



The diatom *Chaetoceros* spp. as a potential contributing factor to fish mortality events in Cockburn Sound, November 2015

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This report has been prepared for the  
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by

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## Executive Summary

During November and December 2015 there was a fish mortality event within Cockburn Sound, Western Australia. The first fish deaths were reported on November 19<sup>th</sup> 2015 with dead fish being reported until December 4<sup>th</sup> 2015. It is estimated that over 2 000 fish and invertebrates, representing more than 15 species, were affected by this series of mortality events.

Following the reporting of high concentrations of the diatom *Chaetoceros* (a potentially harmful phytoplankton taxon) in Cockburn Sound during the fish mortality event by an external laboratory, this report was commissioned to 1) identify the species of *Chaetoceros* present when the *Chaetoceros* cell density was highest; 2) provide expert advice on the potential role of the species identified in contributing to the fish mortality event and 3) to provide a historical context of levels of *Chaetoceros* spp. observed during the fish kill event in Cockburn Sound based on analysis of available historic data.

Light and Scanning Electron Microscopy were used to identify the dominant species of *Chaetoceros* present in the samples analysed. Scientific literature was reviewed and analysed to confirm the status of the dominant species of *Chaetoceros* with respect to its potential role in fish mortality events within Australia and World-wide and to determine a potential cause citing evidence. Historic phytoplankton data from Cockburn Sound was analysed with respect to *Chaetoceros* concentrations to provide historical context for the concentrations recorded during the fish mortality event.

*Chaetoceros danicus* was identified as the dominant *Chaetoceros* species present and its role as a potentially harmful alga confirmed in the literature reviewed. *Chaetoceros danicus* is potentially harmful due to the presence of heavily silicified and barbed setae which can irritate or damage fish gills when concentrations are high enough.

According to the literature reviewed, which included data from reported fish mortality events and experimental data, the cell densities of *Chaetoceros* reported during the Cockburn Sound fish mortality event were more than sufficient to stress fish to the point where death is likely.

A review of historic data from Cockburn Sound indicated that the peak *Chaetoceros* cell density recorded in the Cockburn Sound fish mortality event was unprecedented prior to 2005 and indicated that similar or higher peaks in *Chaetoceros* cell density since 2005 are increasing in both intensity and frequency. Increases in the abundance of diatoms (*Chaetoceros* included) are consistent with changes in phytoplankton assemblages as a result of climate change globally and have been demonstrated locally. Other evidence of changes in phytoplankton assemblages, harmful algal blooms in particular, as a result of climate change are discussed to support this also being the cause of the increases in *Chaetoceros* abundance in Cockburn Sound.

Whilst not the primary cause of the Cockburn Sound fish mortality event, the high cell density of *Chaetoceros danicus* and the resulting mechanical irritation (and possibly damage) to fish gills was most likely a contributing factor to a “perfect storm” of conditions, together with low dissolved oxygen concentrations and unseasonably high water temperatures, leading to the mortality event.

Recommendations were made regarding the need for ongoing phytoplankton monitoring of Cockburn Sound and the coastal waters of Perth and the most appropriate analytical methods. These were presented in a separate document.

## Brief from the Department of Fisheries

This report was commissioned by the Department of Fisheries to address the following key requirements:

- To identify the species of *Chaetoceros* present at high abundance in plankton samples obtained during the Cockburn Sound fish kill event;
- To provide expert advice on the potential role of the species identified in contributing to the fish kill event; and
- To provide a historical context of levels of *Chaetoceros spp* observed during the fish kill event in Cockburn Sound based on analysis of available data.

## Background

During November and December 2015 there was a fish mortality event within Cockburn Sound, Western Australia. This mortality event appears to have been restricted to the southern section of Cockburn Sound. The first fish deaths were reported on November 19<sup>th</sup> 2015 with dead fish being reported until December 4<sup>th</sup> 2015. It is estimated that over 2 000 fish and invertebrates, representing more than 15 species, were affected by this series of mortality events. Significantly, more than 250 WA Pink Snapper (*Pagrus Auratus*) were affected, these fish congregate in Cockburn Sound during late spring and summer each year for spawning.

Initial investigations conducted by a multi-agency incident management team found no evidence of contamination by chemical substances, viruses or other disease, potentially toxic algae (based on data collected throughout the mortality period as part of the Western Australian Shellfish Quality Assurance Program from two locations within Cockburn Sound), or any impact due to a spill of canola grain from the Kwinana Grain Terminal between November 18<sup>th</sup> and November 22<sup>nd</sup> 2015 being responsible for the event. A widespread and persistent algal bloom (defined herein as an unusual and exceptionally large biomass of algae significant enough to cause adverse water quality conditions including discolouration and deoxygenation) was also ruled out as a potential cause of the fish mortality.

Although low dissolved oxygen levels are relatively common in the southern part of Cockburn Sound, routine annual water quality monitoring, conducted by the Cockburn Sound Management Council (CSMC), does not begin until December 1<sup>st</sup> each year so no water quality data are available covering the period of the fish mortality event. However, the Department of Fisheries does consider low dissolved oxygen concentrations in the area affected by the fish mortality events to have been a likely contributing factor. This was supported by an independent review of oceanographic conditions at the time conducted by UWA scientists which proposed a mechanism that may have resulted in likely upwelling of deoxygenated water in the affected area. Data collected by the Department of Fisheries on December 3<sup>rd</sup> 2015 during an extensive water quality survey of the southern part of Cockburn Sound showed no evidence of prolonged low dissolved oxygen conditions.

Data collected by temperature loggers which are permanently deployed in Cockburn Sound by the Department of Fisheries indicate that water temperature in Cockburn Sound during the mortality event was higher than in previous years. This is supported by satellite imagery of sea surface temperatures in Cockburn Sound. The Department of Fisheries considered that this may have been a contributing factor to the increase in algal levels observed.

Results from histopathological examination of gill tissues conducted by the Department of Fisheries on freshly dead fish showed distinct inflammation, haemorrhage and excess mucous production. These symptoms indicate the presence of an external irritant, or an acute exposure to poor water quality, and result in decreased respiratory ability of the fish. This irritant, combined with the low dissolved oxygen levels and the warmer water temperatures, is likely to have contributed to the fish kill.

As discussed above, as part of their investigations, the Department of Fisheries WA requested access to data collected as a part of the Western Australian Shellfish Quality Assurance Program (WASQAP). As a part of this program, fortnightly water samples are collected from two locations in Cockburn Sound, Kwinana Grain Terminal and Southern Flats (the locations of mussel aquaculture leases, Figure 1). These samples are analysed for the presence and abundance of phytoplankton taxa which are potentially capable of producing shellfish toxins.

The data initially reviewed by Fisheries, and subsequently made available to the author of this report, were from samples collected on the following dates:

September 22<sup>nd</sup> 2015 (Report PE101998 RO)

October 6<sup>th</sup> 2015 (Report PE102298 Ro)

October 20<sup>th</sup> 2015 (Report PE102709 Ro)

November 2<sup>nd</sup> 2015 (Report PE103000 Ro)

November 17<sup>th</sup> 2015 (Report PE103397 Ro)

November 23<sup>rd</sup> 2015 (Report PE103554 Ro)

December 1<sup>st</sup> 2015 (Report PE103754 Ri)

The samples collected on November 2<sup>nd</sup> 2015 were indicated to be the samples containing the highest *Chaetoceros* cell density (~ 740 000 cells L<sup>-1</sup>).

These samples were initially analysed only for the presence and abundance of phytoplankton taxa which are potentially capable of producing shellfish toxins however, WA Fisheries had requested that they be reanalysed and a report provided detailing the presence and abundance of all phytoplankton taxa.

**NOTE 1: *These samples are routinely analysed for the presence and abundance of phytoplankton taxa known to potentially produce shellfish toxins. Shellfish toxins, whilst non-toxic to marine life (excluding marine mammals) are toxic to humans if enough of the toxins accumulate in the flesh of the shellfish and this flesh is then consumed. The suite of phytoplankton taxa monitored for does not include any ichthyotoxic species or species that can be harmful to marine life, including fish.***

The initial analyses indicated that no potentially toxic phytoplankton taxa were recorded in significant numbers. Subsequent analyses of the entire phytoplankton assemblage indicated that the dominant taxon in the samples was diatoms of the genus *Chaetoceros* (there was no species level identification undertaken as a part of these analyses).

Unfortunately, the reports provided to the author were for the initial analyses only so the concentrations of *Chaetoceros* recorded in these samples, other than those collected on November 2<sup>nd</sup>, is unknown and cannot be discussed herein.

*Chaetoceros* density was relatively high throughout October and November reaching a peak of around 740 000 cells L<sup>-1</sup> in early November. The analysts reported that several species of *Chaetoceros* were present throughout the duration of the fish mortality event but these were not identified to species level or enumerated separately. It is assumed, but not known for certain, that *Chaetoceros* spp. was the dominant phytoplankton taxon in these samples.

*Chaetoceros* was identified by the analysts as the only phytoplankton taxon present that could potentially be harmful to fish. This is a view that is supported by the author with the caveat that the author did not analyse the samples and is not aware of what other phytoplankton taxa may have been present.

As a result of these analyses, species of *Chaetoceros* will be the only phytoplankton taxa discussed herein.

**NOTE 2:** *The method used for the analysis of these samples by the analytical laboratory concerned, whilst appropriate for a surveillance program such as WASQAP where a fast turnaround time (24 hours) and low detection limit (<100 cells L<sup>-1</sup>) are required (necessitating the use of rapid filtration methods to concentrate the sample), they are not appropriate for routine monitoring of phytoplankton assemblages or the investigation of events such this mortality event.*

*This rapid filtration method passes a large volume of water through a filter paper with the material retained on the filter paper then being re-suspended, into a smaller volume of water. This method is considered to be, at best, a semi quantitative method of preparation of phytoplankton samples (Hötzl and Croome, 1999). This is because the filtration and re-suspension process damages fragile phytoplankton cells and breaks up colonies of larger cells (particularly colonies of chain-forming diatoms such as *Chaetoceros*) but more-so because it is not possible to recover 100% of the phytoplankton from the filter paper especially in the case of fragile taxa and those taxa with spines and setae (such as *Chaetoceros* spp.) that will adhere to the filter paper.*

*Such a method invariably results in the non-detection or underestimation of certain taxa, some of which are potentially significant.*

## Current Investigation

Following the flagging of species of *Chaetoceros* as being potential contributors to the fish mortality events in Cockburn Sound, the author of this report was contacted by the Department of Fisheries WA to undertake some additional analyses specifically to identify the species of *Chaetoceros* present during the fish mortality events and, if possible, to provide some historical context to the cell densities of *Chaetoceros* recorded during that period.

