



**The role of phytoplankton as pre-cursors for disinfection by-product formation
upon chlorination**

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Statement of Originality

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Abstract

Exposure to halogenated organic compounds known as disinfection by-products (DBPs) has been associated with congenital birth defects and increased occurrences of bladder and colorectum cancers. The suite and hence toxicity of the DBPs produced upon disinfection is best understood by the chemical composition and concentration of the reactive natural organic matter (NOM), which in turn is determined by the biogeophysical conditions of the catchment. Local meteorological conditions influence the relative allochthonous (external) and autochthonous (internal) organic matter loads contributing towards the total carbon budget of the aquatic system. This thesis investigates the correlation of autochthonous organic matter with DBP formation by focusing on role of phytoplankton precursors towards the formation of DBPs.

The relative contribution of allochthonous and autochthonous NOM sources towards DBP formation was investigated by comparing winter inflows during a rainfall event and summer phytoplankton blooms. Winter inflows increased the relative contribution of allochthonous organic matter, correlating with the highest measured concentration of dissolved organic carbon (DOC). However, this did not coincide with the highest DBP formation recorded. Instead, summer phytoplankton blooms were significantly more reactive per milligram of DOC producing significantly higher concentrations of DBPs than winter inflows. Further, summer phytoplankton blooms increased the concentration of unknown DBPs (UTOX) and produced more genotoxic nitrogenous DBPs (N-DBPs).

Phytoplankton organic precursors are often associated with a higher composition of hydrophilic organic matter, which is often recalcitrant to conventional treatment. This could potentially result in higher DBP formation after conventional treatment during periods where autochthonous NOM loads account for significant proportion of the carbon budget. The correlation between hydrophobic and hydrophilic properties was investigated via a linear model. This analysis highlighted the significant contribution of highly recalcitrant, hydrophilic neutrals towards the formation of DBPs, UTOX and towards specific categories of DBP compounds.

The relative hydrophilic and hydrophobic composition of phytoplankton is determined by the cells chemical composition and biological processes, which vary significantly

between each species. Simulated phytoplankton blooms of *Dolichospermum circinale* (cyanobacteria) and *Ankistrodesmus* sp. (green algae) were compared to determine their relative DBP formation upon chlorination. It was determined that *Ankistrodesmus* sp. contributed significantly more towards total DBP formation than *D. circinale* relative to their cellular biovolume. However, *D. circinale* produced higher concentrations of N-DBPs which increases the relative genotoxicity of the DBPs formed.

Finally, the removal efficiency of DBP precursors was investigated during a substantial surface phytoplankton bloom. The bulk of the phytoplankton bloom did not enter the treatment plant during this investigation due to thermal stratification of the reservoir and alteration in offtake height. The conventional treatment was efficient at removing the bulk of the organic matter however; the concentration of charged neutrals remained insignificantly affected. The product water contained significantly higher concentrations of DBPs during the phytoplankton bloom in comparison to historic values however; the treated water would comply with the drinking water guidelines. Total unregulated DBP formation was still significant and was comprised of 44.8% of unknown DBPs.

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Abbreviations

ADWG	-	Australian Drinking Water Guidelines
AIC	-	Akaike Information Criterion
AOM	-	Algal Organic Matter
AOX	-	Adsorbable Organic Halogen
AQC	-	Analytical Quality Control
C-DBP	-	Carbonaceous Disinfection By-product
CHA	-	Charged Hydrophilic Acids
CP	-	Chloropicrin
DBP	-	Disinfection By-Product
DBPFP	-	Disinfection By-product Formation Potential
DHAA	-	Dihaloacetic Acid
DCAA	-	Dichloroacetic Acid
DHAN	-	Dihaloacetonitrile
DO	-	Dissolved Oxygen
DOC	-	Dissolved Organic Carbon
DON	-	Dissolved Organic Nitrogen
DPD	-	N, N-diethyl-p-phenylenediamine
EOM	-	Extracellular Organic Matter
HAA	-	Haloacetic Acid
HAN	-	Haloacetonitrile
HK	-	Haloketone
IOM	-	Intracellular Organic Matter

LRV	-	Log10 Reduction Value
N-DBP	-	Nitrogenous Disinfection By-product
NDMA	-	N-nitrosodimethylamine
NEU	-	Hydrophilic Neutral
NLA	-	National Lake Assessment
NOM	-	Natural Organic Matter
NTU	-	Nephelometric Turbidity Unit
PES	-	Polyethersulphone
SHA	-	Slightly Hydrophobic Acids
SUVA	-	Specific Ultraviolet Absorbance
TCAA	-	Trichloroacetic Acid
tHAA	-	total Haloacetic Acids
THAA	-	Trihaloacetic Acid
tTHM	-	total Trihalomethanes
THM	-	Trihalomethane
THMFP	-	Trihalomethane Formation Potential
TOC	-	Total Organic Carbon
TOX	-	Total Organic Halide
TP	-	Total Phosphorus
US EPA	-	United States Environmental Protection Agency
UTOX	-	Unknown Total Organic Halide
UV	-	Ultraviolet
VHA	-	Very Hydrophobic Acids
WHO	-	World Health Organisation

WTP - Water Treatment Plant

Chapter 1

General Introduction

The separation of drinking water from wastewater is considered one of the biggest contributors to the advancement of human health, free from the burden of waterborne disease (Brookes and Carey 2015). The introduction of chlorinated water in the early 1900s enabled the successful disinfection of drinking water whilst maintaining a disinfectant residual throughout the distribution network. The disinfection of water is necessary to form a chemical barrier to combat pathogens (Centers for Disease Control and Prevention 2012). However, it was later discovered that chlorine reacts with natural organic matter (NOM) to form chlorinated by-products known as disinfection by-products (DBPs) (Bellar *et al.* 1974a; Rook 1974). Since this discovery, over 600 disinfection by-products (DBPs) have been identified in drinking water samples and via experimental laboratory analysis (Hebert *et al.* 2010). Epidemiological studies have associated the exposure of DBPs via ingestion, dermal adsorption and inhalation with several cancers and congenital malformations (Villanueva *et al.* 2004; Wright *et al.* 2004; Hinckley *et al.* 2005; Richardson *et al.* 2007; Villanueva *et al.* 2007; Hwang *et al.* 2008; Cantor *et al.* 2010). Although numerous studies have identified associations between excessive DBP exposure and increased risk of serious health conditions, the causal association is still unknown as the attribution of toxicology to human health outcomes is difficult (Hrudey 2009; Nieuwenhuijsen *et al.* 2010; Hrudey *et al.* 2015). Therefore, investigation into contributing factors of DBP formation is required to minimise the potential health risks associated with their exposure.

The use of alternative disinfectants such as chlorine dioxide, chloramine, ozone and UV changes the suite of DBPs formed without resolving the fundamental issue of their formation (Hua and Reckhow 2007b). In fact, alternative disinfectants can increase the genotoxicity of the DBPs formed (Hua and Reckhow 2007b; Richardson *et al.* 2007). As such, the crux of the problem is identified as the concentration and chemical composition of the NOM reacting with the disinfectant. Therefore, identifying the reactivity of NOM loads and improving methods to reduce the concentration of NOM exposed to the disinfectant post-treatment needs to be the focus of future research into DBPs. This focus will improve DBP management and therefore reduce risk of exposure. NOM is often

defined by its origin as catchment organic matter (allochthonous) or internally produced carbon from phytoplankton, macrophytes and bacteria (autochthonous). Allochthonous NOM is often comprised of a high aromatic content, humic acids and fulvic acids, characterised as hydrophobic (Hwang *et al.* 2001; Leenheer and Croué 2003; Bond *et al.* 2011a). Alternatively, autochthonous NOM has a higher composition of carboxylic acids, polyuronic acids, amino acids, peptides, proteins and carbohydrates, which are commonly characterised as primarily hydrophilic (Leenheer and Croué 2003; Bond *et al.* 2011a).

A large compilation of literature has investigated the correlations between NOM and DBP formation, providing critical insight into this complicated relationship summarised by Sadiq and Rodriguez (2004) and Chowdhury *et al.* (2009) and references therein. Numerous studies have identified that the bulk hydrophobic NOM fraction is strongly correlated with the formation of DBPs, indicating that terrestrial NOM is the primary driver of DBP formation (Singer 1993; Kitis *et al.* 2002; Hua and Reckhow 2007a; Soh *et al.* 2008; Hua *et al.* 2015). Substantial influxes of terrestrial NOM are often introduced to the aquatic environment during a rainfall event which causes catchment runoff. Therefore, high hydrophobic NOM loads are often expected during a typical South Australian winter, when rainfall is higher. Correlations between hydrophobic NOM and DBP formation has provided crucial information regarding the significance of removing the bulk hydrophobic NOM fraction during treatment to ensure reduced DBP concentrations. Fortunately, hydrophobic NOM is readily removed during conventional treatment whereas hydrophilic fractions are less prone to coagulation and are therefore generally more recalcitrant to conventional treatment (Singer and Harrington 1993; Kim and Yu 2005; Matilainen *et al.* 2010; Lui *et al.* 2011). The recalcitrant nature of hydrophilic NOM could increase the potential of DBP formation post-treatment within systems with a higher relative contribution of autochthonous carbon. The contribution of phytoplankton towards the total organic carbon load of a reservoir can be substantial; contributing 25-50% of the total dissolved organic carbon (DOC) load of Myponga Reservoir during a period of low annual rainfall (Linden 2008). Further, the eutrophication of freshwater environments has resulted in the apparent increased frequency of phytoplankton blooms worldwide (Paerl and Huisman 2008; Brookes and Carey 2011) which could potentially have severe consequences given the increased concentration of recalcitrant hydrophilic NOM and resultant DBP formation.

The extensive literature relating to DBP formation has also focused on the risks of exposure to DBPs via epidemiological studies. Research has identified the increased genotoxicity and cytotoxicity of nitrogenous disinfection by-products (N-DBPs) compared with carbonaceous DBPs (Plewa *et al.* 2004a; Plewa *et al.* 2004b; Richardson *et al.* 2007; Plewa *et al.* 2008). A potential increase in N-DBP formation could occur in environments with elevated contribution of autochthonous carbon due to significantly lower carbon to nitrogen ratios observed in freshwater autotrophs than terrestrial autotrophs (Elser *et al.* 2000). Furthermore, phytoplankton are a major source of dissolved organic nitrogen, capable of exuding up to 45% of their total fixed nitrogen, further increasing the risk of elevated N-DBP formation (Nguyen *et al.* 2005b; Zhang *et al.* 2014).

There is notable literature that investigates the relationship between phytoplankton organic matter and DBP formation (Graham *et al.* 1998; Nguyen *et al.* 2005b; Hong *et al.* 2008; Huang *et al.* 2009; Fang *et al.* 2010; Lui *et al.* 2011; Wei *et al.* 2011; Li *et al.* 2012; Zhang *et al.* 2014). Extending this research; this thesis further investigates the contribution of autochthonous phytoplankton NOM towards the formation of DBPs. This was achieved by initially investigating the role of phytoplankton as pre-cursors for DBP formation via a comprehensive literature review in Chapter 3. This review considers the chemical composition of phytoplankton, recalcitrance of hydrophilic NOM to conventional treatment, the increased occurrence of phytoplankton blooms, intracellular and extracellular NOM loads and how this all relates to DBP formation. The summary of the review highlights the necessity to further investigate the risk of DBP formation in relation to phytoplankton NOM. The thesis then compares the DBP formation potential of allochthonous and autochthonous NOM loads in Chapter 4. This was achieved by comparing a substantial winter rainfall event and summer phytoplankton blooms to provide insight into the reactivity of both allochthonous and autochthonous sources of NOM with chlorine to produce DBPs. Chapter 4 also highlighted the lack of correlation between the concentration of DOC, and DBP formation potential. To better understand how organic carbon correlates with DBP formation, the relationship between hydrophobic and hydrophilic NOM fractions and resulting DBP formation is investigated in Chapter 5. A linear model was utilised to investigate how each fraction and their interactions correlate with DBP formation. Chapter 6 aimed to investigate the contribution of phytoplankton towards DBP formation by comparing formation potential

of raw water samples with samples injected with known cell biovolumes of cultured phytoplankton species. The two species investigated were *Dolichospermum circinale* (cyanobacteria) and *Ankistrodesmus Sp.* (chlorophyta), chosen for their common occurrence in the system sampled. The different species and variations of cell biovolumes were cross compared to determine their influence on concentration and speciation of DBPs formed. The final data chapter investigates the removal efficiency of DBP precursors via conventional treatment was analysed during a substantial cyanobacterial bloom in Chapter 7. Finally, the findings of this thesis are concluded in Chapter 8.

Chapter 2

Materials and Methodology

This chapter highlights the general materials and methodology used throughout this thesis, providing a general overview of the site description as well as field and laboratory methods. Specific methods relating to experiments performed in subsequent chapters are detailed within those chapters.

2.1 Site Description

The sampling site for this thesis was Myponga Reservoir, located south east of Adelaide, South Australia (Figure 1). The reservoir contains 26,800 ML at capacity, with a maximum depth of 42 metres. The Myponga catchment spans an area of approximately 124 km², with mixed land use of dairy pastures, beef and hay farming and patchy remnant native vegetation. The primary sources of carbon within Myponga Reservoir are derived from the catchment, with the majority of flows entering the system via Myponga River (Linden *et al.* 2004). Artificial destratification is implemented within the reservoir in an attempt to reduce the autochthonous nutrient load by reducing internal phosphorus release from sediments by creating oxic conditions and mixing thermal bands; however, cyanobacterial blooms are still common during summer (Lewis *et al.* 2004; Linden *et al.* 2004).

Currently, there is no public access to Myponga Reservoir which minimises anthropogenic pressures affecting the treatability of the water. Water from Myponga Reservoir is treated on site at the Myponga Water Treatment Plant, where it is then stored and distributed. Key sampling locations have been highlighted as the water treatment plant (A); the dam wall where surface samples and intake samples are collected (B); a secondary inflow, Barclay Rd. Creek (C); and the main inflow, Myponga River (D) (Figure 1).

