a day. Features of the major flagellates observed in the Western Port samples are described below.

The most abundant flagellates in samples were species of **cryptomonads** (Cryptophyceae). These tiny (5-15µm) biflagellate cells are asymmetric in shape. They may be red, brown, blue or green in colour due to accessory pigments in their chloroplasts. The accessory pigments allow them to survive and grow in environments where light is low. Cryptomonads can be found in widely differing habitats, are common in estuarine environments, and thrive in turbid conditions. Dominant cryptomonads in the Western Port samples were *Hemiselmis*, *Plagioselmis* and *Teleaulax*.

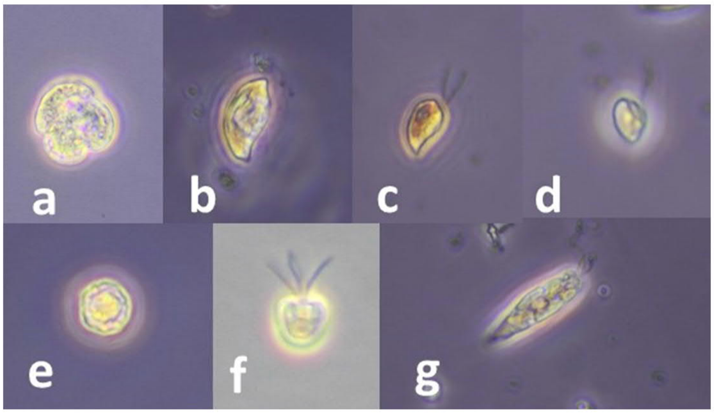

Figure 3. Common small flagellates
a) Dinophyceae (unarmoured gymnodinioid)
b-d) Cryptophyceae *Teleaulax, Plagioselmis* and *Hemiselmis*
e) Prymnesiophyceae *Emiliania*
f) Prasinophyceae *Pyramimonas*
g) Euglenophyceae *Eutreptiella*

The **dinoflagellates** (Dinophyceae) are a large group that contains both photosynthetic and non-photosynthetic forms. The majority of dinoflagellates observed in Western Port samples were small (15-30um) 'unarmoured' species lacking the rigid cellulose thecal plates found on many larger dinoflagellates. The samples contained both pigmented photosynthetic species, and colourless heterotrophic species. *Heterocapsa rotundata*, a small (15um), lightly-armoured dinoflagellate was also abundant in the samples. *Heterocapsa*, and other small unarmoured dinoflagellate species are widely distributed and commonly observed in near-shore estuarine samples. While the smaller

dinoflagellate species were quite abundant in the Western Port samples, larger armoured dinoflagellates were quite rare. Similarly, large benthic and epiphytic dinoflagellate species were encountered infrequently.

The **haptophytes** (*Prymnesiophceae*) were another important group of small flagellates in the samples. They have golden-brown chloroplasts and are distinguished by the presence of organic surface scales and a feeding appendage called the haptonema. Haptophytes usually prefer clean, rather than turbulent, water. Under favourable conditions they can form massive blooms along shorelines or in the open ocean. *Emiliania* (5-7um), a coccolithophorid with distinct $CaCO_3$ surface scales is ubiquitous in coastal waters around the world. The most abundant haptophyte in Western Port samples was *Chrysochromulina* (5-7um), a mixotrophic organism which can use its haptonema to feed voraciously on bacteria.

Like the haptophytes, the **ochromonads** (Chrysophyceae) can be photosynthetic, but may also obtain nutrition externally. Photosynthetic forms have golden-brown chloroplasts. Mixotrophic forms can combine photosynthesis with the ability to actively prey on bacterial cells and small organic particulate matter. Ecologically, they are more prolific and probably better known in freshwater habitats, but are also common in marine waters, often accumulating as other bloom species decline. This is presumably in part a response to an increase in bacterial levels as other cells decompose. Cells in Western Port samples were in the size range 10-15um.

Small **prasinophytes** (Prasinophyceae) were frequently observed in the samples. Prasinophytes are easily distinguished by their bright green colour, shape, number of flagella and layers of minute scales on the surface of cells. These marine flagellates are widely distributed and often abundant. The genus *Pyramimonas*, which was common in our samples (5-15um), is rarely absent from marine samples taken from coastal waters at any location.

Another group of bright green flagellates present in the samples was the **euglenoids** (Euglenophyceae). Euglenoids can be found in the plankton and can also live in sediments. The main euglenoid found in marine waters, *Eutreptiella*, is photosynthetic, but can also graze on bacteria and cyanobactieria. It is widespread in coastal environments and is often found in high nutrient eutrophic waters. Other flagellate groups such as the **raphidophytes** and **dictyochophytes** were present in Western Port samples, but were only observed sporadically and in very low numbers. These groups contain quite delicate species, and it is possible that the turbulent nature of the region precluded their presence.

The phytoplankton community

The samples we examined from Western Port contained a wide range of phytoplankton species. Most of the species observed are considered cosmopolitan, and are widespread in the coastal regions of south-east Australia (Hallegraeff *et. al.* 2010, Tomas 1997). Population levels varied between sites and with time, but phytoplankton community composition did not change markedly throughout the study. The dominant species persisted throughout the year, with the main changes occurring in less abundant species.

The primary species of the Western Port phytoplankton community were small, robust, chain-forming diatoms. The dominant diatoms, *Skeletonema*, *Asterionellopsis*, *Chaetoceros* and *Thalassiosira*, are all capable of forming massive blooms under favourable environmental conditions (Ajani *et. al.* 2019). Levels of these diatoms were, however, relatively consistent – with no dramatic order-of-magnitude changes observed throughout the period. This suggests that conditions, while

favourable for the maintenance of a population, were not optimal for massive growth or bloom formation.