The remaining samples corresponding to the dates listed above were acquired from the analytical laboratory concerned. Unfortunately, all samples obtained comprised of only 5 ml or less of sample which made any additional analyses quite difficult. Samples from two locations, Kwinana Grain Terminal and Southern Flats (Figure 1) were provided for each of the seven sampling occasions.

The following investigations were undertaken:

- Microscopic analysis using light microscopy and scanning electron microscopy to determine the dominant species of *Chaetoceros* present during the peak in its abundance;
- Analysis of historic data from Cockburn Sound collected by the author from 1992 to 2013 to provide an historical context for the densities of *Chaetoceros* recorded during this event; and
- Literature reviews regarding the involvement of *Chaetoceros* in fish mortality events elsewhere and the potential impact of climate change as it relates to marine phytoplankton and harmful algal blooms in particular.

## Limitations of the Current Investigation

Due largely to the manner in which the provided samples were prepared (see Note 2) and the small volume of sample provided (maximum of 5 ml per sample), the following limitations should be noted:

- **Microscopy** – the sample provided for microscopic investigation to determine the dominant species of *Chaetoceros* present during the period of greatest cell density (Sample PE103000.001, Kwinana Grain Terminal, November 2<sup>nd</sup> 2015, reported cell density ~ 740 000 cells L<sup>-1</sup>) contained less than 5 ml of sample. This was pooled with the Southern Flats sample collected on the same date (Sample PE103000.002, Southern Flats, November 2<sup>nd</sup> 2015, reported cell density - unknown) providing about 8 ml of sample for preparation.

In order to prepare marine phytoplankton (diatom) samples for high magnification light microscopy (1 000 x magnification) and for Scanning Electron Microscopy (SEM), the sample material needs to be repeatedly rinsed in deionised water to flush out all salt crystals and then cleared of organic material (leaving just the silica diatom frustule) by heating in a weak acid solution.

The small volume of sample provided did not allow for any rinsing to remove salt crystals and only allowed for limited clearing of organic material. Whilst this did not preclude identification of the dominant *Chaetoceros* to species level, the photomicrographs obtained were of inferior quality primarily due to the presence of salt crystals in the sample.

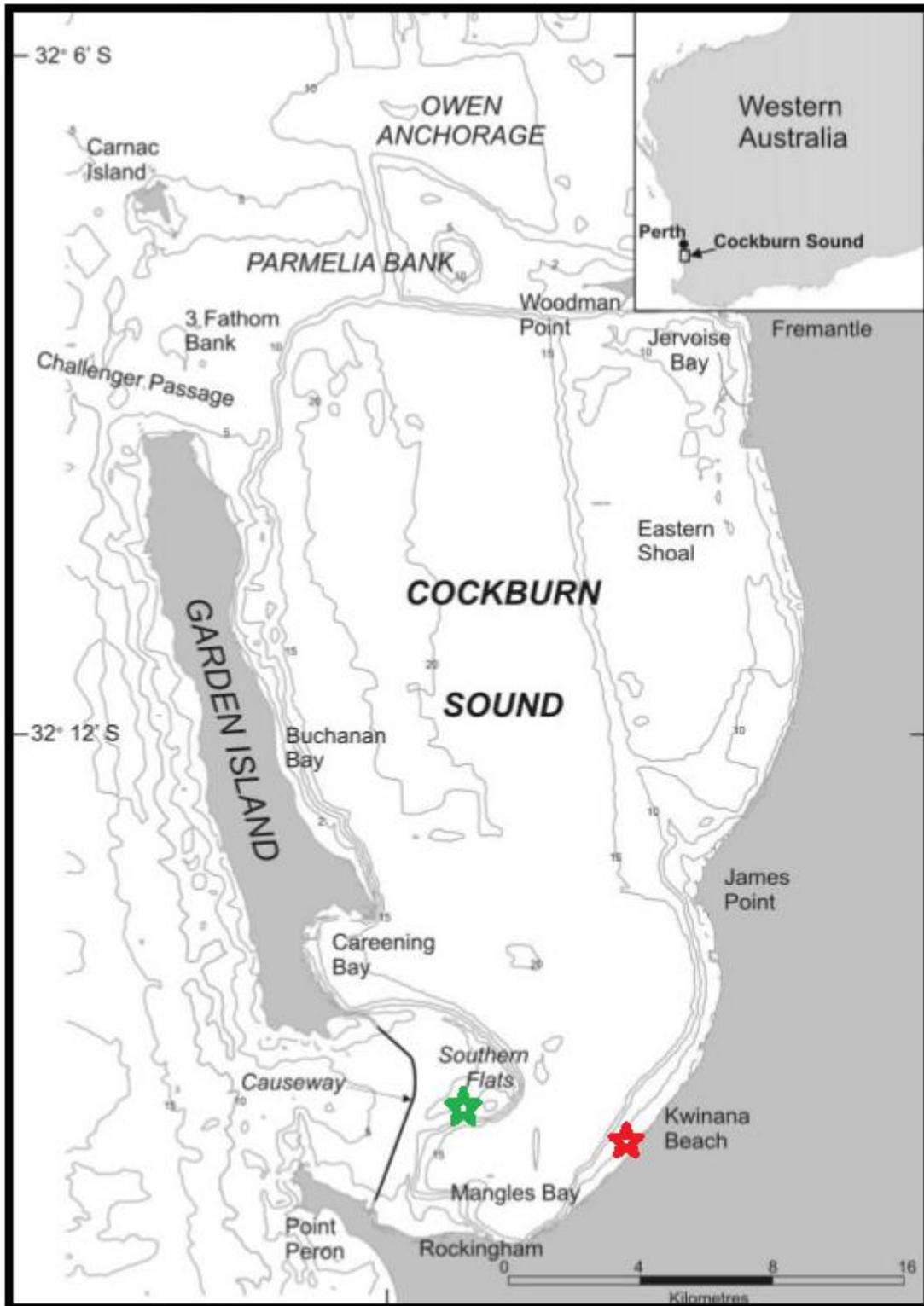


Figure 1. Cockburn Sound showing the approximate locations of the WASQAP Kwinana Grain Terminal (red star) and Southern Flats (green star) sampling locations. Modified from Skene *et al.* (2005).

- **Reported Cell Density** – It should be noted that any cell density determinations cited herein were not the result of analyses conducted by the author and, as such, cannot be vouched for by the author.
- **Inappropriate sample preparation method** – as highlighted in Note 2, the rapid filtration method of concentration used to prepare the samples by the analytical laboratory concerned is not appropriate for routine phytoplankton monitoring and/or investigative analyses.

As a result of this, it is likely that any cell density determinations, particularly those for species of *Chaetoceros* (see Note 2), are an underestimate of the actual cell density due to the retention of phytoplankton cells on the filter paper.

## Methods

### Identification of Dominant *Chaetoceros* species

All samples provided were of sub-optimal volume for preparation for high magnification light microscopy and scanning electron microscopy, the methods of choice for accurate species identification. The preparation method required for microscopy is destructive such that, after preparation, there is no useable sample left. Whilst it would have been possible to pool all the samples to increase the sample volume for preparation, the resulting volume (< 70 ml) still would have been inadequate and there would have been no remaining sample if subsequent analyses were required. In addition to this, pooling the samples would have diluted the concentration of the target *Chaetoceros* species and made them harder to locate during analysis, particularly using SEM. It was therefore decided to use the samples with the highest recorded *Chaetoceros* cell density, as above.

Samples for high magnification (oil immersion) light microscopy and scanning electron microscopy were prepared by Dr Jacob John of the Department of Parks and Wildlife using the methods described in John (2015). As discussed above, marine phytoplankton samples require a rinsing stage prior to sample preparation to remove salt from the sample, this vital step was not undertaken for this sample. Permanent diatom slides were prepared for the high magnification light microscopy after mild acid treatment to remove organic materials. Cleared diatom material was evaporated onto a square coverslip and then mounted using Hyrax, a high refractive index mounting medium. These slides will be archived in the diatom section of the Western Australian Herbarium, Department of Parks and Wildlife. Cleared diatom material was also evaporated onto a round coverslip which was coated with platinum after mounting onto a metal stub for Scanning Electron microscopy.

The resulting photomicrographs, and observations made during analysis, were used to determine the dominant *Chaetoceros* species present in the sample analysed. Where possible, photomicrographs were also taken to highlight specific morphological features of the species identified (this was hindered by the presence of salt in the samples).

## Compilation and analysis of historic phytoplankton data from Cockburn Sound

The author of this report has been undertaking phytoplankton analyses in Cockburn Sound since 1992. In order to put the reported cell densities of *Chaetoceros* recorded during the fish mortality events into historical perspective, this data has been compiled into a single data set.

This represents a large amount of data, much of which was in pdf format and had to be transcribed from the original reports.

The data used are as follows:

1992-1994	Southern Metropolitan Coastal Waters Study
	4 – 5 sites in Cockburn Sound
	47 sampling occasions
	548 samples
1999-2013	Western Australian Shellfish Quality Assurance Program
	2 sites in Cockburn Sound
	344 (Kwinana Grain Terminal) and 313 (Southern Flats) sampling occasions
	657 samples

The gaps in these data represent a period where there was no routine phytoplankton monitoring being undertaken in Cockburn Sound. From 2014 onwards, phytoplankton analyses for the Western Australian Shellfish Quality Assurance program ceased recording data for all phytoplankton species and focused only on a limited suite of phytoplankton taxa known to potentially produce shellfish toxins.