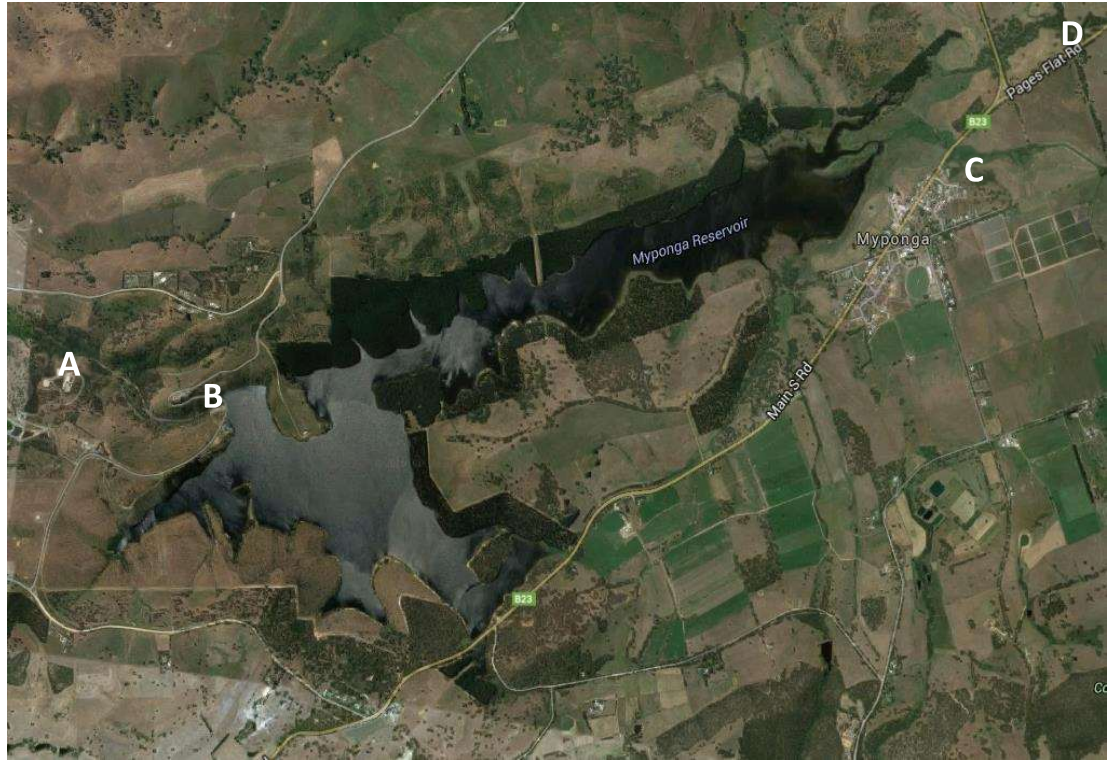


Figure 1. Satellite image of Myponga Reservoir with key locations highlighted: Myponga Water Treatment Plant (A), the dam wall (B), Barclay Rd. Creek (C) and Myponga River (D).

2.2 Field Methods

2.2.1 Sample Collection

The method of sample collection varied between locations and the type of analysis that was required. Samples from the water treatment plant, intake and Myponga River were collected via an access tap. The taps were flushed for several minutes prior to collection. Samples from the surface and Barclay Rd. Creek required submerging a container for collection. For surface samples, collection was achieved by dropping a container over the dam wall and hauling it up. All samples were collected in triplicate to minimise bias from sampling technique and to ensure that the results were statistically sound.

All containers used for sampling were initially rinsed thoroughly with Milli-Q water from the laboratory, and were rinsed again with the sample prior to filling in the field. Samples for water quality and disinfection by-product (DBP) analysis were collected in 10 L polypropylene containers and capped with no air gap. Samples for chlorophyll *a* analysis were collected in dark 1 L plastic bottles to ensure minimal degradation of the chlorophyll *a* pigment. Upon collection, samples were transported immediately to the laboratory in cool conditions and then refrigerated at 4°C.

2.2.2 Field Data Collection

Water quality parameters such as temperature, turbidity, conductivity, chlorophyll *a*, dissolved oxygen and blue green algae counts are routinely monitored via an automated vertical profiling system at Myponga Reservoir. This system provided detail relating to the thermal profile of the reservoir and pre-emptive notification of phytoplankton bloom occurrences, allowing for a structured sampling regime to capture significant events and conditions.

2.3 Laboratory Methods

2.3.1 Sample Filtration

Polyethersulfone (PES) membrane filtration was required to investigate dissolved organic carbon (DOC) concentrations, UV₂₅₄ and colour₄₅₆ absorbance and for the removal of phytoplankton cells. PES filter papers with a pore size of 0.45µm were chosen to remove particulates from the raw water. Filtrations were performed with a vacuum apparatus, a Buchner flask and a Milli-Q filter holder. Filter papers were initially rinsed with 500 mL of Milli-Q water to remove any particulates or contaminants from the filter paper. The maximum volume of raw water filtered through each individual membrane was limited to 100 mL to minimise the influence of caking. The volume filtered through each PES membrane was reduced if caking was observed which generally occurred for samples with high turbidity.

2.3.2 Sample Dilution

The dilution of samples was required to ensure that the measurements taken were within the range of low and high analytical quality control (AQC) standards. Samples that required dilution were diluted with Milli-Q water in volumetric glassware.

2.4 Organic matter Characterisation

2.4.1 Dissolver Organic Carbon

Sample DOC was measured using the Sievers 900 Laboratory TOC Analyser. Samples were analysed in batches alongside Milli-Q water blanks, AQC blank, low and high AQC's, a 25 ppm organic carbon standard, a 25 ppm inorganic carbon standard and a standard containing 25 ppm of both organic and inorganic carbon standards. All samples were filtered through a 0.45 µm polyethersulfone membrane filter to ensure the removal of particulates. The results were validated when all AQC's were reported within two standard deviations of the prepared standards.

2.4.2 Rapid Fractionation

Organic matter was further characterised by the implementation of the rapid fractionation process, established by Chow *et al.* (2004) (Figure 2). The organic matter was fractionated into four distinct groups based on its hydrophobic and hydrophilic properties; very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), hydrophilic charged (CHA) and hydrophilic neutrals (NEU). Samples were initially filtered through a 0.45 µm polyethersulfone membrane to remove particulate organic matter and then pH adjusted to < 2 with 1M HCl. Fractionation was achieved by the sequential filtration of samples through DAX-8, XAD-4 resins at 3mL/min to retain the VHA and SHA fractions respectively, ensuring filtrate aliquots were taken for DOC analysis after filtration through each resin. The remaining filtrate was then pH adjusted to 8 with 1M NaOH and filtered through the IRA-958 resin at 3 mL/min to retain the CHA fraction. An aliquot of the IRA filtrate was taken for DOC analysis. The concentration of each hydrophobic and hydrophilic fraction was back calculated from the DOC results.

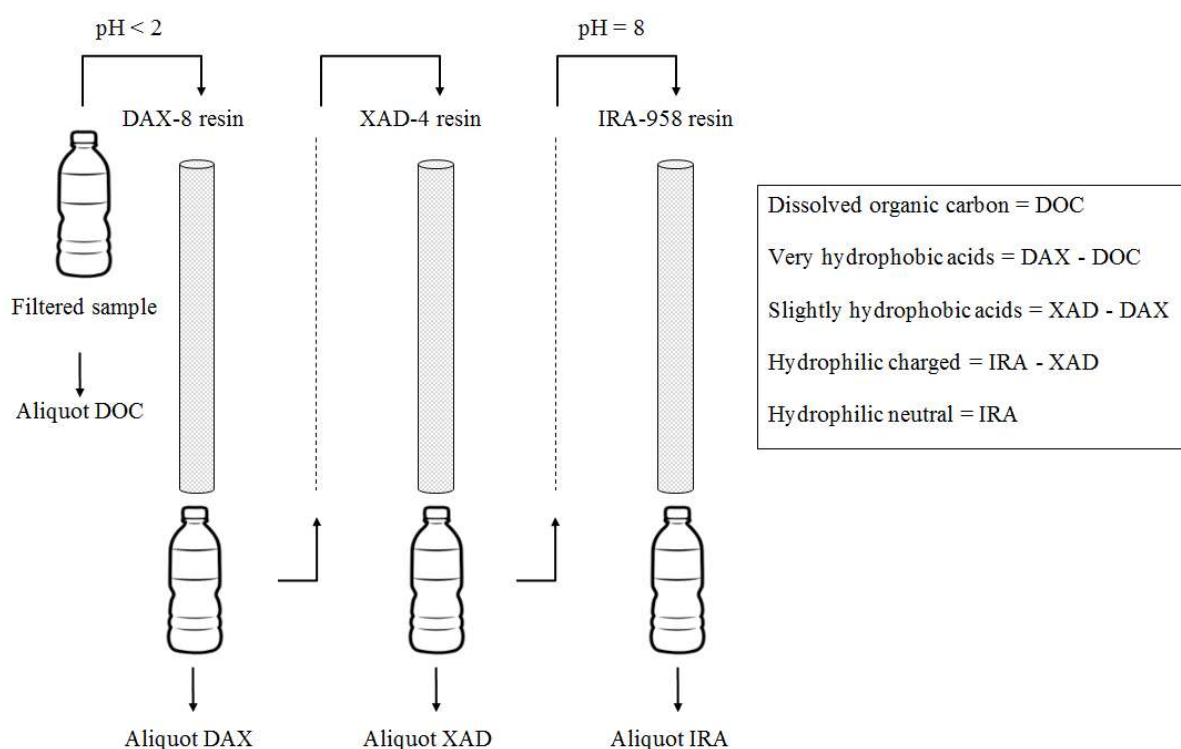


Figure 2. Schematic of the rapid fractionation method

2.4.3 Temperature and pH

Temperature and pH were measured with a WTW ProfiLine pH 3110 portable meter. Temperature and pH measurements were recorded when the pH had stabilised. pH values were also checked against acidic, neutral and alkaline pH buffers as well as a pH AQC. All pH results were validated by ensuring the AQC sample was within two standard deviations from the prepared standard.

2.4.4 UV₂₅₄ Absorbance

The UV₂₅₄ absorbance was measured using the Thermo Scientific Evolution 60 spectrophotometer. The instrument was set to read at wavelength 254 nm and zeroed with Milli-Q water blank with a 1 cm path length quartz cuvette. Samples were then filtered through a 0.45 µm polyethersulfone membrane filter and then analysed. The cuvette was rinsed with Milli-Q water and with the sample prior to analysis.

2.4.5 True Colour₄₅₆

True colour₄₅₆ was measured using the Thermo Scientific Evolution 60 spectrophotometer. The instrument was set to read at wavelength 456 nm and zeroed with Milli-Q water blank with a 5 cm path length quartz cuvette. The instrument was calibrated against a standard prior to use, and the results were validated if both the low and high AQC standards were within two standard deviations of the prepared standards. Samples were then filtered through a 0.45 µm polyethersulfone membrane filter and then analysed. The cuvette was rinsed with Milli-Q water and with the sample prior to analysis.

2.4.6 Turbidity

Turbidity was determined using a Hach 2100AN Turbidimeter. Sample vials were handled with lint free Kimwipes tissue paper to minimise risk of interference during analysis. The vial was rinsed with Milli-Q water and with the sample prior to analysis. Results were validated when the low, intermediate and high turbidity AQC's were all within two standard deviations from the prepared standards.

2.4.7 Chlorophyll a

Chlorophyll a analysis was performed using the Agilent Technologies Cary 60 UV-Vis Spectrophotometer. Samples were filtered through a 47 mm Whatman glass microfiber filter (grade GF/C) to retain any phytoplankton present in the solution. The filter paper was then dried under vacuum and placed in a vial containing 10 mL of ethanol (96%) to extract the chlorophyll a pigment. The vial was sealed and vortex mixed at 1800 RPM for one minute. Eluted samples were then placed into a freezer at -24 °C for at least 12

hours. After the elapsed time, samples were removed from the freezer and allowed to return to room temperature (~20 °C). Samples were then syringe filtered through a 0.45 µm membrane filter and analysed at 750 nm, 665 nm and 649 nm with a 1 cm path length quartz cuvette. Chlorophyll a concentration was then determined by the following equations:

$$E_{665} = \frac{OD_{665} - OD_{750}}{P}$$

$$E_{649} = \frac{OD_{649} - OD_{750}}{P}$$

$$\text{Chlorophyll } a \text{ (}\mu\text{gL}^{-1}\text{)} = \frac{13.7E_{665} - 5.76E_{649}}{V_F}(V_E)$$

Where OD665 is the optical density at 665 nm, OD649 is the optical density at 649 nm, OD750 is the optical density at 750 nm, P is the path length of the cuvette, VF is the volume of sample filtered and VE is the volume of ethanol used during the extraction.

2.4.8 Chlorination

2.4.8.1 Chlorine Demand

The chlorine demand of each sample was determined to ensure that the doses given retained a chlorine residual of approximately 0.5 mg/L after a contact time of 72 hours, simulating the maximum retention time and conditions within the local distribution network. Replicates of each sample were dosed with concentrations of chlorine in increasing increments of 5 mg/L and placed in a room absent of light for 72 hours at room temperature (~20°C). After the elapsed time, the residual chlorine concentrations were measured with the DPD colourimetric titration method (Harp 1995; APHA *et al.* 2005). The replicate that produced a residual of $\leq 5\text{mg/L}$ had sufficiently satisfied the largest DBP formation potential and was used to calculate the appropriate dose for the chlorination experiments. Samples with chlorine concentrations $> 5\text{mg/L}$ were outside of the calibration curve and were therefore disregarded. Achieving the appropriate chlorine dose for each sample was critical to ensure that sufficient chlorine was present to react

with the organics for DBP analysis without significantly skewing the outcome by overdosing or under dosing the samples.

2.4.8.2 Chlorine Dosing

Samples were then dosed with the predetermined chlorine doses and stored in a room absent of light for 72 hours at room temperature ($\sim 20^{\circ}\text{C}$). After the elapsed time, the residual chlorine concentrations were checked to ensure the concentrations were indeed approximately 0.5 mg/L. Residual chlorine was then quenched with ammonium chloride and the samples were stored in vials with no air gap in preparation for DBP analysis.