Large, slow-growing diatoms were generally not abundant in samples, although numerous species were observed. Some of the larger diatoms such as Dactyliosolen and Ditylum were present throughout the year, while others were more seasonal. Guinardia and Helicotheca were common in the cooler months, while Pseudo-nitzschia, Leptocylindrus and Bacteriastrum were more abundant from spring to autumn. These large diatoms normally favour open clean water, and can become dominant after a decline in smaller diatom species. It is possible that the turbulent and turbid environment of the Lower North Arm may favour the more robust small species over the larger diatoms, which can be easily damaged. The persistent presence of benthic diatoms and sediment in samples provides further evidence of disturbance, suggesting continuous mixing between the bottom sediments and water column. The presence of high levels of sediments in samples would contribute to sub-optimal conditions for phytoplankton growth.

In general, the flagellates observed in Western Port samples were also all quite small and robust. Most were cosmopolitan organisms that can be commonly found in shallow, dynamic sites with disturbed sediments. They are generally fast-growing organisms capable of reproducing daily, and many are considered 'weed' species that are capable of thriving in a wide range of environmental conditions. The dominant group, the cryptophytes are adapted to thrive in low light conditions which commonly occur in disturbed environments. Many of the flagellate species are also capable of assimilating external nutrition, through ingestion of bacteria, other microalgae or suspended organic material. Such nutritional modes are beneficial in conditions where available light may be low, or in high nutrient environments where alternate food sources may be available.

Large, armoured dinoflagellates such as Gonyaulax, Tripos, Scrippsiella and Alexandrium are common and often abundant in temperate, coastal samples. In the Western Port, however, armoured dinoflagellates were observed only sporadically, and at very low levels. It is possible that the dynamic nature of the region may preclude their presence. Most large, armoured dinoflagellates are recruited from resting cysts which normally accumulate in gyres, sheltered bays or inlets. With the high level of mixing caused by tidal currents, and the disturbance of bottom sediments, it is unlikely that cyst beds are present in the Lower North Arm. In fact, an early University of Melbourne survey of dinoflagellate cysts in Western Port failed to detect any cysts in samples from seven different sites (D.Hill pers. comm.).

Seagrasses, which form an extremely important component of the biota in Western Port, normally act as a substrate for epiphytic dinoflagellates. Considering the persistent sediment load and the presence of seagrass beds within the Lower North Arm, there was a surprising lack of benthic/epiphytic armoured dinoflagellates in the water column samples.

The composition of the phytoplankton assemblages in the Western Port samples was quite consistent across the thirteen months of this study. There were no massive increases in cell numbers to indicate algal blooms, nor were there major changes in the dominant species that would suggest successional changes. Phytoplankton levels and community composition were relatively stable from summer into autumn, and then levels increased steadily from winter to spring. Such increases are commonly associated with increased nutrient inputs during the wetter months, combined with increasing daylength after the winter solstice. A substantial drop in phytoplankton numbers occurred in October, most likely associated an increase in numbers of grazing invertebrates (see main report).

The major findings of this short-term study indicate:

- Composition of the phytoplankton community did not change markedly throughout the year with the dominant species persisting through all seasons
- The flora was dominated by small, robust chain-forming diatoms
- Flagellates were generally small, robust cosmopolitan species
- Large diatoms were less abundant and large armoured dinoflagellates were rare
- Fragile species were rare
- Benthic diatoms and sediment were always present in samples, suggesting mixing of the water column and sediments
- The community composition leans toward species that are capable of surviving in dynamic, well mixed environments

The community dynamics observed here appear to differ somewhat from that reported for other open coastal and estuarine phytoplankton communities. The current study, however, is limited in scope and provides only a brief snapshot of phytoplankton composition and dynamics in one section of Western Port. Normally, it is important to study phytoplankton communities over a much longer period than a single year to accurately understand the seasonal changes and long-term trends. The waters of Western Port present an extremely diverse range of environments - indicating that much wider ranging and more comprehensive work is necessary to more fully understand the phytoplankton flora of Western Port at a catchment or regional level.

References

Ajani P., Eriksen R and Rissik D (2019) Coastal and marine phytoplankton: diversity and ecology. In 'Plankton: A Guide to Their Ecology and Monitoring for Water Quality' (I. Suthers, D. Rissick and A. Richardson eds.) CSIRO Publishing, Melbourne. 236pp.

EPA (2011) Western Port Condition Report - 2009. Publication 1371. Environment Protection Authority, Victoria.

Hallegraeff G.M., Bolch C.J.S., Hill D.R.A, Jameson I., LeRoi J.-M., McMinn A., Murray S., de Salas M.F., Saunders K. (2010) 'Algae of Australia: Phytoplankton of Temperate Coastal Waters' ABRS Canberra, CSIRO Publishing, Melbourne. 421pp.

Keough M. (ed.) (2018) 'Understanding the Western Port Environment' Melbourne Water Corporation, Melbourne. 226pp.

Shapiro M.A. (ed.) (1975) Westernport Bay Environmental Study, 1973 -1974. Environmental Study Series No. 502. Ministry for Conservation, Melbourne.

Sparkes G. (2016) 'State of the Bays' Commissioner for Environmental Sustainability, Victoria. Melbourne. 212pp.

Tomas C.R. (1997) 'Identifying Marine Phytoplankton' Academic Press, London. 858pp.