Data from a total of 1 205 samples was organised and compiled into a single data set, a very time consuming process. A time-series plot of *Chaetoceros* cell density covering these two time periods was created from the data.

## Literature Reviews

Online university catalogues and electronic scholarly databases were searched for literature relating to the implication of *Chaetoceros* (particularly *Chaetoceros danicus*) in fish mortality events to provide context and supporting evidence for the implication of *Chaetoceros* in the current fish mortality event.

Given the lack of adequate phytoplankton monitoring in Cockburn Sound over the last 20 plus years, literature was also consulted regarding the impacts of climate change on phytoplankton assemblages, harmful algal blooms in particular, globally. This was related to potential changes which may have occurred, and are most likely still occurring, locally in Cockburn Sound and the broader coastal waters of Perth and cross-referenced against the limited data currently available from these waters.

## Results

### Identification of Dominant *Chaetoceros* species

Examination of material from samples PE103000.001 and PE103000.002 (November 2<sup>nd</sup> 2015) using light microscopy (analysis of fresh samples at 400x magnification and analysis of prepared permanent slides at 1 000x magnification) and scanning electron microscopy indicated that there were two species of *Chaetoceros* present, *Chaetoceros danicus* and *Chaetoceros lorenzianus*, with *Chaetoceros danicus* being the dominant taxon.

As is the case with all diatoms, the cell wall of *Chaetoceros* is made out of silica, effectively a cell made of glass. It is because of this, that some species of diatom are considered harmful and capable of causing irritation and/or damage to the soft tissue gills of fish especially when the cells themselves are long, thin and spindle-shaped (e.g. *Cylindrotheca*) or where there are similarly shaped setae (e.g. *Chaetoceros*). *Chaetoceros danicus* (along with several other species of *Chaetoceros*) is considered to be a harmful species of phytoplankton (Guiry & Guiry, 2016) due to the rigid (heavily silicified) nature of its setae (see below) which can irritate the gills of fish causing excessive mucous production or, in extreme cases, cause direct physical damage to the gills. The setae of many of these harmful species of *Chaetoceros*, *Chaetoceros danicus* included, contain barbs along their length exacerbating the amount of irritation and/or damage they can cause.

*Chaetoceros lorenzianus* is not considered to be a harmful species and, as such, will not be discussed any further in this context herein.

A description of the genus *Chaetoceros* as well as a description of *Chaetoceros danicus* are given below. Figure 2 has been provided to give a basic understanding of diatom morphology and Figure 3 has been provided to assist with understanding of the terminology used in the description of *Chaetoceros danicus*.

Diatoms are generally divided into two groups according to the symmetry exhibited by the valve (cell). Centric diatoms (Figure 2, a) typically exhibit a radial symmetry and pennate diatoms typically exhibit a bilateral symmetry (Figure 2, b). The diatom cell is made up of two halves (valves), the upper valve is called the epi-valve (Figure 2, E) and the bottom valve is called the hypo-valve (Figure 2, H) with the epivalve being slightly larger and fitting over the top of the hypovalve. A simple way to picture this is to think of how the two halves of a petri dish fit together.

The two halves of the diatom cell are held together by a series of bands called girdle bands or copulae, collectively these are referred to as the cingulum (Figure 2, C). The girdle bands closest to the valves are called valvocopulae (Figure 2, vc). The valve surfaces of both centric and pennate diatoms are ornamented to varying degrees with punctuations or pores (which may form lines called striae), raphe slits (in some pennate diatoms) and an assortment of other structures.

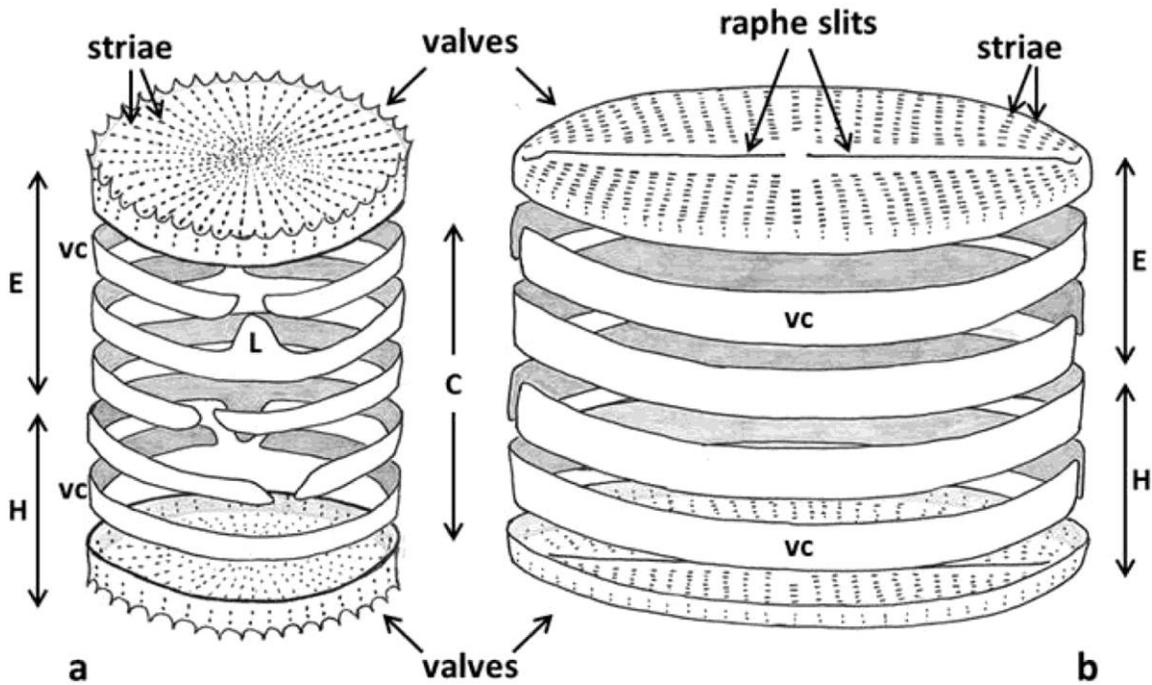


Figure 2: Morphology of a typical centric (a) and pennate (b) diatom.

The diatoms illustrated in Figure 2 are shown in an oblique view where both the valve surface and the valve mantles (the sides of the valves) and cingulum can be seen at the same time. Typically, however, diatoms are viewed in either valve view where only the epivalve or hypovalve surface can be seen (i.e. top or bottom view) or in girdle (or cingular view) where only the valve mantles and cingulum can be seen (i.e. side view).

Figure 3 shows two different chain-forming species of *Chaetoceros* in girdle view (Figure 3, 1) and a single cell of *Chaetoceros* in valve view (Figure 3, 2).

A typical *Chaetoceros* cell is oval (sometimes to almost circular) in shape when viewed in valve view (VS = valve surface) with two, usually long, protuberances called setae (S) emerging from a common point on the “sides” of the cell. When viewed in girdle view, the individual cell is rectangular (cells may be longer than wide, or wider than long) to almost square with the setae emerging from each “corner” of the cell.

In colonial (chain-forming) species of *Chaetoceros*, the setae on the terminal cells (TC), called terminal setae (TS) are usually oriented differently to the setae of the intercalary cells (IC). The gap between cells in the chain is called the aperture (A) and its shape is determined by the distance between adjacent cells, the shape of the valve face (some may be concave, some may be convex) and whether or not there are any protuberances on the valve face, such as structures called rimoportulae (Rp).

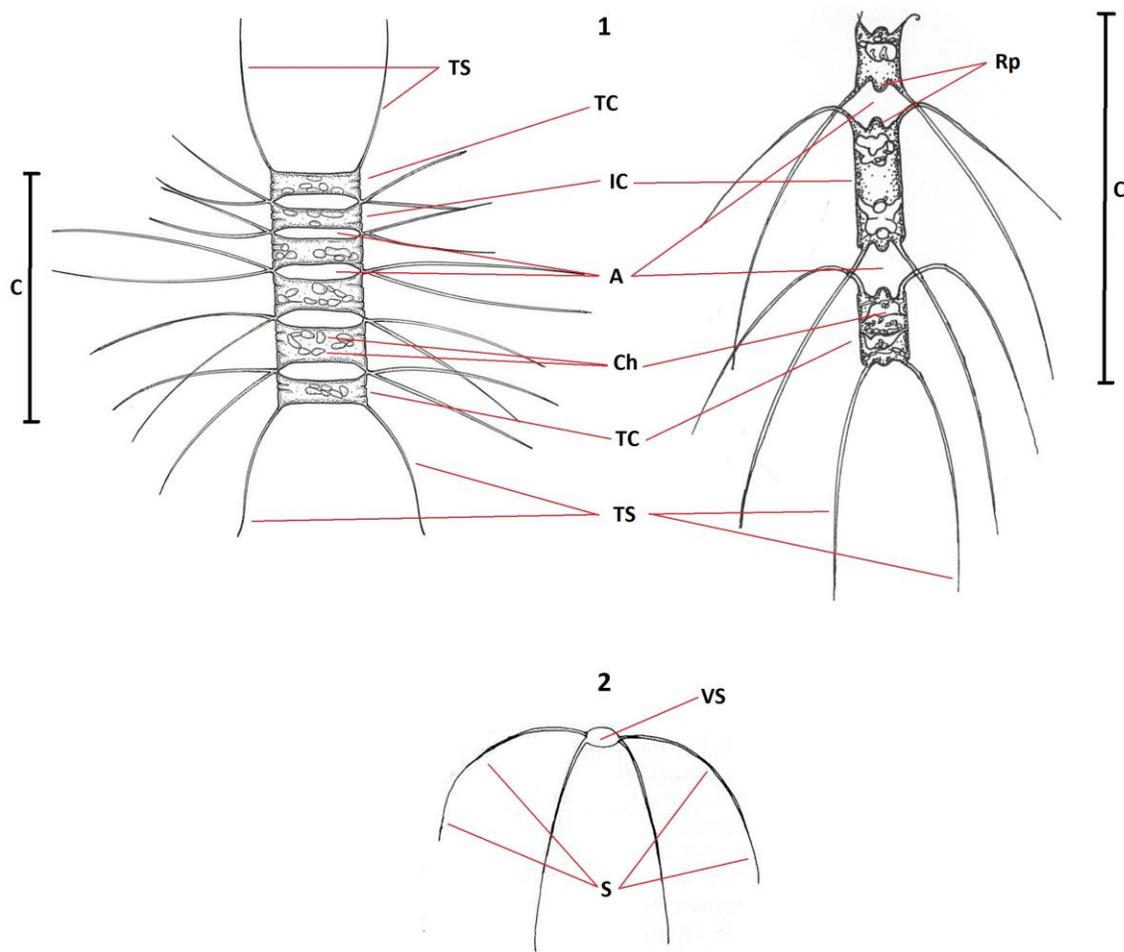


Figure 3: The morphology of a typical chain-forming (C) *Chaetoceros* in (1) girdle or cingular view and (2) valve view.