2.5 Disinfection By-Product Formation

2.5.1 Adsorbable Organic Halogens

Total DBP formation was determined by measuring the concentration of adsorbable organic halogens (AOX) formed in chlorinated samples. This was achieved with the Mitsubishi Chemical Analytech Total Organic Halogen Analyser. Samples were filtered at a rate of 3.3 mL/min through two sequential packed carbon columns. Organic halogens were extracted from the sample onto the activated carbon. The columns were then washed with 10 mL of KNO_3 solution to remove inorganic halogens prior to analysis. The activated carbon from the columns was then injected into a furnace at 950°C . The carbon was then combusted in oxygen flow and converted into hydrogen halide. Vapours were then dehydrated within a dehydrating tube containing concentrated H_2SO_4 . The vapours then flowed into a titration cell causing a potential difference to be detected and then titrated against. The energy required to complete the titration was converted into the quantity of halogens present ($\mu\text{g/L}$) using Faraday's Law. Results were validated by analysing blanks and trichlorophenol standards with every analytical run performed. An electrolyte cell check was also performed by injecting a known volume of 0.01M HCl into the titration cell prior to analysis to ensure that the electrode produced an appropriate response curve, and accurately estimated the concentration of HCl injected.

2.5.2 Haloacetic Acids

Analysis of haloacetic acids (HAAs) determined the formation of bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid, dibromoacetic acid, dichloroacetic acid, bromoacetic acid, chloroacetic acid, tribromoacetic acid and trichloroacetic acid (APHA *et al.* 2005). This was achieved by pH adjusting chlorinated samples to 0.5 to extract acids via a liquid-liquid extraction method with methyl tert-butyl ether. Upon reaction with diazomethane, the extracted acids were converted to their methyl esters. Excess diazomethane was then removed and the remaining methyl esters were analysed via gas chromatography using the Varian GC/ECD with dual column configuration.

2.5.3 Trihalomethanes

Analysis of trihalomethanes (THMs) determined the formation of bromodichloromethane, bromoform, chloroform and dibromochloromethane (APHA *et al.* 2005). THM formation was determined using the Perkin Elmer head space/ECD single column. Samples were transferred and sealed in vapour tight vials. The vials were heated and agitated to ensure that the vial contained volatile components in equilibrium between the liquid and vapour phases. A defined volume of the headspace was then transferred to the column within the gas chromatograph. The components were then detected by an electron capture detector to determine the concentration of each THM species.

2.5.4 Disinfection By-product Method 551

Analysis of DBP method 551 determined the formation of haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones (US EPA 1990). Chlorinated water samples were extracted with methyl tert-butyl ether. The extract was then analysed quantitatively by gas chromatography using the Varian GC/ECD with dual column configuration. Confirmation of the eluted compounds was obtained from dual column and electron capture detection sensor. Aqueous calibration standards were also extracted and analysed in order to compensate for any extraction losses.

2.5.5 Unknown Total Organic Halogens

The unknown total organic halogen (UTOX) formation was calculated by subtracting the summation of HAA, THM and DBP 551 from AOX. This provided insight into the concentration of DBPs that were not detected by the methods used within this thesis.

Publication Associated with this Thesis

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The role of phytoplankton as pre-cursors for disinfection by-product formation upon chlorination

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Performed analysis, interpreted data, wrote manuscript and acted as corresponding author.

Overall Percentage: 60%

I hereby certify that the statement of contribution is accurate

Signed,...

.....Date.. 27/11/2018

M. Drikas

Supervised development of work, helped in data interpretation and manuscript evaluation

Overall Percentage: 20%

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed..

.....Date 24/7/18

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Supervised development of work, helped in data interpretation and manuscript evaluation

Overall Percentage: 20%

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed.....

.....Date 27 July 2018

Chapter 3

The role of phytoplankton as pre-cursors for disinfection by-product formation upon chlorination: a literature review

Water safety remains one of the greatest concerns with regards to human health. Advances in science and technology have resulted in highly efficient water treatment plants, significantly reducing diseases related to waterborne pathogenic microorganisms. While disinfection is critical to mitigate pathogen risk to humans, the reactions between the disinfectant and dissolved organic compounds can lead to the formation of chemical contaminants called disinfection by-products (DBPs). DBPs have been related to numerous health issues including birth defects and cancer. The formation of disinfection by-products occurs due to the reaction of oxidants and natural organic matter. DBP precursors are derived from anthropogenic sources including pharmaceuticals and chemical waste, the breakdown of vegetation from external catchment sources (allochthonous) and internally derived sources including phytoplankton (autochthonous). Current literature focuses on the contribution of allochthonous sources towards the formation of DBPs; however, the recalcitrant nature of hydrophilic phytoplankton organic matter indicates that autochthonous organic carbon can significantly contribute to total DBP concentrations. The contribution of phytoplankton to the formation of DBPs is also influenced by cellular exudation rates, chemical composition, environmental conditions and the physical and chemical conditions of the solution upon disinfection. Formation of DBPs is further influenced by the presence of cyanobacteria phyla due to their notoriety for forming dense blooms. Management of DBP formation can potentially be improved by reducing cyanobacteria as well as other DBP precursors derived from phytoplankton.

3.1 Introduction

Chemical disinfection is vital for the continued protection from bacterial, viral and some protozoan pathogens, and the common disinfectant chlorine is effective against a range of these pathogens (Table 1). While disinfection is critical to mitigate pathogen risk to humans, the reactions between the disinfectant and dissolved organic compounds can lead to the formation of chemical contaminants called disinfection by-products (DBPs). The formation of DBPs results in a residual, unintended health hazard (Richardson 2003).

3.1.1 Disinfection By-product Formation

Understanding how DBPs are produced is essential for determining the mechanisms of how phytoplankton may contribute to their formation. In addition to effectively killing pathogens, disinfectants are strong oxidising agents, able to oxidise complex NOM molecules into simpler moieties (Richardson 2003). This is often exploited to improve the treatability of the organic carbon pool prior to coagulation/flocculation, often termed ‘pre-oxidation’. However, the disinfectant can react with readily available NOM and/or inorganic constituents to yield DBPs during the disinfection process and throughout the distribution network. Therefore, it is intuitive that the formation and yield of DBPs is dependent on the availability of NOM, choice of disinfectant, the presence of inorganic compounds and the physical conditions of the reaction.

Table 1. Examples of pathogens with evidence of health significance, indicating chlorine resistance and expected time for minimal removal during chlorination. Pathogen minimum removal data collected from (Centers for Disease Control and Prevention 2012) and references therein.

	Pathogen	Health Significance	Resistance to Chlorine ^a	Minimal Removal (CT ₉₉)
Bacteria	Overall	High	Low	0.04-0.08 min.mg/L (5°C, pH 6-7)
	<i>E. coli</i>	High	Low	<0.25 min.mg/L (23°C, pH 7)
	<i>Campylobacter jejuni</i>	High	Low	0.5 min.mg/L (25°C, pH 8)
	<i>Salmonella Typhi</i>	High	Low	1 min.mg/L (20-25°C, pH 7)
Viruses	Overall	High	Moderate	2-30 min.mg/L (0-10°C, pH 7-9)
	Poliovirus	High	Moderate	6.36 min.mg/L (5°C, pH 6)
	Hepatitis A Virus	High	Moderate	<0.41 min.mg/L (25°C, pH 8)
	Rotavirus	High	Moderate	0.05 min.mg/L (4°C pH7)
	Coxsackie A	High	Moderate	0.14-0.15 min.mg/L (5°C, pH 6)
Protozoa	Overall	High	High	25-245 min.mg/L (0-25°C, pH 7-8)
	<i>Cryptosporidiumhominis/ parvum</i>	High	High	15,300 min.mg/L (25°C, pH 7.5)
	<i>Entamoeba histolytica</i>	High	High	20 min.mg/L (27-30°C, pH 7)
	<i>Giardia intestinalis</i>	High	High	15 min.mg/L (25°, pH 7)

^a General indication of CT times for each pathogen group

Although there are a range of disinfectant agents (chloramine, ozone, chlorine dioxide) chlorine is commonly utilised for its low cost and capability to retain a disinfection residual. The chemical structure of the DBP formed is also influenced by the presence of inorganic constituents, such as bromide, iodide, nitrites and nitrates and the physical conditions of the reaction (Figure 3). Disinfection by-products were discovered with the identification of trihalomethanes (THMs) in 1974 by Bellar *et al.* (1974a) and Rook (1974); since then there have been over 600 DBPs identified in drinking water or simulated in laboratory experiments (Deborde and von Gunten 2008; Hebert *et al.* 2010). Given the imperative to disinfect, mechanisms are required to minimise formation of these chemical contaminants. Considering the range of possible chemical interactions and DBPs that may be formed, the removal of DBP precursors prior to chlorination is the preferred approach and has received the most attention in recent literature (Bond *et al.* 2011a). This can be achieved either by preventing DBP precursors entering the water body or removing them from the source water prior to disinfection.

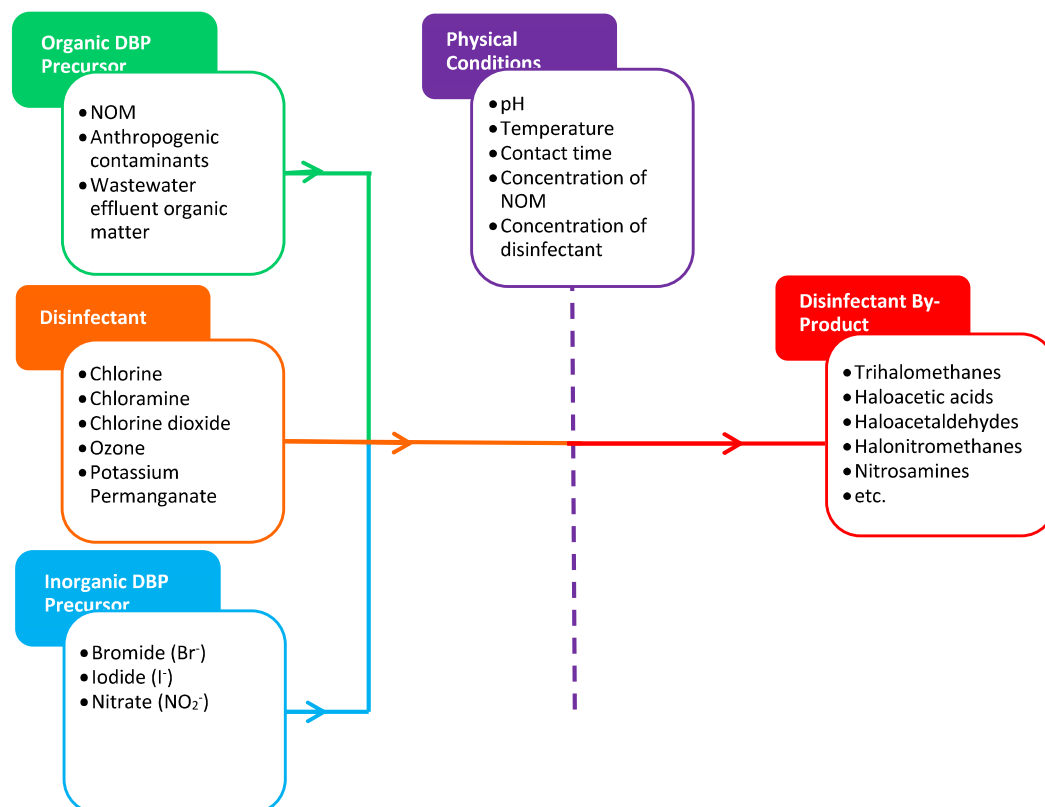


Figure 3. General schematic of disinfection by-product (DBP) formation; the reaction of a disinfectant agent with an organic precursor and/or an inorganic precursor forms a suite of DBPs. The rate and yield of the reaction is governed by a range of physical conditions.

Disinfection by-product precursors are derived from anthropogenic compounds, the breakdown of vegetation from external catchment sources (allochthonous) and from internal sources including the phytoplankton (autochthonous). Anthropogenic sources of DBP precursors include pharmaceuticals and chemical wastes, which can accumulate in waterways due to their difficulty to remove during treatment. The transformation of pharmaceuticals to DBPs during the disinfection process has been detailed by Postigo and Richardson (2014). The contribution of catchment derived-allochthonous NOM towards DBP formation varies between catchments depending on local climate, soil type, vegetation and the morphology of the watershed. The relative contribution of autochthonous carbon will be a function of nutrient load and phytoplankton growth. Allochthonous organic matter often exceeds autochthonous carbon as the dominant energy source in humic and oligotrophic lakes, whereas autochthonous organic carbon is often the dominant energy source in productive, eutrophic lakes (Jonsson *et al.* 2001). It

is expected that autochthonous NOM will also be the dominant source of DBP precursors in environments exposed to low or intermittent rainfall events (Soh *et al.* 2008). The organic matter is removed during the coagulation process; however, autochthonous organic matter can be harder to treat due to higher concentrations of hydrophilic compounds (Bond *et al.* 2011a; Lui *et al.* 2011). Therefore, phytoplankton dominated systems may cause problematic DBP formation within the water treatment plant and distribution network.

The majority of the literature pools various sources of NOM or focuses only on allochthonous contributions to DBP formation, with minimal studies considering the contribution from phytoplankton (Hong *et al.* 2008; Fang *et al.* 2010; Li *et al.* 2012). This review is necessary given the limited availability of comprehensive DBP literature reviews, highlighting the significance of phytoplankton derived organic matter as a viable DBP precursor. As algal-derived organic carbon is generally more recalcitrant to conventional treatment it is imperative that the total contribution of phytoplankton to the formation of DBPs is thoroughly understood for improved management. This review aims to assess the potential for phytoplankton-derived DOC to form DBPs by determining the phytoplankton contribution to the organic carbon load in reservoirs and identify the cellular constituents of phytoplankton that may react with the chlorine.

3.1.2 Disinfection By-product Toxicity

Currently only 15 of DBPs are regulated by the World Health Organisation (WHO) as these compounds have sufficient toxicological evidence of carcinogenicity, genotoxicity or adverse reproductive incidences (Richardson *et al.* 2007; Krasner 2009; World Health Organisation 2011). Less than 100 of the 600+ known and emerging DBPs have undergone quantitative or toxicology studies (Hebert *et al.* 2010). Although many of the studied DBP chemicals produced harmful effects, attribution of toxicology to human health outcomes is difficult (Hrudey 2009). Furthermore, there is not a consistent approach by which DBPs are regulated and the key authoritative organisations adopt/set unique lists of DBPs with significant variation in guideline values (Table 2).