The following formal description of the genus *Chaetoceros* is taken from Guiry & Guiry (2016).

*Generally colonial bipolar centric diatoms. Forms cylindrical cells with an oval or circular base that contain 1, 2 or more chloroplasts (labelled Ch in Figure 3). The chloroplasts appear to be quadrangular in cingular view and elliptic or circular in valve view. The mantle is vertical and the cingulum is more or less developed. Processes, called setae, that are of varying thickness emerge from each apex. In the colonial species the terminal setae, those emerging from the last valve in the colony, are distinguished from intercalary setae, those of the internal valves that cross each other and/or fuse at some point, allowing the formation colonies of differing lengths. Specialised intercalary setae are found in some species which are different in size and shape from the other setae in the colony. An aperture that varies in size and shape and is delimited by the valve faces and the proximal part of the setae separates the cells of a colony. The valve surface generally has radial costae originating from a central hyaline\transparent annulus. The rimoportula, when present, is located centrally or subcentrally, usually with a short external tube. In the cingulum, the intercalary bands and the pleurae are of the open or segmented type. The setae have a variable ornamentation, often consisting of rows of spines or pores of different sizes.*

The following formal description of *Chaetoceros danicus* is taken from Horner (2002).

*Cells relatively small, 8-20 μm wide; often solitary or joined in short chains of 2-4 cells, frequently appearing in valve, or slightly oblique, valve view. Valve surface flat, apertures between cells in chains narrow. Setae originating at the valve margin, long, stiff, perpendicular to the perivalvar axis.*

Figures 4 and 5 show light photomicrographs of *Chaetoceros danicus*. Figure 4 is photomicrographs that the author has taken from samples from Cockburn Sound on various occasions and Figure 5 is from other sources as acknowledged.

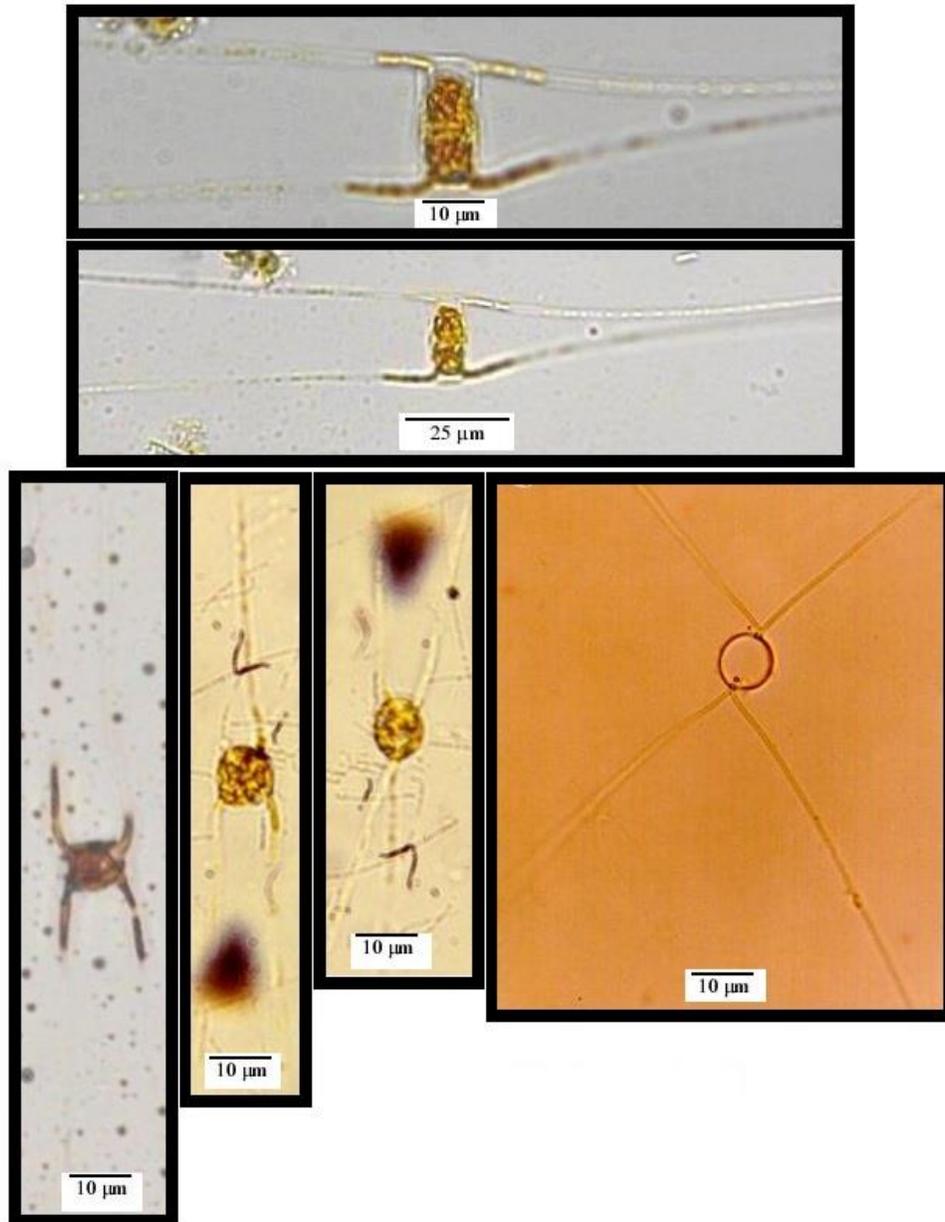


Figure 4: Photomicrographs of *Chaetoceros danicus* from samples collected from Cockburn Sound between 1992 and 2013.

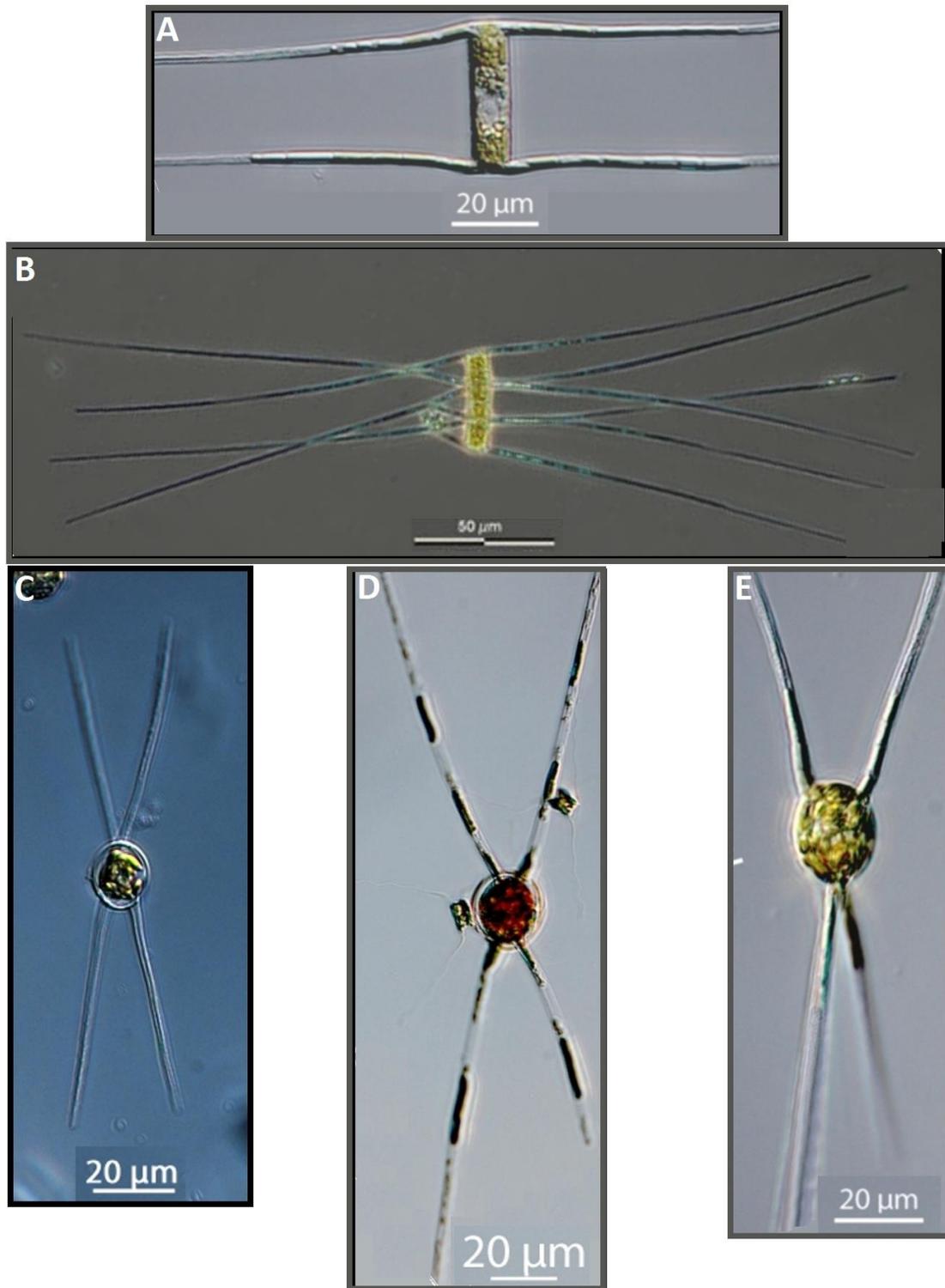


Figure 5: Photomicrographs of *Chaetoceros danicus* sourced from Plankton Net (2016). A) a single cell in girde view; B) a small chain of three cells in girde view; C-D) single cells in valve view.

Figures 6, 7 and 8 are SEM photomicrographs showing the ultrastructure of *Chaetoceros danicus*, in particular the spines located on the setae. Figures 6 and 7 are from samples analysed for this report and Figure 8 is micrographs from other sources (as referenced) showing this detail more clearly.

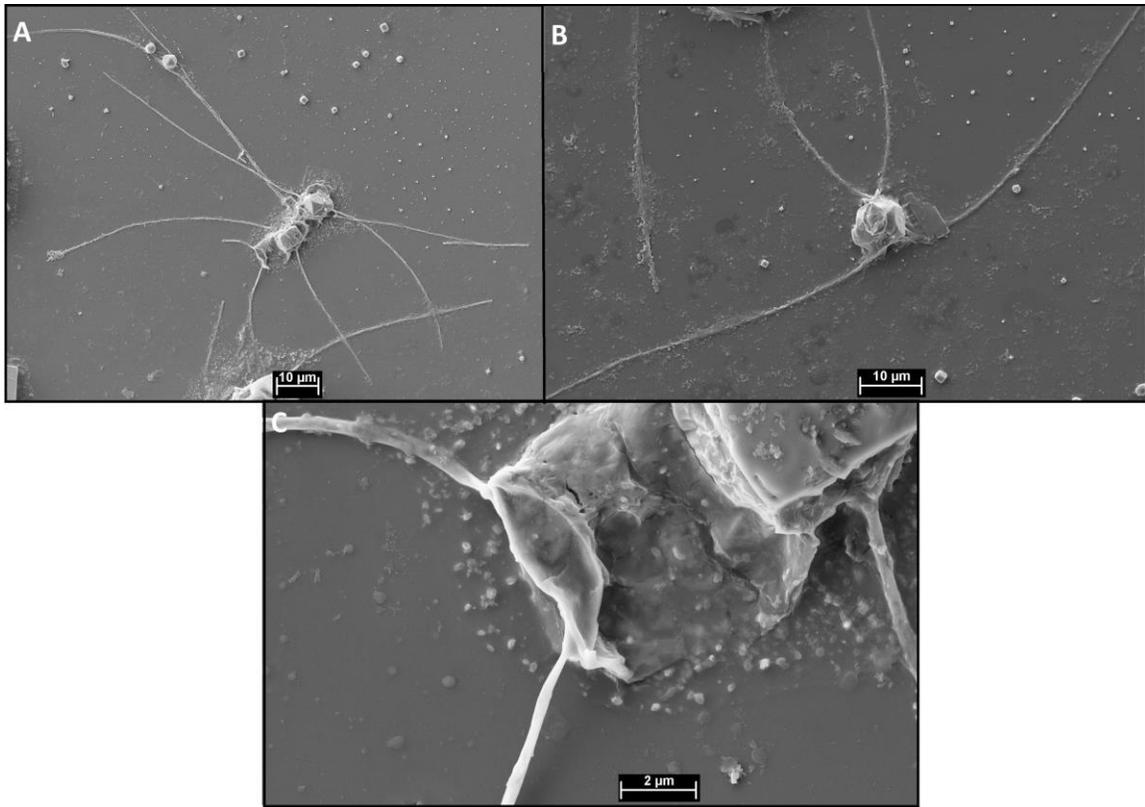


Figure 6: *Chaetoceros danicus* from samples provided for this report under SEM showing a small chain of two cells (A) and individual cells (B and C). The impact of salt crystals on the quality of sample and the subsequent micrographs is quite evident.

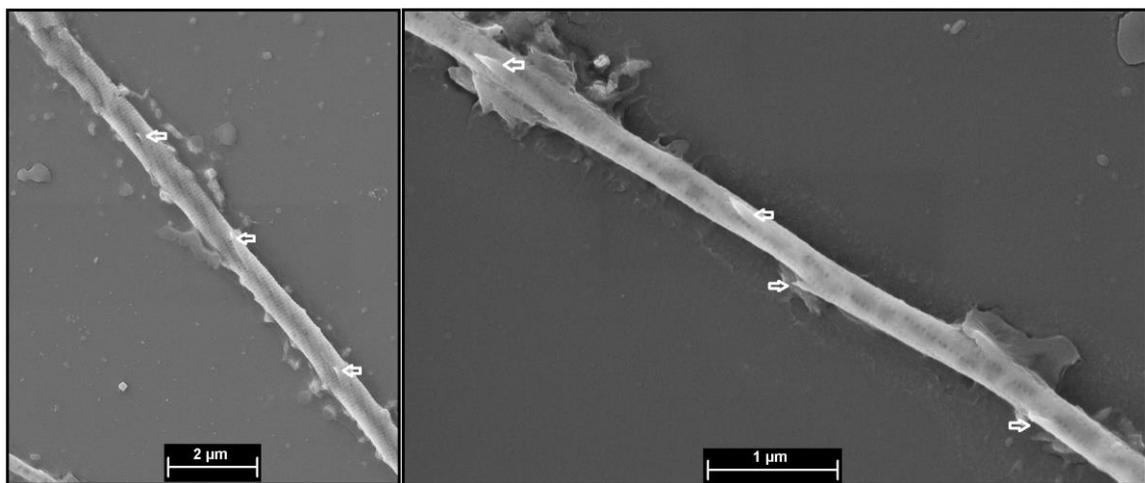


Figure 7: Setae of *Chaetoceros danicus* from samples provided for this report under SEM showing spines (arrows).

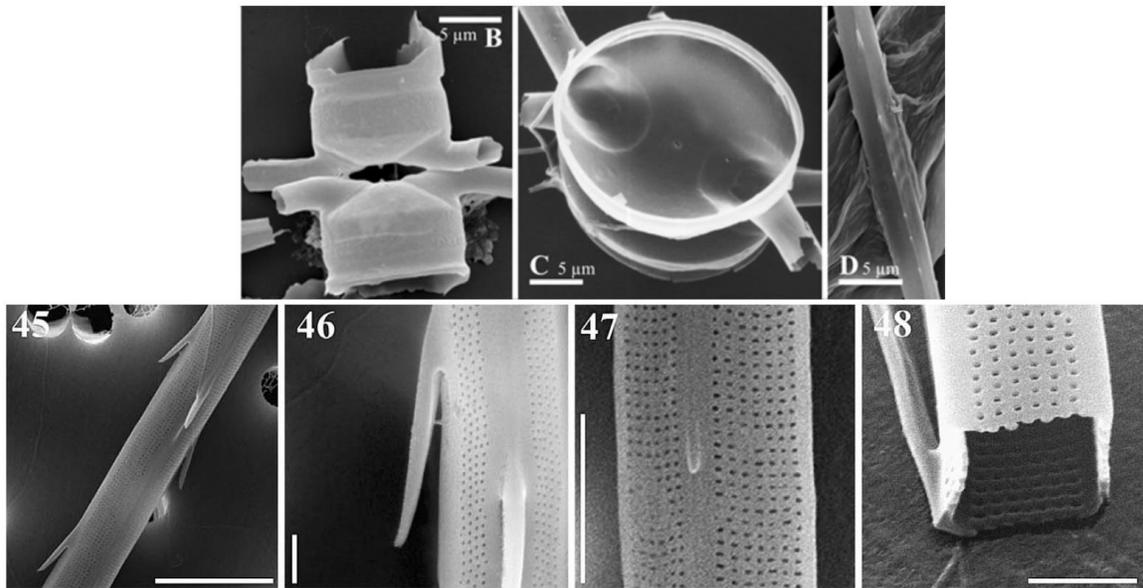


Figure 8: SEM images of *Chaetoceros danicus* from Sunesen, Barcena, and Sar (2009) (top row) and Lee *et al* (2014) (bottom row). Top Row – B) two adjacent cells in a chain, note the very narrow aperture, C) interior view of a hypovalve, D) a single seta showing spines. Bottom Row – 45-47) ultrastructure of setae showing spines, 48) cross section of a seta.

The spines on the setae of *Chaetoceros danicus* are quite evident in the figures above. The setae themselves have a thickness of about 0.25  $\mu\text{m}$  (the minimum width of a human hair is about 17  $\mu\text{m}$ ), the spines are about one third to one half the thickness of the setae and are about 0.25  $\mu\text{m}$  long, the spines are raised about 0.1  $\mu\text{m}$  above the surface of the setae.

### Compilation and analysis of historic phytoplankton data from Cockburn Sound

As discussed earlier, data representing 1 205 phytoplankton samples from Cockburn Sound collected between 1992 and 2013 has been compiled into a single data set and, from this, a time series of *Chaetoceros* abundance has been extracted (Figure 9).

*Chaetoceros* species were present in 1 111 (92%) of the 1 205 samples. The highest recorded *Chaetoceros* density during the 1992-1994 period was 681 000 cells  $\text{L}^{-1}$ , the peak *Chaetoceros* density recorded for the samples provided for the current study was 740 000 cells  $\text{L}^{-1}$ . The highest recorded *Chaetoceros* density for the 1999-2013 period was 4 135 500 cells  $\text{L}^{-1}$ , the 740 000 cell  $\text{L}^{-1}$  peak density recorded for the current study was exceeded in 18 samples (out of 657 samples) on 12 occasions (out of 350 occasions) during the 1999-2013 period.