Comprehensive genotoxicity experiments assessed the *in vitro* cytotoxicity on Chinese hamster ovary cells when exposed to various classes of DBPs (Plewa *et al.* 2004a; Plewa *et al.* 2004b; Plewa *et al.* 2008) These experiments provide evidence that the toxicity for

various substituted DBP halogenated functional groups is, $I > Br > Cl$, and that nitrogenous disinfection by-products (N-DBPs) are generally more genotoxic than carbonaceous disinfection by-products (C-DBPs). This suggests that regulated carbonaceous and chlorine substituted DBP classes have lower genotoxic activities than other emerging DBP classes (Richardson *et al.* 2007). Therefore, some classes of DBPs that have higher associated health risks are not being routinely monitored under current guideline standards. The higher genotoxicity of N-DBPs is of concern given that phytoplankton are significant contributors to dissolved organic nitrogen (DON) and are known to promote the formation of N-DBPs (Nguyen *et al.* 2005b; Bond *et al.* 2011b; Zhang *et al.* 2014). A potential increase in more genotoxic N-DBPs may give rise to associated health risks with DBP exposure include; the potential association with bladder cancer, as well as links to miscarriages and birth defects (Thomson and Sarkar 2014).

Table 2. List of regulations on disinfection by-products (DBPs) with associated guideline values from the US EPA, WHO, the European Union and, Australia and New Zealand.

WHO guideline values		US EPA mandatory standards		European Union mandatory standards		Australian drinking water guidelines	
Regulated DBPs	µg/L	Regulated DBPs	µg/L	Regulated DBPs	µg/L	Regulated DBPs	µg/L
Total THM	*	Total THM	80	Total THM	100	Total THM	250
Bromate	10	Total HAA (5)	60	Bromate	10	Chloroacetic acid	150
Bromodichloromethane	60	Bromate	10			Dichloroacetic acid	100
Bromoform	100	Chlorite	1000			Trichloroacetic acid	100
Chlorate	700					Chloral hydrate	100
Chlorite	700					NDMA	0.1
Chloroform	300					Bromate	20
Dibromoacetonitrile	70					Chlorite	800
Dibromochloromethane	100					2-chlorophenol	300
Dichloroacetate	50					2,4-dichlorophenol	200
Dichloroacetonitrile	20					2,4,6-trichlorophenol	20
Monochloroacetate	20					Cyanogen chloride	80
NDMA	0.1					Formaldehyde	500
Trichloroacetate	200						
2,4,6-Trichlorophenol	200						

^a Sum of the ratio of the concentration of bromoform, dibromochloromethane, bromodichloromethane and chloroform to its respective guideline value can't exceed 1.

N-nitrosodimethylamine (NDMA) is a N-DBP of significant concern, given nitrosamines are classified as carcinogenic, mutagenic and teratogenic (Choi and Valentine 2002). NDMA is predominantly formed from reactions between chloramine and dimethylamine, whilst also forming in chlorinated water in the presence of ammonia (Choi and Valentine 2002). A report by Mitch (2009) found that significant NDMA precursors are only dominant in wastewater samples, whilst algal dominated and pristine water samples were insufficient in generating NDMA concentrations under typical chloramine disinfection conditions. In contrast, NDMA formation from phytoplankton indicated that extracellular organic matter (EOM) and intracellular organic matter (IOM) are capable of producing NDMA concentrations above local Californian public health goal of 3ng/L (Li *et al.* 2012). Further investigation is required due to contradictions on NDMA formation potential from phytoplankton precursors.

3.2 Phytoplankton Contribution to Total NOM Pool

To determine how much carbon phytoplankton can contribute to the NOM pool it is necessary to obtain an estimate of the proportion of autochthonous and allochthonous NOM within a lake or reservoir ecosystem. A large-scale assessment of a broad range of aquatic environments has to be completed. A meta-analysis of the U.S. EPA National Lake Assessment (NLA) dataset of 1326 total sample points in 1076 U.S. lakes, provided a snapshot of a range of physical, chemical and biological lake properties (Rigosi *et al.* 2014). The lakes used in the NLA were selected from the U.S. National Hydrographic dataset using a generalised random tessellation stratified survey design (Stevens and Olsen 2004). All surveyed lakes located across the lower 48 U.S. states had a minimum depth of 1 meter and a minimum surface area of 0.01 km². Sampling for the NLA was conducted during the summer of 2007 to minimise the influence of seasonal variation. Total organic carbon (TOC) and chlorophyll a concentrations were recorded, allowing for a snapshot estimate of the phytoplankton derived organic matter relative to the total organic carbon pool. This was achieved by using a comparative ratio between total chlorophyll a and TOC concentrations. The carbon to chlorophyll a ratio varies due to species composition and light exposure, with numerous studies reporting ratios between 27:1 and 83:1 (C:Chla) (Reynolds 1984; Riemann *et al.* 1989; Yacobi and Zohary 2010).

The Reynolds estimation (C:Chla 50:1) is used as an general prediction of carbon based on values of chlorophyll a of a general phytoplankton pool, whereas other estimations are species specific. The chlorophyll a concentrations from the EPA database were multiplied by the carbon to chlorophyll ratio (C:Chla 50:1) to estimate how much carbon was found within the phytoplankton (Figure 4). Autochthonous carbon estimations from the US EPA National Lake Assessment indicated that in 520 of the sampling locations, or 39.2% of samples, phytoplankton biomass contributed >10% to the total carbon pool (Figure 4). This analysis provides an estimate of the standing pool of TOC within each lake; however, the TOC in phytoplankton is continually turning over as cells fix atmospheric CO₂ converting it to organic carbon. As the phytoplankton cells lyse the organic carbon enters the dissolved fraction of the carbon pool.

An analysis of carbon sources in Myponga Reservoir, South Australia, identified that phytoplankton contributed 25-50 % of the total dissolved organic carbon (DOC) to the NOM pool during a period of low annual rainfall when allochthonous inputs were reduced (Linden 2008). The contribution of phytoplankton to the total DOC pool is dependent on the type of lake. For instance; Bade *et al.* (2007) measured phytoplankton production of two oligotrophic lakes at ~20 % whereas; Carpenter *et al.* (2005) made reference to a eutrophic lake where phytoplankton production was accountable for as much as 40% of the total DOC pool. Therefore, phytoplankton could be a significant DBP precursor in in similar euphotic systems, and during periods of low rainfall. Several species of phytoplankton form blooms in eutrophic water bodies resulting in water quality degradation and an increased risk to DBP formation (O'Neil *et al.* 2012). To gain more insight into how much autochthonous carbon phytoplankton contribute to the total NOM load it is necessary to consider phytoplankton chemical composition, growth and mortality rates, cellular exudation, cell lysis and loss of settling of cells. This would require sophisticated modelling beyond the scope of this review; however, both the Myponga Reservoir example and the US EPA Lake analysis suggest that phytoplankton can contribute a significant amount of organic carbon to lakes.

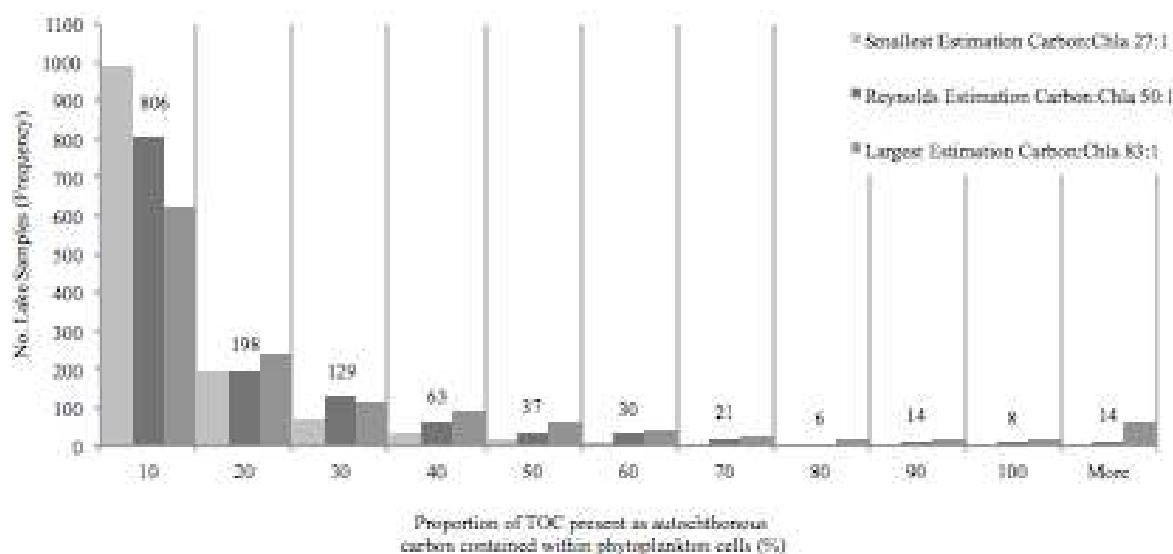


Figure 4. Analysis of the autochthonous carbon load from phytoplankton from the U.S. National Lake Assessment. Phytoplankton carbon contribution was estimated from the known chlorophyll a concentration using predictive ratios from the literature. The Reynolds Estimation (Carbon:Chla, 50:1) is an accurate average estimation of carbon based on total species composition of phytoplankton.

3.2.1 Influence of Natural Organic Matter on DBP Formation

The chemical composition of allochthonous NOM is defined by local climate and catchment characteristics, including the soil and vegetation type (Frimmel 1998; Findlay and Sinsabaugh 2003). The characterisation of NOM into operationally defined fractions (Leenheer and Croué 2003) can aid in the prediction of DBP formation potential (DBPFP) post chlorination (Figure 5). Humic and fulvic acids, hydrocarbons, tannins and aromatic amines are contained within the hydrophobic fraction. Terrestrial NOM is commonly derived from lignin and contains a high aromatic content; hence allochthonous NOM tends to be hydrophobic in character (Hwang *et al.* 2001; Bond *et al.* 2011a). Alternatively, carboxylic acids, polyuronic acids, amino acids, peptides, proteins and carbohydrates are commonly contained within the hydrophilic fraction. Autochthonous NOM is derived from phytoplankton, macrophytes and bacterial sources, consisting of low aromatic and high nitrogen content; indicating that autochthonous NOM tends to be predominantly hydrophilic in character (Bond *et al.* 2011a).

The hydrophilic organic carbon fraction is less prone to coagulation and as a result is partially recalcitrant to conventional treatment methods (Singer and Harrington 1993; Kim and Yu 2005; Matilainen *et al.* 2010; Lui *et al.* 2011). Eutrophic systems dominated by phytoplankton species can provide NOM with high hydrophilic content. Li *et al.* (2012) analysed the relative hydrophobicity of *Microcystis aeruginosa* using XAD and IRA resin fractionation technique. They demonstrated that hydrophilic organic matter accounts for 86 % of IOM and 63 % of EOM from *M. aeruginosa*. This has implications for the water treatment process as NOM from phytoplankton will be partially recalcitrant to conventional treatment methods. Furthermore, Lui *et al.* (2011) reported that hydrophilic NOM derived from algal protein can increase the DBPFP, in comparison to hydrophobic proteins. The research suggested that hydrophilic proteins were 35 times more effective as precursors of chloroform. Due to a high prevalence of hydrophilic content, the DOC from autochthonous phytoplankton production can significantly increase the DBPFP, even after conventional treatment.

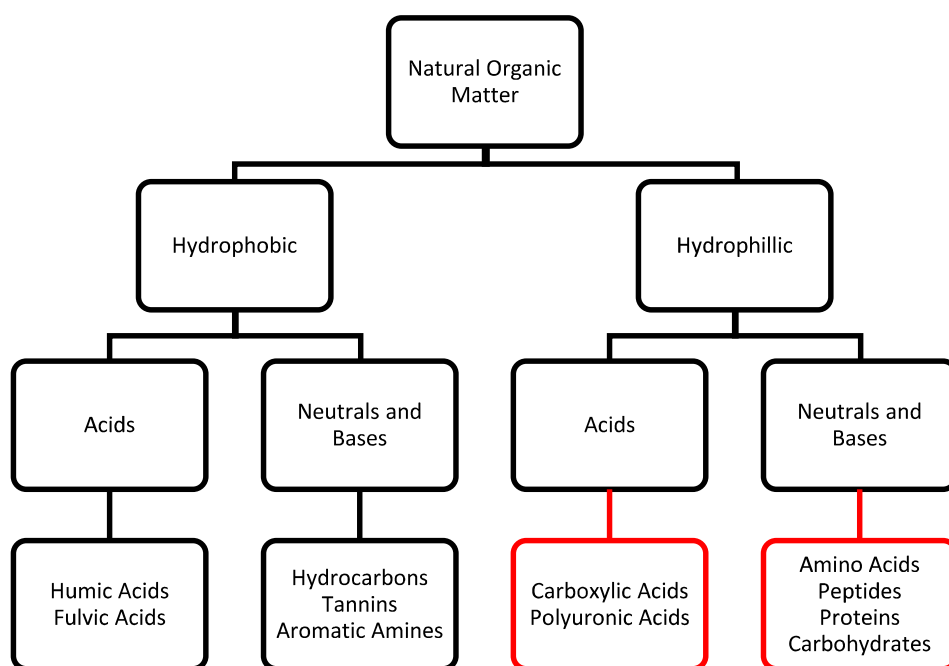


Figure 5. General classification of natural organic matter (NOM) by hydrophobicity and acidity into specific chemical groups. The red boxes highlight the major constituents of phytoplankton (Leenheer and Croué 2003)

3.2.2 Growth and Mortality Rates

The contribution of phytoplankton to the NOM pool, and resulting DBPFP can be further quantified with an understanding of population dynamics. Comprehension of species composition and distribution involves knowledge of phytoplankton growth and mortality rates. Population dynamics provide insight into how rapidly autochthonous organic matter derived from phytoplankton can enter the system. Phytoplankton are capable of rapid growth, with individual organisms expressing doubling rates between 6 hours to 10 days (Harris 1986). Smaller cells generally replicate faster than larger algal cells. The fast growth rate of phytoplankton results in the rapid turnover of autochthonous NOM within the lake and results in a large pool of carbon that could react to form DBPs. During the life cycle of phytoplankton, the release of DOC can be substantial, ranging from 9-67 % of total primary production (Hwang 1995).