These exceedances of the current peak value were all recorded from 2005 onward, there was one exceedance occasion in 2005, two exceedance occasions in 2006, three exceedance occasions in 2007, two exceedance occasions in 2008, one exceedance occasion in 2009, no exceedance occasions in 2010 or 2011 and 3 exceedance occasions in 2013. The highest *Chaetoceros* density recorded was in 2012. Five of these exceedance occasions were during autumn, five (including the occasion with the highest abundance) were during winter and two were during summer.

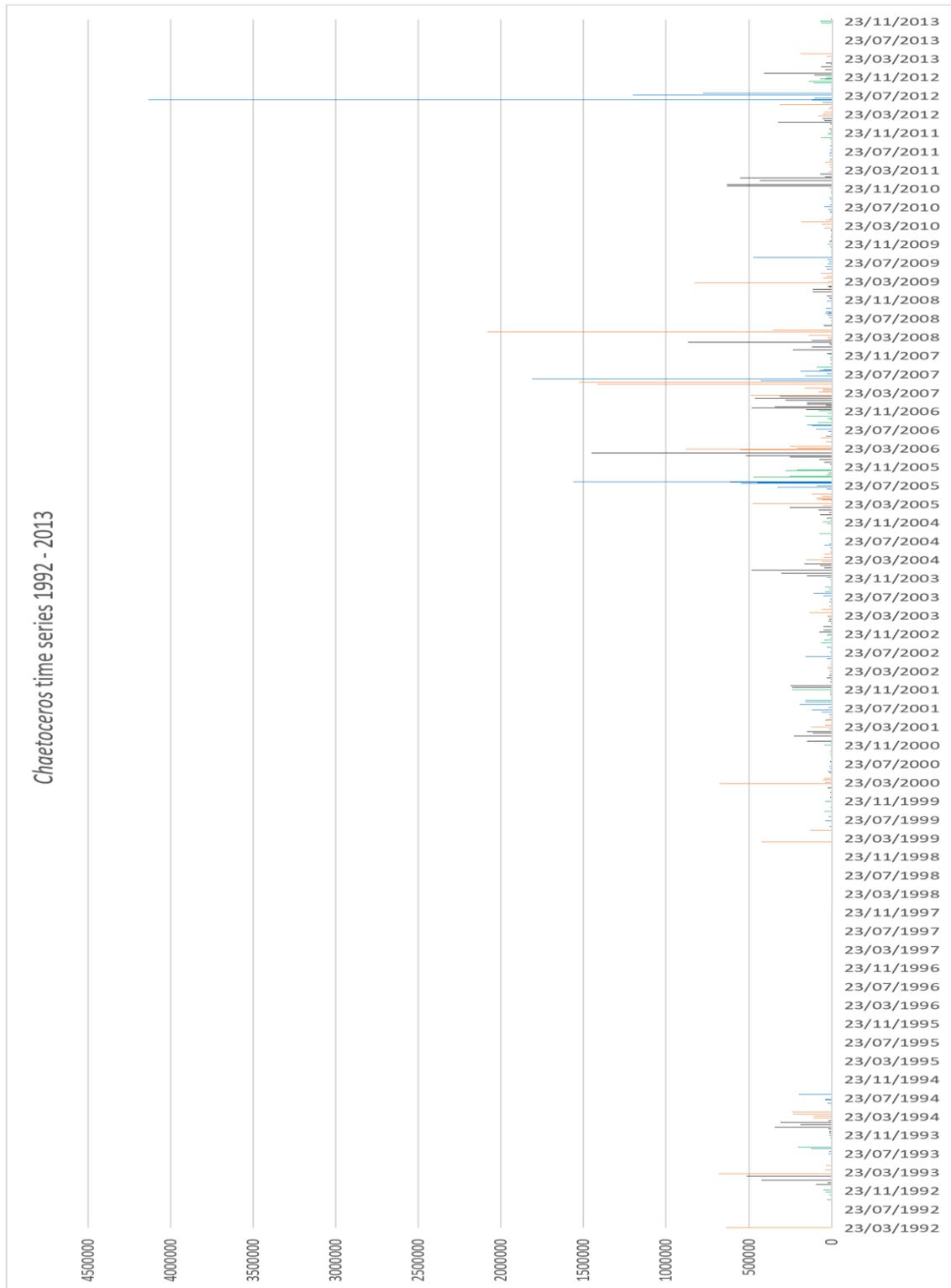


Figure 9. Time-series plot of *Chaetoceros* cell density (cells L<sup>-1</sup>) from 1992 to 2013. Cell density (cells L<sup>-1</sup>) is on the vertical axis, date is on the horizontal axis. Bar colours indicate season (black = summer, orange = Autumn, blue = winter and green = spring).

Figure 9 suggests that peaks in *Chaetoceros* abundance in Cockburn Sound are showing an increasing trend since the surveys in the early 1990s and particularly since 2005 with high abundances most likely to occur during autumn and winter.

## Discussion

Histopathological examination of the fish gills using light microscopy by the Fisheries Department showed clear evidence of inflammation, haemorrhage and excess mucous production as a result of an external irritant. There was no visible evidence however of this external irritant. These symptoms however (excessive mucous production, inflammation, haemorrhaging of damaged tissue, and blood hypoxia) are all considered to be the symptoms of exposure to external irritants such as the setae of species of *Chaetoceros* (Rensel, 1993; Rensel, Haigh and Tynan, 2010).

The setae of *Chaetoceros danicus*, and most of the other potentially harmful *Chaetoceros* species, are brittle and it is broken off setae or segments of setae that typically are responsible for causing irritation and/or damage to fish gills rather than intact cells or colonies of cells. This is particularly the case when cell densities are high (Figure 10). Given the sub-micron width of these setae and the fact that under light microscopy they would have been close to transparent (silica cell walls), it is not surprising that they were not observed, assuming of course that setae fragments were responsible for the observed pathology.

As discussed earlier, *Chaetoceros danicus* is considered to be a harmful species (Rensel, 1993; Sunesen, Barcena, and Sar 2008; Sunesen, Barcena, and Sar 2009; Rensel, Haigh and Tynan, 2010; Guiry and Guiry, 2016). *Chaetoceros danicus* is considered to have a cosmopolitan distribution. Within Australia it has been recorded from sub-tropical to temperate waters along both the east and west coasts. *Chaetoceros danicus* has been implicated in fish mortality events globally, and, within Australia, it has been a component of the phytoplankton assemblage when other species of *Chaetoceros* have been implicated in fish mortality events.

Whilst several species of *Chaetoceros* (*Chaetoceros concavicornis*, *Chaetoceros convolutus*, *Chaetoceros danicus*, *Chaetoceros densus*, *Chaetoceros eibonii*, *Chaetoceros pendulum*, *Chaetoceros wighami*) have been reported as the primary causative factor in many fish mortality events globally, these mortalities are generally associated with caged or penned fish in aquaculture farms (Bell, 1961; Treasurer, Hannah and Cox, 2003; Rensel and Whyte, 2003). In natural environments, species of *Chaetoceros* are often implicated in fish mortality events but are not the sole causative factor. Fish mortality events in natural environments where species of *Chaetoceros* have been implicated typically involve a combination of other mitigating factors such as low dissolved oxygen concentrations, abnormally high water temperatures, pollution and or pathogens. It is a combination of two or more of these factors that lead to the mortality events rather than any one in isolation.

An example of this was a prolonged mortality event in South Australia during the summer of 2013-2014. This mortality event affected multiple marine species but predominantly benthic reef fish, abalone and dolphins (PIRSA, 2014). It was determined that the likely primary factor involved in the mortality event (at least for the fish and abalone) was unusually high water temperature, which caused heat stress, together with harmful algal blooms which included *Chaetoceros coarctatus*. The dolphin mortalities were associated with dolphin morbillivirus infection.

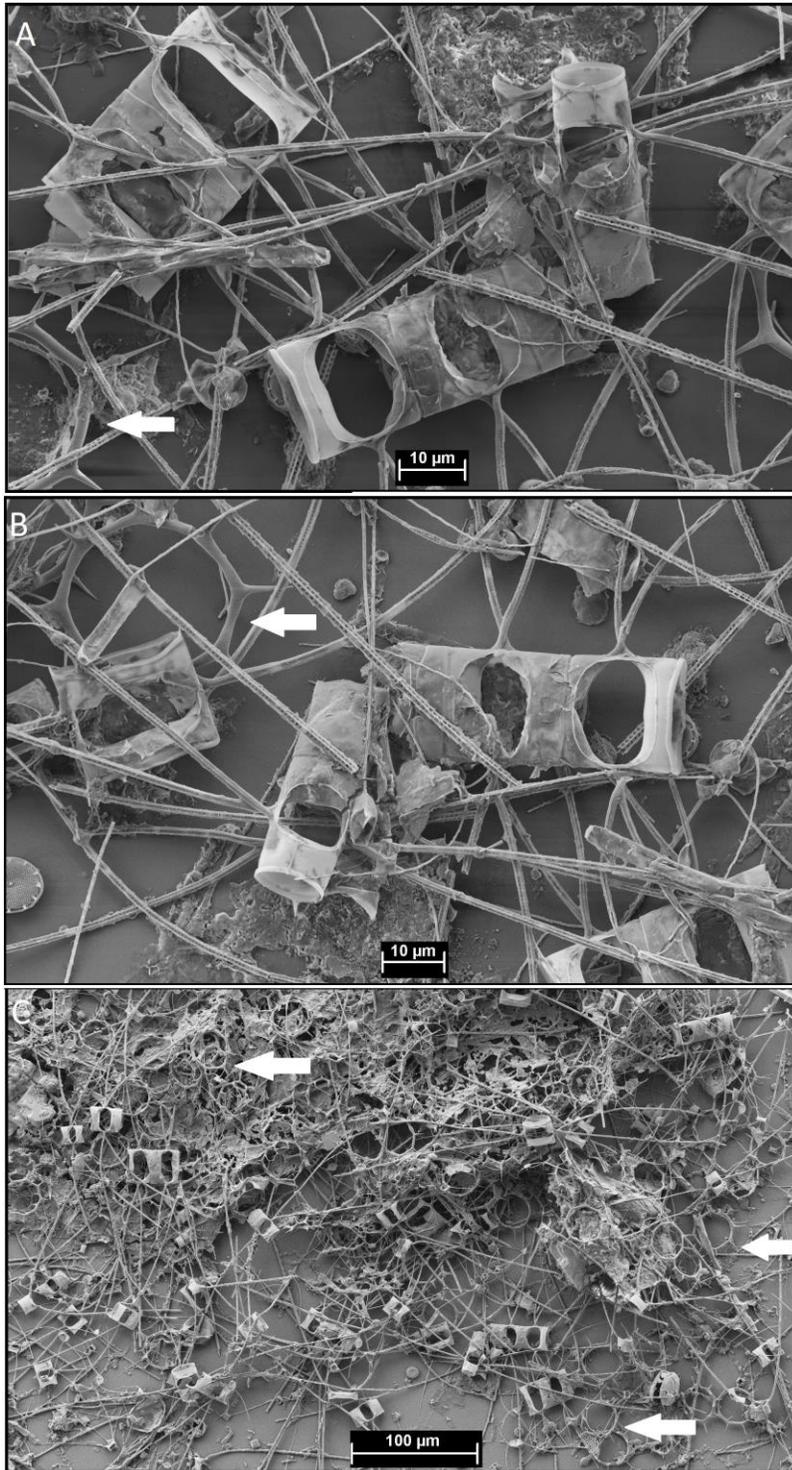


Figure 10. SEM micrographs showing high densities of *Chaetoceros* from Cockburn Sound (not during the current mortality event). Broken setae and parts of other diatoms are evident as is the silicoflagellate *Dictyocha octonaria* (white arrows), also a HAB species implicated in fish mortality events globally. *Dictyocha octonaria* was abundant in Cockburn Sound during the 1991-1994 survey.

Other examples of species of *Chaetoceros* being responsible for or implicated in fish mortality events include mortality events of farmed Atlantic salmon in several areas of Scotland (Bruno, Dear and Seaton, 1989), mortality of farmed Pacific salmon in Canada (Margolis and Evelyn, 1987) and the mortality of both farmed and wild fish populations in the Salish Sea (British Columbia, Canada) (Bell, 1961; Albright, Yang and Johnson, 1993; Rensel and Whyte, 2003)

Whereas some reports cite peak *Chaetoceros* cell densities of up to 460 000 cells L<sup>-1</sup> during mortality events (Treasurer, Hannah and Cox, 2003), others report densities as low as 5 000 cells L<sup>-1</sup> as being sufficient to restrict dissolved oxygen concentration to an extent that fish mortalities may occur (Bruno, Dear and Seaton, 1989) and densities as low as 400 cells L<sup>-1</sup> as increasing mortality rates of two species of salmon due to vibriosis and/or bacterial kidney disease (Bruno, Dear and Seaton, 1989).

Rensel (1993) conducted an interesting experimental study in which Atlantic Salmon (*Salmo salar*) were exposed to varying cell densities of *Chaetoceros concavicornis* to determine the minimum cell density at which these fish are adversely impacted. The means by which impact on the fish was assessed included partial pressure of gasses in the blood (O<sub>2</sub> and CO<sub>2</sub>), behavior of the fish (including “coughing” and swimming behavior/orientation) and histopathological examination.

The cell densities of *Chaetoceros concavicornis* to which the fish were exposed to ranged from 10 000 cells L<sup>-1</sup> to 400 000 cells L<sup>-1</sup>. Short (7 hour) and long (96 hour) bioassays were conducted to determine the impact of exposure time. Even at the lowest cell density, the fish began to exhibit a periodic “cough” immediately after the introduction of *Chaetoceros* cells to the experimental aquaria.

Fish exposed to the highest cell density of *Chaetoceros* (this density was only used in the 7 hour bioassay) began to die within three hours after initial exposure. Death was preceded by increased “cough” rates, gasping at the water-air interface and partial, and eventually total, loss of self-righting ability. There were observed increases in the partial pressure of blood CO<sub>2</sub> and decreases in the partial pressure of blood O<sub>2</sub> at all concentrations for both bioassays.

For the 7 hour bioassay, observed “cough” rates declined after initial exposure but these did not do so for the 96 hour bioassay suggesting that the duration of exposure is important and that, at some point, a point of no return with respect to the damage caused is reached. Histopathological examinations showed clear evidence of *Chaetoceros* cells and, mostly, setae contained within the mucilage secretions but no evidence of penetration into the gill tissue itself.

This is consistent with the histopathological examinations of fish gills for the current mortality events where no evidence of setae or other irritants were found in the gill tissue.

It is very likely that the high density of *Chaetoceros* recorded during the reported mortality events was a contributing factor. It is also evident, based on the historic data analysed, that such high densities of *Chaetoceros* in Cockburn Sound are a relatively recent event, post 2005, and that the frequency and magnitude of peaks in *Chaetoceros* density appear to be increasing. However, although this historic data covers a large time period (1992-1994 and 1999-2013), the bulk of it, 1999-2013, covers only two sites in Cockburn Sound and is the result of analyses that are considered by the author as inappropriate for phytoplankton monitoring programs.

The last comprehensive survey of the phytoplankton of the coastal waters of Perth was in 1991-1994 as part of the Southern Metropolitan Coastal Waters Study. Four to five sites in Cockburn Sound and four to five sites in Warnbro Sound were monitored fortnightly throughout this period for water quality, sediment quality, seagrass health, zooplankton assemblages and phytoplankton assemblages. The author of this report was responsible for the phytoplankton and zooplankton components of this study.

Since the completion of the Southern Metropolitan Coastal Waters Study, the only ongoing water quality monitoring in Cockburn Sound has been the routine phytoplankton monitoring undertaken by the shellfish aquaculture industry (WASQAP monitoring) at a small number of locations (typically 2 locations) and the annual summer-time water quality monitoring undertaken by the Cockburn Sound Management Council which incorporates a large number of sites within Cockburn Sound but which does not include phytoplankton analysis.

As discussed earlier, the historic data from Cockburn Sound presented herein also indicates that peaks in the abundance of *Chaetoceros* are increasing over time having become more prevalent in the last 10 years (Figure 9). This is unlikely to be the only broad scale change, but this data set has not been analysed in any more detail. Climate change is a likely explanation for this and is supported by data from the coastal waters of Perth from other studies that the author has been responsible for and from other studies world-wide as will be discussed below.

Broad-scale changes in the phytoplankton assemblages of the coastal waters of Perth outside of Cockburn Sound have been noticed by the author over the last 20 years with a shift in community structure towards increased dominance by diatoms and decreased dominance by dinoflagellates and small flagellates such as prasinophytes (personal observation). It is likely that the same changes are occurring in Cockburn Sound, as evidenced, in part, by the increased *Chaetoceros* abundances, although small flagellates are not represented in current or historic Cockburn Sound phytoplankton analyses.

There is an ever increasing body of research which indicates that phytoplankton assemblages are being affected by global climate change (Reid *et al.*, 1998; Vogt, 2015) and that this is happening at an unprecedented rate (Vogt, 2015). These changes will affect phytoplankton assemblages at several levels ranging from broad taxonomic group (e.g. Class) to species level.

Rivero-Calle *et al.* (2015) report that coccolithophorid (Prasinophyceae) abundance in the North Atlantic has increased significantly, by an order of magnitude, in the five decades leading up to their study and suggest that increased carbon dioxide is a likely factor although earlier studies of the same data set suggested that increased temperature was the main driver of the increase (Beaugrand *et al.*, 2013). This study by Rivero-Calle *et al.* (2015), which has now been supported by a significant number of other studies, is a contradiction to the general consensus that calcifying phytoplankton would be adversely affected by increased dissolved oceanic carbon dioxide (ocean acidification) and suggests that increased dissolved carbon dioxide actually stimulates the growth of this group of phytoplankton which is of considerable importance for marine and atmospheric carbon cycling. However, this study is based on recorded carbon dioxide concentrations over the last 50 years whereas other studies, supporting the adverse impacts on calcifying phytoplankton, are based on predicted carbon dioxide concentrations for the remainder of this century.

Rivero-Calle *et al.* (2015) suggest that coccolithophore abundance will continue to increase before stabilizing at a CO<sub>2</sub> concentration of around 500 ppm and that the trends which they observed represent a global trend.

McMahon *et al.* (2015) conducted a palaeoecological study in the North Pacific subtropical gyre over the past 1 000 years and identified three major shifts in the phytoplankton assemblages (regime) each coinciding with major climatic changes. The most significant of these is the most recent, at around 1850 – the onset of the industrial era, where the generally eukaryotic phytoplankton assemblage transitioned back to a cyanobacteria-dominated assemblage as it was prior to the Little Ice Age (~1400-1850), but, in contrast, the cyanobacteria post ~1850 were predominantly nitrogen-fixing (diazotrophic), a condition that continues today but is unprecedented in the 1 000 years covered by the study. The authors suggest that this most recent change is likely due to increased sea surface temperature, increased stratification and decreased nutrient availability and highlight both the unprecedented nature of the composition of the phytoplankton community compared to the past 1 000 years and the rapidity (165 years) at which this change has occurred.

In a study spanning 50 years (1960-2009) in the northeast Atlantic Ocean and the North Sea, and consistent with observations made by the author regarding the phytoplankton assemblages in the coastal waters of Perth cited above, Hinder *et al.* (2012) recorded a decline in dinoflagellate abundance, particularly since 2006. This decline was across the board for all dinoflagellate taxa including both HAB (Harmful Algal Bloom) and non-HAB taxa but there was no similar decline in diatom abundance which resulted in a marked increase in the relative abundance (relative dominance) of diatoms versus dinoflagellates. The abundance of some common diatom taxa, including HAB and non-HAB taxa showed an increase in abundance over the time frame of this study (Hinder *et al.*, 2012).