Phytoplankton mortality rates also greatly impact total contribution to the autochthonous organic matter pool. Losses within the phytoplankton community occur as a result of sedimentation, natural cell lysis, flushing, parasitism and predation (Crumpton and Wetzel 1982; South and Whittick 1987). In the event of phytoplankton mortality, intracellular content is released into the water column raising the available NOM content for DBP formation. In a study of phytoplankton mortality rates by (Crumpton and Wetzel 1982), grazing was considered the dominant cause of phytoplankton mortality. During ingestion by zooplankton, 16-37 % of algal carbon content can still be released as available NOM, susceptible to DBP formation upon chlorination (Lampert 1978; Strom *et al.* 1997). Alterations in phytoplankton species dominance, growth and mortality rates provide evidence of a boom and bust lifecycle, resulting in NOM accumulation that is susceptible to DBP formation upon chlorination.

3.2.3 Cellular Exudation

Phytoplankton contribution to the NOM pool is also increased by natural exudation of dissolved organic matter. Phytoplankton excretion of NOM is theorised to occur continuously, or as a result of environmental stressors (Malinsky-Rushansky and Legrand 1996). The rate of cellular exudation is enhanced by an increase in UV radiation closer to the surface of the water (Köhler *et al.* 2001). The increased extracellular release has been linked to the accumulation of excess photosynthates or products of photosynthesis

(Fogg 1983). Experiments have closely related the rate of exudation to the rate of primary production (Mague *et al.* 1980; Descy *et al.* 2002). To estimate percentage of extracellular release, (Baines and Pace 1991) assessed published results based on 225 observations of phytoplankton extracellular release, particulate primary production and biomass values. The meta-analysis determined that approximately 13 % of total carbon fixed by phytoplankton is exuded by cells and found extracellularly (Baines and Pace 1991). This indicates that the phytoplankton continuously contribute a significant NOM load to their surrounding environment, further increasing the risk of DBP formation.

There is significant variation in extracellular release between individual species and phyla. A study compared NOM production per unit of chlorophyll a, per hour for a species of cyanobacteria (*Oscillatoria prolifera*), green algae (*Scenedesmus quadricauda*) and diatom (*Chaetoceros muelleri*) (Nguyen *et al.* 2005b). The study indicated that cyanobacteria had the highest rate of DOC exudation ($9.0 \mu\text{g C } (\mu\text{g Chl a})^{-1}\text{h}^{-1}$), followed by green algae ($3.6 \mu\text{g C } (\mu\text{g Chl a})^{-1}\text{h}^{-1}$) and diatom species ($1.1 \mu\text{g C } (\mu\text{g Chl a})^{-1}\text{h}^{-1}$). The continuous rate of cellular exudation equates to a large autochthonous carbon input, particularly in eutrophic systems where a large biomass of phytoplankton is usually present. Increased autochthonous DOC input from continuous cellular exudation can potentially further increase the DBP formation upon chlorination. The natural cellular exudation of organic matter is of even greater concern to water treatment in the event of an algal bloom.

3.2.4 Phytoplankton Blooms

Excess nutrient supply and adequate exposure within the euphotic zone can result in a phytoplankton bloom event. Eutrophication of freshwater environments from urban, agricultural and industrial development has resulted in an increased frequency of phytoplankton blooms (Paerl and Huisman 2008). Numerous phytoplankton genera are known to form blooms; however, cyanobacteria are most notorious (Paerl *et al.* 2001). Measurements of *M. aeruginosa* blooms by (Oudra *et al.* 2001) have indicated cell densities exceeding 10^6 cells/mL. Cyanobacteria blooms often occur as surface blooms, due to the presence of gas vesicles that provide cyanobacterial cells with buoyancy and promote the formation of a thick scum across the surface of the water (Oliver *et al.* 2012). Phytoplankton blooms significantly increase the concentration of autochthonous NOM

due to increased cell biomass resulting in accelerated rates of cell lysis, parasitism, predation and cellular exudation. During a bloom event, rapid carbon turnover significantly increases autochthonous organic matter content. As the hydrophilic fraction is more recalcitrant to removal by coagulation, organic matter will carry through the distribution system resulting in the increased chlorine demand and DBP formation (Lui *et al.* 2011).

Phytoplankton are important DBP precursors, indicated by a boom and bust lifecycle, rapid cellular exudation, hydrophilic dominant cellular composition and the formation of highly concentrated blooms. Research conducted by Graham *et al.* (1998) indicated that cellular exudation and DBP formation increased with the age of the culture. There was a spike in yield of DBPs during the late stationary death phase of the cell lifecycle. The correlation between DBP yield and age of the phytoplankton culture occurs due to the breakdown of storage products into more chemically reactive compounds and the consequential release of these compounds (Graham *et al.* 1998).

3.2.5 Chemical Composition of Phytoplankton

Differences in the chemical composition of phytoplankton between individual species results in the alteration of cellular production rates, structural characteristics, chlorine reactivity and the biological lability of NOM synthesised (Nguyen *et al.* 2005b). Phytoplankton have three major classed biomolecules; proteins, lipids and carbohydrates. The concentration of these major biomolecules can be measured to determine the cellular composition of phytoplankton. Surrogate compounds bovine serum albumin (BSA), fish oil and starch can be used to investigate how variations in phytoplankton composition can influence the formation of DBPs (Hong *et al.* 2008; Wei *et al.* 2011). The model compounds are considered to be statistically reliable surrogates due to chemical similarities between BSA, fish oil and starch and the respective algal derived proteins, lipids and carbohydrates. Hong *et al.* (2008) determined the DBPFP upon chlorination of the three model compounds (chlorine dose = 10 mg Cl₂/ mg⁻¹ C, contact time = 96 hour, temperature = 20 °C, pH = 7). This work identified that lipids and proteins were more effective precursors of the THM chloroform and that proteins are also a dominant precursor for two haloacetic acids (HAAs); dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). Starch was not identified as a major contributor to the

formation of DBPs (Hong *et al.* 2008) (Table 3). The use of model compounds to predict total DBPFP of phytoplankton is based on two assumptions; (1) that algal cellular content is 100 % comprised of proteins, lipids and carbohydrates, and (2) that carbon percentages of proteins, lipids and carbohydrates are 53 %, 76 % and 40 % respectively (Hong *et al.* 2008). Results obtained from the chlorination of model compounds were then used to predict the DBPFP of 49 species across three phyla based on their known chemical compositions; cyanobacteria (8 species), green algae (15 species) and diatom (26 species) (Table 4). Estimations of chloroform formation closely matched experimental data; however, DCAA and TCAA concentrations were significantly underestimated. It is likely that the presence of RNA, DNA and aromatic compounds resulted in higher than anticipated haloacetic acid concentrations (Kitis *et al.* 2002; Hong *et al.* 2008). The results identify that phytoplankton chemical composition changes the formation potential of DBPs during chlorination.

Table 3. Disinfection by-product formation as a result of chlorination of model compounds (Hong *et al.* 2008).

Model Compounds	CHCl ₃ (µg mg ⁻¹ C)	DCAA (µg mg ⁻¹ C)	TCAA (µg mg ⁻¹ C)
BSA	27.1	25.9	22.8
Fish oil	50.0	3.36	1.27
Starch	3.06	4.91	0.09

Table 4. Total disinfection by-product formation potential (DBPFP) based on a comparison of protein, carbohydrate and lipid concentrations from cyanobacteria (8 species), green algae (15 species) and diatom (26 species) (Hong *et al.* 2008)

	Protein (%)	Carbohydrates (%)	Lipids (%)	CHCl ₃ (µg mg ⁻¹ C)	DCAA (µg mg ⁻¹ C)	TCAA (µg mg ⁻¹ C)
Cyanobacteria	61.5	25.2	13.3	24.1	17.6	14.2
Green algae	50.5	21.7	27.8	28.3	15.1	11.9
Diatoms	42.9	17.9	39.2	31.8	13.3	10.3

Although chemical variation exists between individual species, general trends in cellular constituents are evident across cyanobacteria, green algae and diatom phylum. A meta-analysis of the chemical composition of phytoplankton species indicates that cyanobacteria are generally comprised of more protein (41-69 %) than diatoms (12-50

%); however diatoms generally accumulate more lipids (5-43 %) in comparison to cyanobacteria and green algae (2-30 %) (Hong *et al.* 2008). Higher concentrations of proteins within cyanobacteria species may cause problematic DBP formation within the water treatment plant due to higher efficiency of protein to form THM and HAA species. Growth experiments have also indicated that diatom and cyanobacteria cell cultures produced in excess of 20 mg/L of DOC which was significantly more in comparison to the green algae cell culture which produced between 10-12 mg/L of DOC (Nguyen *et al.* 2005b). Phytoplankton are also a major source of DON in natural waters, with some species of cyanobacteria capable of excreting up to 45 % of their total fixed nitrogen as organic nitrogen (Nguyen *et al.* 2005b; Zhang *et al.* 2014). The chlorination of phytoplankton enriched with organic nitrogen resulted in an increased formation of N-DBPs (Fang *et al.* 2010). This has major implications for water quality within the water treatment plant due to the higher genotoxicity associated with N-DBPs (Richardson *et al.* 2007). Therefore, cyanobacteria species are of significant concern with regards to DBP formation due to higher protein concentrations, increased DOC formation, high DON contribution and notoriety of forming blooms.

3.2.6 Intracellular vs Extracellular Organic Matter

Phytoplankton derived organic matter arises from two sources, the metabolic excretion forming extracellular organic matter (EOM) or via cell lysis, where intracellular organic matter (IOM) is released from a break in the cell wall (Henderson *et al.* 2008). The extracellular release of organic matter from phytoplankton is dominated by proteins and carbohydrates (38 % < 1kDa) as waste and excess photosynthetic derivatives (Reynolds 2007; Li *et al.* 2012). A high concentration of proteins would result in substantial formation of DBPs (Hong *et al.* 2008). A comparison of EOM and IOM allows for increased precision when estimating the total DBPFP within the water treatment plant. A study by Li *et al.* (2012) assessed cyanobacteria *M. aeruginosa*, to compare total contribution of EOM and IOM to organic matter yield and DBP concentrations. EOM contributed significantly less organic matter than IOM, 29.7 and 100.5 mg/L respectively. However, assessment of DBP formation indicates that EOM contributed more to the formation of both THMs and NDMA per mg of carbon when water samples were subjected to chlorination and chloramination (Figure 6) (Li *et al.* 2012). In comparison to IOM, EOM is represented as a significant contributor to the formation of DBPs,

inferring that species with a large surrounding mucilage component and high cellular exudation rate will have a greater contribution to the formation of DBPs (Nguyen *et al.* 2005b; Li *et al.* 2012). A corresponding study by (Huang *et al.* 2009), identified that specific yield from EOM resulted in a slightly higher total THM and HAA yield compared with the IOM for *Anabaena flos-aquae*. However, the opposite trend was observed for *M. aeruginosa*, contradictory to (Li *et al.* 2012). Variability in species strain, light and nutrient availability are known to alter the production of EOM and are a likely explanation for variations between the two studies (Mague *et al.* 1980; Reynolds 1984). Both studies identified, using a mass specific comparison, that IOM was the main contributor to the formation of DBPs due to the significantly larger contribution of NOM (Huang *et al.* 2009; Li *et al.* 2012). This mass specific comparison of EOM and IOM indicated that intracellular content contributed 77.2, 80.9, 63.3 and 77.2 % of the total organic matter, THMFP, HAAFP and NDMAFP respectively (Li *et al.* 2012). The autolysis of phytoplankton cells will release excess DOC that is comprised of up to 86 % hydrophilic matter remaining recalcitrant during conventional water treatment. Therefore both IOM and EOM contribute significantly to the formation of DBPs.

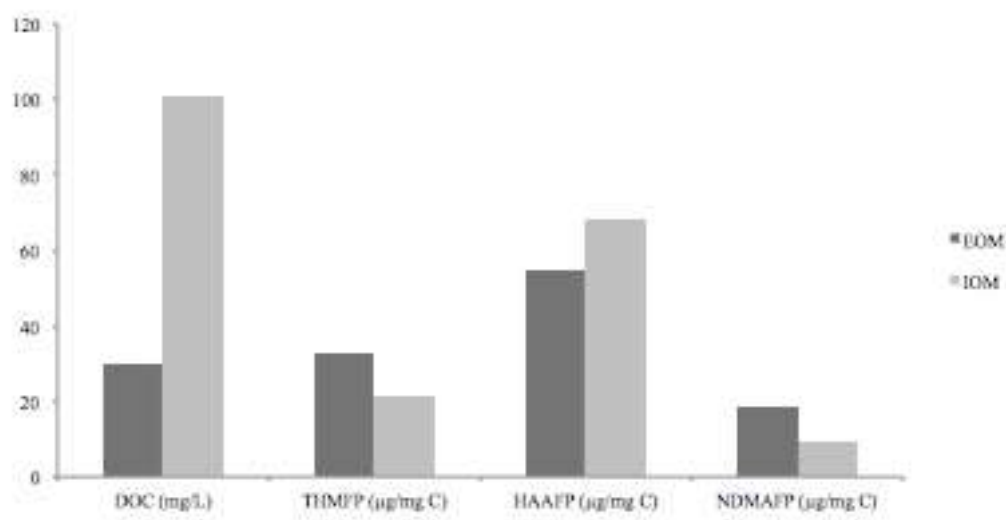


Figure 6. Comparison of intracellular organic matter (IOM) and extracellular organic matter (EOM) contribution to DOC, trihalomethane formation potential (THMFP), haloacetic acid formation potential (HAAFP) and nitrosodimethylamine formation potential (NDMAFP) (Li *et al.* 2012).

3.3 Other Contributing Factors to DBP Formation

Disinfection by-product formation is influenced by environmental conditions, the choice of disinfectant, concentration of inorganic moieties such as bromide and iodide, and the physical conditions of the chemical reaction including; temperature, pH, dosage of disinfectant and contact time of the reaction.

3.3.1 Environmental Conditions

To enable adequate growth and proliferation of phytoplankton the physical, chemical and biological conditions of the lake have to be suitable. The impact of climate dramatically alters community composition with variation of species dominance depending upon the mixing/stratification regime and nutrient availability (Lund 1965). Typically green algae and diatoms rely on vertical mixing of the water column to remain entrained and ensure adequate exposure within the euphotic zone to satisfy their light requirements (Brookes *et al.* 2003; Oliver *et al.* 2012). Warm conditions that enable stratification to develop can favour the gas vacuolated cyanobacteria. Climate change scenarios indicate that freshwater systems will be exposed to increased temperatures, more intense and longer periods of thermal stratification and altered nutrient loads potentially favouring cyanobacteria over other phytoplankton groups (Carey *et al.* 2012). Increase in cyanobacteria production due to the effects of climate change is of concern given their chemical composition, contribution to DOC and notoriety of forming blooms.

Nutrient availability is fundamental for phytoplankton growth, with limiting nutrients reducing the growth. Carbon, nitrogen and phosphate have often been considered to restrict phytoplankton growth (Hecky and Kilham 1988). Reviews by Hecky and Kilham (1988) and Guildford and Hecky (2000) on nutrient limitations have identified that phosphorus concentration is the critical limiting nutrient that regulates algal biomass and growth rates within most freshwater systems (Nagar *et al.* 1974); although nitrogen limitation can occur in freshwater systems (Baker *et al.* 2000). In an evaluation of historical data from Myponga Reservoir, South Australia (Linden *et al.* 2004) compared the maximum annual total phosphorus (TP) and the maximum chlorophyll *a* found in the following growth period in the years between 1985 and 2000 (Figure 7). They showed chlorophyll *a* concentrations, as a measure of phytoplankton population, increased as total phosphorus increased supporting earlier works (Sakamoto 1966; Vollenweider and

Dillon 1974; Jones and Lee 1982). This indicates the significant role that phosphorus plays in determining both the rate of phytoplankton growth and the carrying capacity of a lake, which would determine the yield of organic matter produced.

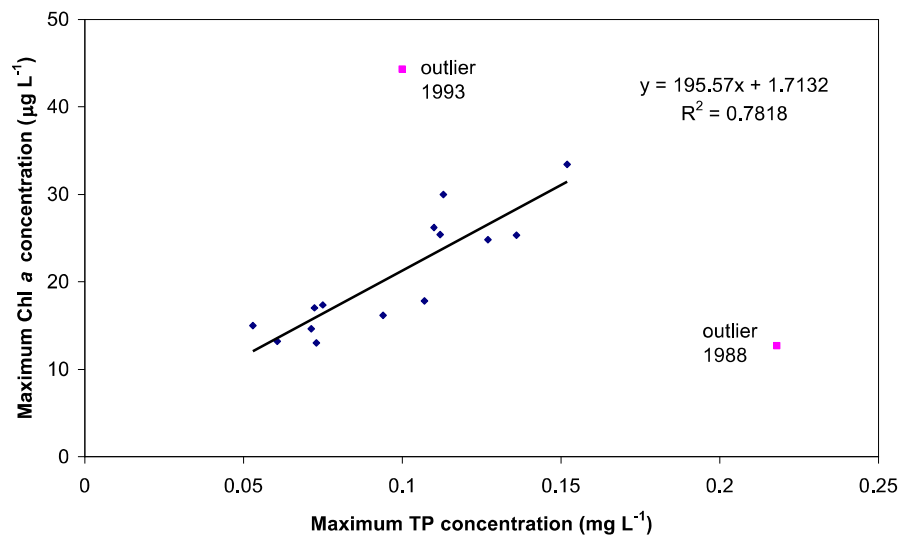


Figure 7. Positively correlated relationship between maximum total phosphorus (TP) and maximum chlorophyll a concentration (Linden et al. 2004)

3.3.2 Disinfection Agent

The choice of disinfectant is important in determining what DBPs can be formed in the presence of NOM and inorganic matter. Chlorine is predominantly used as a disinfectant for water treatment due to its low cost and stability, as it provides a residual to prevent microbial regrowth throughout the distribution network (Bond *et al.* 2011a; Fabris *et al.* 2012; Zhang *et al.* 2014). At a pH < 7.5 chlorine dissolves in water to form a strong oxidising agent, hypochlorous acid, capable of oxidising NOM. Due to public health concerns regarding DBP formation, there has been an increasing interest in the substitution of chlorine by other disinfectants.

Hua and Reckhow (2007b) used natural surface waters collected from Newport News Virginia to study the DBP formation potential using five oxidants; chlorine, chloramine, both with and without pre-ozonation, and chlorine dioxide. To minimise variation between experiments the temperature (20°C), pH (7) and reaction time (48 hours) were

held constant. A range of DBPs were monitored, including four chlorinated/brominated THMs, nine chlorinated/brominated HAAs, three dihaloacetonitriles, two haloketones, chloropicrin and total organic halide (TOX). There were several notable outcomes from this experiment; each disinfection scenario was capable of producing a unique range of DBPs with a large percentage of unknown halogenated compounds (UTOX) formed. The use of Ozone and chloramines as disinfection agents resulted in the increased formation of nitrogenous DBPs that are known to be more genotoxic. When Ozone was used in conjunction with chloramine the concentration of UTOX increased substantially. This increase in UTOX was also achieved when chloramine or chlorine dioxide were used as the sole disinfectant. These results indicated that the issue of DBP formation cannot be resolved simply by using an alternate disinfection agent.

The investigation of DBP formation from various disinfectants by Hua and Reckhow (2007b) can provide an understanding of what DBPs are likely to form when the source water contains higher concentrations of hydrophilic, phytoplankton derived organic matter. Analysis of chloramination and chlorination of phytoplankton by Fang *et al.* (2010) identified significant differences in DBP yields between the two treatments. Chlorination of *M.aeruginosa* culture resulted in increased formation of N-DBPs and haloaldehydes, with reduced C-DBP formation in comparison a dominant humic NOM source. Alternatively, chloramination of phytoplankton culture resulted in a slight reduction of total DBP formation in comparison to a humic NOM source. The use of a strong oxidiser such as ozone can result in an overall increase in DBP formation due to its ability to lyse algal cells, releasing IOM and increasing DBP formation during subsequent chlorination/chloramination (Fang *et al.* 2010).

When choosing a disinfection agent it is also important to consider other issues including; the inability to retain disinfection residual (ozone), inefficiency against taste and odour compounds (chloramine), higher concentration of unknown total organic halide (UTOX) with a potentially higher genotoxicity (chloramine, ozone/chloramine, chlorine dioxide), and higher chemical costs (ozone, ozone/chlorine, ozone/chloramine) (Nikolaou *et al.* 1999).

3.3.3 Dose and Contact Time

Application of chlorine for the efficient disinfection of potable water supplies is driven by the maintenance of a chlorine residual post-treatment, influenced by dose concentration time and the contact time of the reaction. A chlorine concentration of 0.5 mg/L at point of delivery is recommended (World Health Organisation 2011). A reduction in chlorine dose can allow for incomplete removal of pathogens or insufficient chlorine to reach the end of the distribution system. Chlorine residual less than the recommended concentration can allow for microbial regrowth throughout the distribution network, exposing consumers to an increased risk of disease from waterborne pathogens. However, excess chlorine dose can result in an escalated health risk by increasing total DBP formation (Sadiq and Rodriguez 2004). For example, (El-Dib and Ali 1995) observed that upon chlorination of Nile River water total THM formation increased from 70 to 85 µg/L when chlorine dose was increased from 5 to 20 mg/L respectively (contact time = 2 hours, pH = 8, temperature = 20°C). A similar relationship was observed by (Dojlido *et al.* 1999), identifying peak HAA concentrations when chlorine dose was highest.

Contact time with chlorine also influences the formation rates of DBPs (Nikolaou *et al.* 1999). El-Dib and Ali (1995) determined that total THM formation ranged from 30 to 90 µg/L when contact time was adjusted from 30 to 240 minutes respectively (Cl₂ dose = 5 mg/L, pH = 8, temperature = 20°C). A corresponding experiment by Liang and Singer (2003) supports these results, whilst suggesting that HAA concentration also increases with prolonged contact time. However, increased contact time can also result in the decreased concentration of some halogenated DBPs including haloacetonitriles (HANs) and haloketones (HKs) as a result of hydrolysis and further reactions with chlorine (Singer 1994).

An increase in chlorine concentration and contact time during the disinfection of a phytoplankton dominated system has the potential to significantly increase DBP formation with a probable increased production of N-DBP. Chlorine dose and contact time affects phytoplankton cell integrity, releasing intracellular content for further reaction (Daly *et al.* 2007). Further research is required to determine how chlorine dose concentration affects the rate of DBP and more specifically N-DBP formation from phytoplankton derived organic precursors.

3.3.4 Temperature

Disinfection by-product formation is also influenced by the temperature and pH of the water during treatment. Research by Roccaro *et al.* (2008) studied the effects of temperature on chlorine consumption and formation of DBPs from Ancipa Reservoir samples. It was evident that chlorine consumption accelerated as temperature was manipulated from 3 to 34°C and disinfection by-product formation increased as reaction temperature was altered from 3 to 20°C. A further increase in temperature from 20 to 34°C resulted in a shift in DBP speciation to a less brominated pool. Higher temperatures result in increased reaction rate kinetics causing faster and higher yielding formation of DBPs (Fang *et al.* 2010). This has seasonal implications suggesting that DBPFP will be maximised during summer, when ambient temperatures are higher (Nikolaou and Lekkas 2001).

Phytoplankton population densities are strongly influenced by seasonal fluctuations, typically peaking in summer when water temperatures are at a maximum and stratification is most strongly developed (Reynolds 1984). Although phytoplankton are capable of surviving subarctic and arctic climates, their growth rates are substantially diminished (Rautio *et al.* 2011). The optimum temperature and the degree to which growth rate increases with temperature; differ greatly between phytoplankton species. The Q10 temperature coefficient for growth describes the rate of change of growth rate with a 10°C change in temperature. The Q10 for cyanobacteria range between 1.8-4.3 and for chlorophytes 1.1-3.7 (Lurling *et al.* 2013). Therefore warmer temperature will accelerate phytoplankton growth and phytoplankton-derived DOC concentrations. An increase in phytoplankton-derived DOC will result in increased concentrations of hydrophilic organic matter contributing to DBP formation upon chlorination.

3.3.5 pH

The effects of pH on DBPFP is more complex as it chemically alters the speed of the rate determining step of the reaction (Bond *et al.* 2011a). Therefore, the effect of pH on the formation of DBPs is defined by the chemical structure of the precursor. Research by Hua and Reckhow (2008) assessed the rate of formation of THMs, dihaloacetic acids (DHAA), trihaloacetic acids (THAA) and UTOX at pH values of 5, 7 and 10 (DOC = 4.7 mg/L, chlorine dose = 8.1 mg/L, contact time = 72 hours, temperature = 20°C). The yield

of THMs and DHAAs increased as pH was elevated from 5 to 10. However, the opposite effect was observed for the formation of THAAs and UTOX. A decrease in TOX concentration from 930 to 878 and 768 $\mu\text{g/L}$ was also observed as pH increased from 5 to 7 and 10 respectively. The reduction in concentration of some DBPs may result from accelerated hydrolysis and dehalogenation at higher pH values (Singer 1994; Hua and Reckhow 2008). Therefore it would be critical to determine the effect of pH on DBP formation from phytoplankton precursors, to enable a more accurate prediction of DBPFP speciation and toxicity. Phytoplankton are able to modify the pH of the water due to formation of by-products from photosynthesis and respiration. During the day photosynthesis increases with increasing exposure to light, consuming free CO_2 and increasing O_2 production; resulting in an increase in alkalinity. At night the opposite is true, photosynthesis rates decrease and respiration increases, raising CO_2 concentrations; resulting in an increase in acidity (Wetzel 2001). Therefore during a bloom event the time of the day will considerably influence the pH of the water and may indirectly impact DBP formation where pre-oxidation is practised.

3.3.6 Influence of Inorganic Constituents

The chemical speciation of DBP formation upon chlorination is altered by the presence of inorganic constituents, bromide and iodide. Upon chlorination, bromide and iodide are rapidly oxidised to hypobromous acid and hypiodous acid respectively. Hypobromous and hypiodous acids are active oxidising agents that react with NOM to form brominated and iodated DBPs. To investigate the effect of these inorganic constituents on DBP formation Hua *et al.* (2006) analysed raw water samples from drinking water treatment plant intakes at the City of Winnipeg, Manitoba and the City of Tulsa, Oklahoma. Samples were dosed with bromide and iodide at concentrations of 0, 2, 10 and 30 μM prior to chlorination. Chlorination of the samples was conducted to produce a chlorine residual of 0.5 $\text{mg Cl}_2/\text{L}$ after a 48 hour contact time at 20°C with a pH of 7. The experiment concluded that increased concentration of bromide and iodide halogens resulted in a general increase in DBP speciation dominated by bromo- and iodo- moieties by outcompeting chlorine substitution. The addition of 2-30 μM of bromide to Tulsa raw water samples increased the total yield of THMs (four species) by 18-74% and HAAs (nine species) by 2-35% respectively. The addition of 2-30 μM of iodide to Tulsa raw water samples had minimal effect on total THM (10 species) yield; whilst TOX decreased

by 2-35 % respectively. The rate of iodide substitution was also significantly slower than bromide substitution. The formation of bromo- and iodo- substituted DBPs results in higher values of genotoxicity, causing concern for detrimental health outcomes (Plewa *et al.* 2004a; Plewa *et al.* 2004b; Richardson *et al.* 2007). The influence of inorganic constituents on DBP formation and speciation varied depending on the conditions of the NOM precursors in the source water. For example, Cowman and Singer (1996) assessed the effect of bromide on aquatic humic substances and found no correlation between bromide concentrations and HAA formation.

The concentration of inorganic constituents also alters the DBP formation potential of phytoplankton precursors. Results obtained from studies of *M. aeruginosa*, indicated that the addition of bromide shifted DBP formation from HAA to THM dominated compounds (Wei *et al.* 2011). These results are contradictory to results from Hua *et al.* (2006) where whole raw water samples were used. The effects of inorganic constituents on a phytoplankton dominated system could have profound effects on DBP formation, speciation and the resulting associated health risk.