Detailed analyses of the data together with climatological data indicated that the change in phytoplankton community structure was driven by the interaction effect of both increasing sea surface temperatures and increasingly windy conditions during summer. No other phytoplankton groups were investigated during this study.

The opposite is happening in Perth's Swan River Estuary where, over the last few decades, there has been an increase in the relative dominance of dinoflagellates over diatoms (J. John, personal communication, Towie, 2014). A drying climate with decreased river flow is the likely cause of this change in the structure of the phytoplankton community (J. John, personal communication, Towie, 2014). There has also been a dramatic increase in the number of algal blooms in the Swan River Estuary over the past decade (Conservation Council of Western Australia, 2015) as well as unseasonal algal blooms during winter (Leitch, 2012).

In addition to the changes cited above, it is now well accepted that global changes in the abundance and distribution of diatoms and dinoflagellates, as well as increases in the occurrence of HAB species, are underlain by changing hydrological conditions associated with regional climate change, particularly climate warming (Reid *et al.*, 1998; Moore *et al.*, 2009; Hallegraeff, 2010, NCCOS, 2014a, b) and increasing concentrations of dissolved carbon dioxide (ocean acidification) (NCCOS, 2014a, b). This is in addition to existing factors implicated in the global increase of HAB species including ballast water transport, aquaculture development, transport of seed stock and eutrophication of coastal waters due to human activities (Anderson, 1989; Hallegraeff, 1993).

In recent years, scientists have observed that the global occurrence of harmful algal blooms (HABs) have increased due to the effects of climate change (Hinder *et al.*, 2012; US EPA, 2013). These increases have been with respect to the frequency of HAB blooms, the severity of HAB blooms and the geographic distribution of HAB Blooms (US EPA, 2013).

**NOTE 3: It is important to note the distinction in the use of the word “bloom” when describing “algal blooms” and “harmful algal blooms”. Algal blooms, as defined herein previously, typically describe an unusual and exceptionally large biomass of algae which is usually significant enough to cause adverse water quality conditions including discolouration and deoxygenation. Harmful algal blooms, on the other hand, typically describe an occurrence of potentially harmful algae (toxigenic algae or algae that can be harmful in other ways as described herein for *Chaetoceros*) in numbers significant enough for them to pose a threat to other marine life or humans. The density of HAB algae required for them to be a threat can be quite low.**

The alga responsible for the majority of blooms and fish mortality events in the Swan River Estuary, as discussed above, is the HAB dinoflagellate species *Karlodinium veneficum*. *Karlodinium veneficum* is an ichthyotoxic dinoflagellate which is responsible for numerous fish mortality events worldwide (Place *et al.*, 2012) including the Swan River Estuary where it is now considered to be a persistent problem (Adolf *et al.*, 2015). Blooms of *Karlodinium veneficum* were not reported from the Swan River Estuary prior to 2002, although Gyrodinioid/Gymndinioid dinoflagellates (*Gyrodinium estuariale* and *Gynodinium galatheanum*) recorded from the Swan River Estuary prior to 2002 may indeed have been *Karlodinium veneficum* (Adolf *et al.*, 2015).

Again, a drying climate resulting in reduced river flow and an altered salinity regime (toward prolonged marine conditions) is considered to be the primary cause of the increase of this HAB species (J. John, personal communication, Towie, 2014). It is anticipated that the ongoing effects of climate change will result in a continued drying of south western Australia (Australian Academy of Science, 2015; Delworth and Zeng, 2014) which will continue to impact on the Swan River Estuary.

*Karlodinium veneficum* is considered to be a coastal marine/estuarine species. Although not yet reported in significant densities in Cockburn Sound or the coastal waters of Perth, the persistent and increasing blooms of this HAB dinoflagellate in the Swan River Estuary should be of concern with respect to the waters into which the Swan River Estuary flows. *Karlodinium veneficum* is potentially the first of several HAB species to increase in density and/or bloom frequency in the estuarine and coastal waters of Perth as a result of climate change.

The likely threat of increases in the geographic distribution, frequency, magnitude and duration of HABs as a result of climate change is perhaps best summarised by Hallegraeff (2010) who cites increasing temperature, enhanced surface stratification, alteration of ocean currents, intensification or weakening of local nutrient upwelling, stimulation of photosynthesis by elevated CO<sub>2</sub>, reduced calcification through ocean acidification (although this may not be the case, see above study by Rivero-Calle *et al.* (2015)) and heavy precipitation and storm events causing changes in land runoff and micronutrient availability as all being likely to have impacts on the composition of marine phytoplankton communities.

Hallegraeff goes on to cite the likely effects of these climatological changes on phytoplankton communities, in particular HAB species to be: the expansion in range of warm-water species at the expense of cold-water species which will be driven poleward, species-specific changes in the abundance and seasonal window of growth of HAB taxa, the earlier timing of peak production of some phytoplankton, and secondary effects for marine food webs, notably when individual zooplankton and fish grazers are differentially impacted by climate change.

Hallegraeff (2010) warns that the greatest problems for human society will be caused by being unprepared for significant range expansions or the increase of HAB problems in currently poorly monitored areas and calls for increased vigilance and monitoring programs emphasising that changes in phytoplankton communities provide a sensitive early warning for climate-driven perturbations to marine ecosystems.

There are now a multitude of studies that indicate that phytoplankton assemblages, including the distribution, frequency and duration of HABs, are significantly impacted by the ongoing effects of climate change, the evidence is overwhelming and the timeframes involved are alarmingly short – years to decades. Most of these studies suggest that the impact of climate change on phytoplankton assemblages, and particularly HABs, will be greatest in coastal areas and will exacerbate the impacts of those factors that already contribute to HABs.

Although tentative, altered ocean currents (possibly associated with a strong and persistent El Niño period) have already been postulated as a potential cause of altered phytoplankton regimes in Cockburn Sound and Perth's coastal waters (Helleren and John, 1997; Helleren, 2003).

This is not unprecedented in the Australian region; the East Australian Current has an increased southward penetration as a result of changes in surface winds, ocean temperature and ocean salinity (Ridgway, 2007; Australian Academy of Science, 2015) and climate models predict an increase in the frequency and intensity of El Niño events as a result of climate change (Wenju *et al.*, 2014).

It is now 20 years since any significant monitoring of Cockburn Sound which has included phytoplankton analysis and there has never been a broader study (longer than a few weeks) investigating the phytoplankton of Cockburn Sound and the broader coastal waters of Perth concurrently. There is evidence that indicates that both the phytoplankton of Cockburn Sound and Perth's coastal waters have undergone significant change over the last 20 or so years including likely increases in the abundance HAB phytoplankton and the magnitude and frequency of peaks in HAB phytoplankton abundance as has been the case in the Swan River Estuary.

Little is known about the current phytoplankton assemblages within Cockburn Sound (the methods used by WASQAP are inappropriate for a scientifically rigorous monitoring program) and the broader coastal waters of Perth and nothing is known about how these assemblages have changed over the past several decades or in response to climate change.

## Conclusion

It is quite clear that, according to the scientific literature available, the cell densities of *Chaetoceros* observed during the fish mortality events in Cockburn Sound during November and December 2015, particularly the peak recorded density of 740 000 cells L<sup>-1</sup> during early November 2015, would be sufficient to cause widespread fish mortality. However, it should be noted that, most fish mortality events around the world for which *Chaetoceros* species are considered to be responsible, and most of the research consulted and cited herein, have occurred in farmed aquaculture situations where the fish are caged or penned and are unable to escape or avoid the harmful conditions that these high densities of *Chaetoceros* cells present. These fish are also stocked in unnaturally high densities which may make them more prone to the impacts of external stressors than fish in their natural environment would be.

Having said this however, natural fish populations do occasionally succumb to the impacts of harmful algal blooms including the non-toxicogenic, mechanical effects of high densities of silicified micro-algae which contain spines and other protuberances (e.g. diatoms of the genera *Chaetoceros*, *Cylindrotheca*, *Rhizosolenia* etc. and silicoflagellates such as species of *Dictyocha*).

Based on the data made available to the author, (species and cell density data, comments on water quality and comments on the histopathology of affected fish), it is likely that mechanical irritation (and possibly damage) to fish gills as a result of relatively high (but not unprecedented) cell densities of species of the diatom genus *Chaetoceros* were a potential contributing factor to the fish mortality events observed in Cockburn Sound during November and December 2015.

It is clear from the research and data presented herein that quite low cell densities of *Chaetoceros*, as low as 400 cells L<sup>-1</sup>, can stress some species fish sufficiently that they are prone to the, possibly lethal, impacts of other stressors such as disease. It is most likely that multiple stressors, impacting the fish of Cockburn Sound at the same time, including low dissolved oxygen concentrations, higher than usual water temperatures and the high *Chaetoceros* cell densities combined to cause the observed mortality events. Most likely, any one of these stressors alone would not have been sufficient to adversely impact the fish populations of Cockburn Sound, but together they comprised a “perfect storm” of stressors.

It is now well accepted that the impacts of climate change are causing changes in phytoplankton community structure and particularly resulting in an increase in the frequency and magnitude of HABs in marine and estuarine environments globally. Analysis of historic data from Cockburn Sound indicates that the frequency and magnitude of peaks in *Chaetoceros* density have increased, particularly since 2005 and that there also seems to have been a change in seasonality of these peaks from summer to autumn and winter.

Whilst a general increase in the dominance of diatoms is consistent with changes in phytoplankton assemblages globally, the lack of additional information from Cockburn Sound precludes any attempt to link this change in phytoplankton community structure to climate change. On the other hand, an increase in the frequency and intensity of harmful algal blooms and fish mortality events in the Swan River Estuary (also since 2005), has been directly attributed to climate change.

It is certain that there have been and will continue to be impacts on the coastal and estuarine phytoplankton assemblages of the Perth region and of the south western Australian region in general as a result of climate change. These impacts are largely unknown though due to a lack of monitoring programs but prominent researchers in the field warn that the environmental and economic consequences will be serious and that only ongoing monitoring will provide a sensitive early warning for climate-driven perturbations to marine ecosystems.

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