3.4 Mitigation of DBP formation from Phytoplankton Derived Precursors

To mitigate DBP formation it is critical that hydrophilic, autochthonous organic matter is targeted and removed prior to chlorination. This could be achieved by improving catchment management to reduce nutrients and phytoplankton production. Improved catchment management combined with an understanding of the dominant species and populations of phytoplankton within the system will allow for early detection of increased DBP formation potential. More advanced water treatment such as activated carbon, ultrafiltration, or resins can then be utilised to prevent risk of exposure to phytoplankton derived DBPs during a detected increase in phytoplankton population, reducing the concentration of NOM precursors exposed to chlorination.

Developing a greater understanding of the risk of DBPs to human health will allow for improved monitoring of harmful DBPs and tighter regulation. There is still a significant percentage of UTOX compounds being produced with minimal understanding of the short and long term impacts to human health.

3.5 Conclusion

The focus on phytoplankton within water treatment has largely been on phytoplankton cell removal and the removal of toxic compounds. However, only a few species are known to produce toxins or taste and odour compounds that can compromise water quality. In contrast all phytoplankton species fix carbon and contribute to the DOC pool and potential DBP precursors. This can pose a threat to human health when increased concentration of algal derived DOC is exposed to chlorine, increasing the risk of DBP formation. The contribution of phytoplankton towards the formation of DBPs is underestimated or largely ignored. The majority of the literature pools various sources of NOM or focuses only on allochthonous contributions to DBP formation. As algal-derived organic carbon is generally more recalcitrant to conventional treatment it is imperative that the total contribution of phytoplankton to the formation of DBPs is thoroughly understood for improved management and the minimisation of associated health risks.

Phytoplankton derived DBP formation is impacted by the rapid algal growth and turnover rates, cellular composition and biological lability. The contribution of phytoplankton to the formation of DBPs can potentially be heightened due to the notoriety of formation of cyanobacterial blooms, chemical composition and high DON contribution. Reducing phytoplankton populations within the water body is necessary to limit disinfection contact with cells and exudates. Therefore limiting nutrient supply with improved catchment management can mitigate many of the problems associated with algae (Brookes and Carey 2011). Nutrient reduction limits phytoplankton carrying capacity and growth rates reducing the populations of toxic cyanobacteria and concentration of DBP precursors derived from phytoplankton. Reducing the algal concentrations exposed to the disinfection process will reduce DBP formation within the water treatment plant and distribution network.

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Publication to be Associated with this Thesis

Chapter 4 – To be submitted for publication

Winter flows and summer blooms – comparison between organic matter load and disinfection by-product formation potential

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Chapter 4

Winter flows and summer blooms – comparison between organic matter load and disinfection by-product formation potential

Chemical disinfection during water treatment is necessary to inactivate pathogens; however, halogenated organic chemicals known as disinfection by-products (DBPs) can result from the reaction between the disinfectant and natural organic matter (NOM). DBPs have been associated with health issues including cancer and congenital malformations, further heightened by the higher genotoxicity of nitrogenous DBPs. The formation of DBPs is significantly influenced by the chemical composition and concentration of NOM, determined by the relative composition of allochthonous and autochthonous NOM precursors and the prevailing meteorological conditions. This study investigated the relative contribution of NOM from winter flows with elevated composition of allochthonous NOM, and summer phytoplankton blooms with higher composition of autochthonous carbon towards DBP formation. The largest organic matter load occurred during a winter rainfall event due to the large influx of catchment organic matter. The significant increase of catchment NOM correlated with high concentrations of DBPs. However, during periods of low rainfall inflow NOM was characterised with low DOC concentrations but resulted in higher yields of DBPs per milligram of DOC reacted. Further, the NOM associated with summer phytoplankton blooms resulted in the highest reactivity towards the formation of total DBP, unknown DBP and nitrogenous DBP formation. Higher concentrations of potentially genotoxic unknown DBP and more genotoxic nitrogenous DBP formation increases the potential risk to human health.

4.1 Introduction

Disinfection by-products (DBPs) are primarily formed via the oxidation reaction between a chemical disinfectant and natural organic matter (NOM). Exposure to DBPs via ingestion, dermal absorption or inhalation has been linked to numerous health issues including birth defects and cancer (Richardson 2003; Richardson 2005; Chisholm *et al.* 2008; Cantor *et al.* 2010; Villanueva *et al.* 2015; Freeman *et al.* 2017). With over 600 DBPs identified since their discovery by Rook (1974) and Bellar *et al.* (1974a), predicting the quantity and type of DBPs produced can be difficult and requires an in depth understanding of the reaction mechanisms that cause their formation.

DBPs are usually monitored at water treatment plants to ensure compliance with regulatory guidelines. These guidelines and the permissible concentrations vary considerably between regulatory bodies (Tomlinson *et al.* 2016). The vast majority of DBPs have not undergone comprehensive toxicological analysis and hence are not regulated and pose an unknown potential risk. Plewa *et al.* (2008) highlighted that nitrogenous disinfection by-products (N-DBPs) are more genotoxic and cytotoxic than their carbonaceous counterparts and yet there are fewer regulated N-DBPs (Richardson *et al.* 2007). Increased concentrations of organic nitrogen derived from phytoplankton could increase N-DBP formation and consequently the greater formation of N-DBPs would contribute a potentially greater health risk (Nguyen *et al.* 2005b; Richardson *et al.* 2007; Zhang *et al.* 2014; Wagner and Plewa 2017). Compliance with only regulated DBPs means there is a potential risk of exposure to a broader range of DBPs that are not monitored or regulated. For effective treatment there is a need to better understand how NOM influences total concentrations of DBPs and what causes variability in DBP speciation.

A significant cause of variability in DBP formation is the reactivity of the NOM, which is dependent on its chemical structure, which in turn is influenced by its source (Westerhoff *et al.* 2004; Golea *et al.* 2017). NOM is often categorised by its source as externally derived allochthonous organic matter and internally produced autochthonous organic matter (Hein *et al.* 2003). The relative contribution and chemical structure of allochthonous (terrestrial plant detritus and soils) and autochthonous (phytoplankton and macrophytes) organic matter is determined by the biogeophysical conditions of the

catchment including meteorological conditions, internal nutrient availability and catchment characteristics such as soil type, vegetation, catchment morphology and land usage (Finlay and Kendall 2007). Seasonal variation temporally defines the relative contribution of allochthonous and autochthonous organic matter sources to the total carbon budget of a lake or reservoir. Allochthonous NOM is typically washed in during rain event inflows and the greatest concentration of autochthonous NOM will be generated during conditions that generate phytoplankton blooms.

The novelty of this paper is to investigate the seasonal impacts upon DBP formation potential in real water samples to highlight differences in DBP formation between a winter rainfall event and a summer phytoplankton blooms. This was achieved by analysing the organic matter load within Myponga Reservoir during two ‘extreme events’; a rainfall event in winter and a phytoplankton bloom during summer. The organic matter was characterised and reacted with chlorine to determine DBP formation with different carbon character. Understanding the source of carbon and contribution to DBP formation will enable targeted management to control the risk and ultimately lead to improved water quality and human health outcomes. It was hypothesised that summer phytoplankton blooms would be more reactive with chlorine in the formation of DBPs, in particular, N-DBPs. It was also predicted that winter inflows would produce the highest concentrations of DBPs due to the higher dissolved organic carbon (DOC) loads in the reservoir during a rainfall event.

4.2 Methods

4.2.1 Site Description

Samples were collected from Myponga Reservoir, located approximately 63 km south east of Adelaide, South Australia (Figure 1). At capacity, Myponga Reservoir contains 26,800 ML with a maximum depth of 42 metres. Water drains from a catchment area of approximately 124 km². The catchment has mixed land use including improved pasture for dairy, beef and hay production, with patchy remnant native vegetation. Catchment sources often contribute most of the carbon load to Myponga Reservoir; however, autochthonous carbon load can contribute up to 50% of the total carbon budget during elongated periods of low rainfall (Linden *et al.* 2004). Artificial destratification of the

reservoir lowers the autochthonous phosphorus contribution which may reduce the severity of phytoplankton blooms during summer. Myponga Reservoir has a history of summer cyanobacteria blooms and is generally characterized by high DOC (13-15 mg/L), high colour₄₅₆ (55-65 HU) and low turbidity (< 5 NTU) (Lewis *et al.* 2004; Linden 2008). The influence of phytoplankton blooms on the treatability of the water is minimised by the adjustable height of the intake (Hobson *et al.* 2010). During winter the intake is positioned close to the surface at a depth of 5-10 meters; whereas during summer the intake is often 20-30 meters below the surface to avoid the intake of any surface phytoplankton blooms.

4.2.3 Experimental Approach and Logic

Sampling was designed to capture two seasonal events that significantly alter the NOM load within the reservoir; a substantial rainfall event (10 June 2016) and two phytoplankton blooms (7 December 2016 and 8 March 2017). Sampling locations were at the intake to the treatment plant, the surface at the dam wall, the inflow at the V-notch weir in Myponga River and at a secondary inflow at Barclay Rd Creek. The creek was sampled where it ran parallel to pasture and was highly modified to border the farmland. These locations were chosen to highlight the differences between NOM within the inflows, at surface samples where phytoplankton blooms are most prevalent, and water entering the treatment plant. Samples were collected in pre-rinsed new PET bottles or sterilised 10 L polypropylene containers. After collection, samples were transported immediately to the laboratory and preserved by refrigerating at 4°C. Samples were then returned to room temperature (~20°C) prior to analysis and were processed within 48 hours to ensure minimal degradation of the organic matter.

Water quality parameters of UV₂₅₄, colour₄₅₆, SUVA₂₅₄, turbidity, pH, temperature and DOC were measured to aid in organic matter characterisation (APHA *et al.* 2005). Automated sampling of temperature and dissolved oxygen (DO) profiles at the Myponga Reservoir dam wall location was also available during the elapsed time of this investigation. Samples were then chlorinated for a 72-hour period to represent the maximum exposure time within the local distribution network. Residual chlorine was quenched and DBP analysis was performed.

4.2.4 Analytical Methods

4.2.4.1 Phytoplankton Enumeration

Phytoplankton bloom samples were preserved in Lugol's iodine supplied by Sigma Aldrich (Missouri, USA) and enumerated using a Nikon Eclipse 50i microscope (Nikon, Japan) and a Sedewick Rafter Counting Cell (Pyser-SGI, UK). Samples were allowed to settle for 20 minutes after slide preparation and prior to enumeration. Cell counts were performed by using the procedure for randomly selected fields described by Chorus and Bartram (1999).

4.2.4.2 Chlorination

Chlorine demand was calculated by dosing samples from each location with known concentrations of chlorine stock solution at increments of 5 mg L⁻¹ with a contact time of 72 hours. After the 72 hours had elapsed, the resulting chlorine concentration was measured using the DPD colourimetric titration method (Harp 1995; APHA *et al.* 2005). Samples that produced a chlorine residual ≤ 5 mg L⁻¹ were used to calculate chlorine demand. Samples for DBP formation were then dosed with the calculated concentration of chlorine stock solution. After 72 hours of contact time the chlorine residual was measured and ammonium chloride was added to the remaining sample to quench any remaining chloride ions in preparation for DBP analysis.

4.2.4.3 Adsorbable Organic Halogens

Adsorbable organic halogens (AOX) are an indicator of total DBP formation. AOX formation was measured using the Mitsubishi Chemical Analytech Total Organic Halogen Analyser. Chlorinated samples were filtered at a rate of 3.3 ml/min through two carbon columns in sequence. Halogenated species present in the solution adsorb to the activated carbon. The columns were then washed with 10 mL KNO₃ solution to remove any inorganic halogen compounds prior to being injected into a furnace at 950 °C. The AOX and activated carbon were combusted in the oxygen flow and converted into hydrogen halide. The gases formed were dehydrated within the dehydration tube

containing concentrated sulfuric acid. The gases then flowed into a titration cell where a potential difference was measured and titrated against. 'Faraday's Law' was then applied to convert the energy used to complete the titration into the quantity of halogens present. To ensure the results are comparable, trichlorophenol standards were run at the beginning and end of the experiment. An electrolyte cell check was also performed prior to analysis with a known volume of 0.01 M hydrochloric acid to ensure that the electrode was responding appropriately.

4.2.4.4 Haloacetic Acids

This method covers the formation of nine HAA species: bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid, dibromoacetic acid, dichloroacetic acid, bromoacetic acid, chloroacetic acid, tribromoacetic acid and trichloroacetic acid (APHA *et al.* 2005). To determine haloacetic acid (HAA) formation, chlorinated water samples were pH adjusted to 0.5 and acids were extracted via liquid-liquid extraction method with methyl tert-butyl ether. The extracted acids were converted to their methyl esters via reaction with diazomethane. Excess diazomethane was removed and the remaining methyl esters were analysed by gas chromatography using the Varian GC/ECD with dual column configuration.

4.2.4.5 Trihalomethanes

This method covers the formation of four THM species: bromodichloromethane, bromoform, chloroform and dibromochloromethane (APHA *et al.* 2005). Samples were sealed in vapour tight vials. THM formation was determined using the Perkin Elmer headspace/ECD single column. A headspace auto sampler heated and agitated the vial to ensure that the vial contained volatile components in equilibrium between liquid and vapour phases. A defined volume of the vials headspace was then transferred to the column of the gas chromatograph. The components were detected by an electron capture detector and the output was measured by data acquisition software. Constructed calibration curves for each of the THM species present were used to quantify THM concentrations.

4.2.4.6 Disinfection By-product Method 551

DBP method 551 is designed to analyse for the suite of DBPs including haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones (US EPA 1990). Chlorinated water samples were extracted with methyl tert-butyl ether. The extract was then analysed quantitatively by gas chromatography using the Varian GC/ECD with dual column configuration. Confirmation of the eluted compounds was obtained from a dual column and electron capture detection sensor. Aqueous calibration standards were also extracted and analysed in order to compensate for any extraction losses.

4.3 Results

4.3.1 Meteorological Conditions and Organic Matter Characterisation

Seasonal meteorological conditions shape the prevailing hydrology and hydrodynamics and therefore play a key role in determining the relative contribution of allochthonous and autochthonous organic matter to the carbon budget of lakes and reservoirs (Tranvik *et al.* 2009). The influence of rainfall events and phytoplankton blooms alter the chemical composition of the NOM precursors.

In winter, Myponga Reservoir had lower average ambient temperatures and higher rainfall than December (2016) and March (2017) (Figure 8). The first substantial inflow for 2016 occurred on 10 June, peaking at 75.86 ML/day following 21.2 mm of rain on 9 June (Figure 9). Winter flows on 10 June were substantial enough to inundate the creek, which is often dry or stagnant for the majority of the year and hence no data was recorded in December or March. The flow rate recorded on 10 June was not the largest flow within Myponga River during this study; however, it was evidently the first major river inundation of 2016 (Figure 9). Rainfalls increased the concentration of catchment organic matter caused by runoff. Allochthonous organic matter was considered the dominant source in winter as the primary inflow (Myponga River) was characterised by high DOC (25.08 mg/L), high colour₄₅₆ (175 HU), high UV₂₅₄ (0.95 abs cm⁻¹) and high SUVA₂₅₄ (3.78 L/mg-m) measurements (Table 5). Low chlorophyll *a* concentrations were measured across all locations in June due to lower populations of phytoplankton during the onset of winter (Table 5). Inflows began to decline in late October of 2016 as rainfall became more sporadic and less intense resulting in a reduced load of fresh allochthonous

organic matter entering the system. Flow rates of 3.52 ML/day and 1.77 ML/day were recorded on 7 December and 8 March respectively (Figure 9). Catchment organic matter was significantly reduced as indicated by lower inflow DOC, UV₂₅₄ and colour₄₅₆ measurements comparative to winter inflows (Table 5). The DOC and UV₂₅₄ measurements within the inflow in March were the lowest on record at 9.85 mg/L and 0.39 cm⁻¹, which combined with the low flow would indicate low allochthonous carbon loading at that time. However, the character of the organic carbon within the inflows was still defined as allochthonous and aromatic given high SUVA measurements within Myponga River (Table 5).

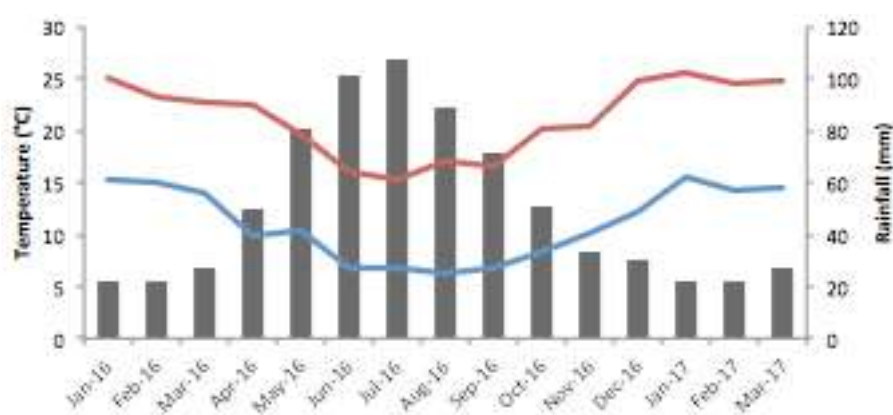


Figure 8. Temperature and rainfall data for 2016 and early 2017 at Myponga Reservoir. The red band represents the mean maximum temperatures and the blue band represents the mean minimum temperatures for each month. The columns indicate the mean rainfall at Myponga Reservoir per month.

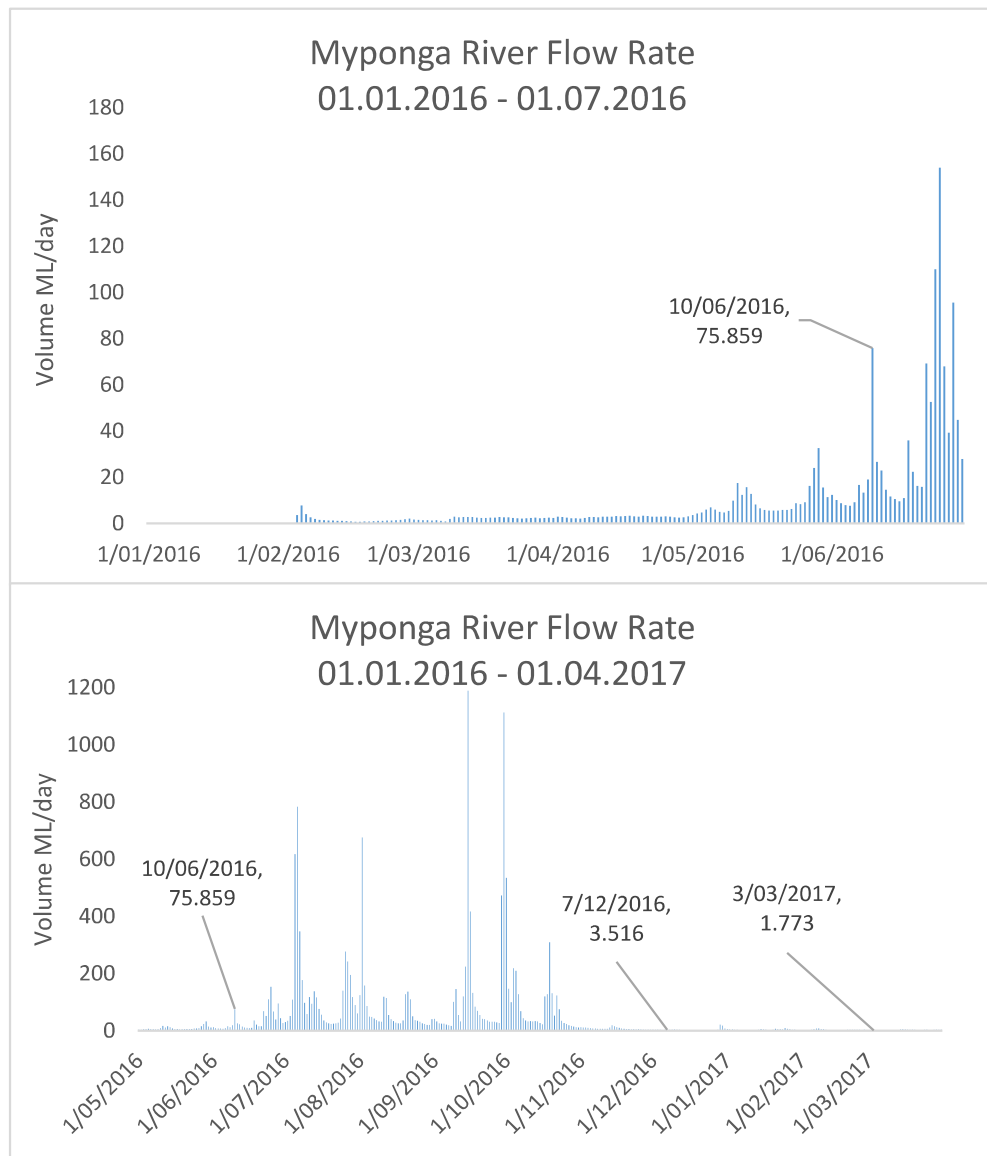


Figure 9: Flow rate in Myponga River for periods 01.01.2016 – 01.07.2016 and 01.01.2016 – 01.04.2017. Graphs indicate that the flow captured was one of the first major flows of 2016 within the river (top); however, this was not the largest flow within the year heavier rainfalls followed later within the season (bottom).

Table 5. Water quality parameters at Myponga Reservoir

Date	Location	DOC (mg/L)	pH	UV ₂₅₄ abs (cm ⁻¹)	SUVA (L/mg-m)	Colour ₄₅₀ (HU)	Turbidity (NTU)	Bromide (mg/L)	Chl <i>a</i> (µg/L)
10 Jun 2016	Intake	11.80*	7.7*	0.34*	2.88	28*	3.1*	0.53*	2.89*
	Surface	11.89*	7.7*	0.34*	2.85	28*	2.5*	0.54*	3.04*
	Inflow	25.08&	7.0&	0.95&	3.78	175&	10.1&	0.39&	3.88*
	Creek	18.48 ⁺	7.3 ⁺	0.74 ⁺	4.00	146 ⁺	35 ⁺	0.28&	4.35*
7 Dec 2016	Intake	16.63*	7.2*	0.67*	4.02	117*	5.7*	0.31*	2.07 ^a
	Surface	17.53&	8.6&	0.69*	3.93	131*	21&	0.31*	131.69&
	Inflow	15.84 ⁺	7.2*	0.81&	5.11	158&	5.3*	0.47&	1.60*
	Creek	NA	NA	NA	NA	NA	NA	NA	NA
8 Mar 2017	Intake	15.31*	6.9*	0.60*	3.91	85*	3.9*	0.33*	9.01*
	Surface	15.80*	7.2*	0.60*	3.79	88*	19&	0.32*	85.33&
	Inflow	9.85&	7.0*	0.39&	3.95	50&	2.8*	0.53&	1.13 ⁺
	Creek	NA	NA	NA	NA	NA	NA	NA	NA

Measurements within each event were assigned arbitrary characters *, &, +, and # to represent statistical significant differences between locations where $p > 0.05$. Significant difference was analysed using a pairwise t test with a Bonferroni P value adjustment method.

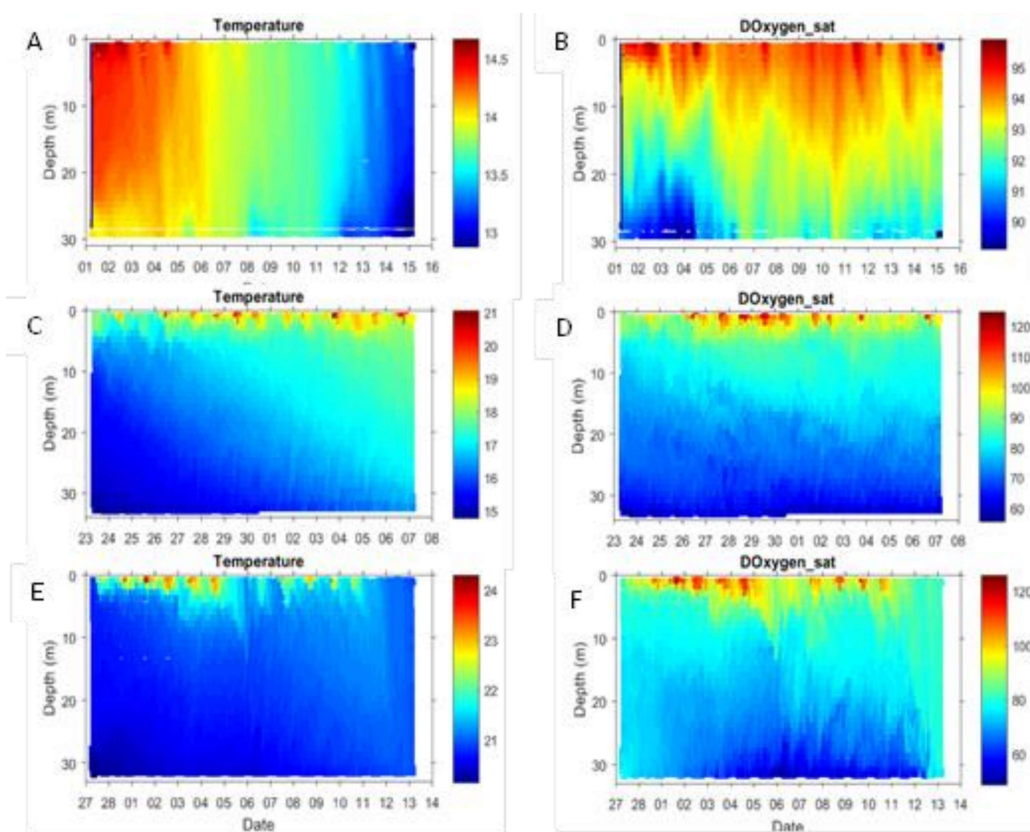


Figure 10. Temperature and oxygen saturation profiles at Myponga Reservoir during June 2016 (A-B), December 2016 (C-D) and March 2017 (E-F). Automated temperature and DO depth profiles are recorded at the dam wall near the surface and intake locations.

In comparison to high concentrations of catchment NOM measured within the winter inflows, reservoir DOC and UV₂₅₄ measurements were significantly lower (Table 5). Lower organic matter loading within the reservoir in June was likely caused by the inflow presenting as a plunging cold riverine intrusion that would have inserted at the bottom of the reservoir (Hobson *et al.* 2010). Low phytoplankton populations represented by low chlorophyll *a* concentrations would imply minimal contribution of internally produced NOM. However, lower SUVA₂₅₄ values would indicate that the characteristics of the DOC within the reservoir in winter was less allochthonous and aromatic comparative to Myponga River water (Table 5). The thermal profile of the reservoir represented a mixed system in June, increasing the homogeneity of the organic matter character at the intake and surface locations (Figure 10). Comparatively, the reservoir was thermally stratified

in December with a temperature difference of $\sim 4.5^{\circ}\text{C}$ from bottom to top, providing optimal conditions for the proliferation of surface cyanobacteria blooms (Figure 10). Although the temperature profile was considerably warmer there was minimal indication of thermal stratification in March (Figure 10). Significantly, higher chlorophyll *a* concentrations of 131.69 $\mu\text{g/L}$ and 85.33 $\mu\text{g/L}$ were recorded within the epilimnion in December and March respectively, signifying an increase in internally produced NOM within the reservoir during the phytoplankton blooms (Table 5). A *Dolichospermum circinale* surface bloom was present from 21 November and captured on 7 December with a surface scum cell count of 169,833 cells/mL (Table 6). A mixed bloom of *Microcystis flos-aquae* and *D. circinale* was observed at the surface on 8 March with surface scum cell counts of 9123 cells/mL and 2226 cells/mL respectively (Table 6). The phytoplankton bloom in March was captured in its infancy, with routine monitoring identifying that the bloom prevailed until 27 March (Table 6). Reservoir DOC and UV_{254} measurements were significantly higher comparative to winter reservoir samples due to the increase of autochthonous organic matter load (Table 5). However, relatively high DOC, UV_{254} , colour_{456} and SUVA_{254} measurements indicated that a significant proportion of the reservoir NOM load was still comprised of allochthonous and aromatic carbon (Table 5). Although characteristically allochthonous, the presence of the dense phytoplankton blooms increased the relative contribution of autochthonous NOM.

Table 6. Representation of surface bloom prevalence during sampling events. Cell counts are based on 5m integrated sample and are therefore lower than surface scum samples analysed on the day of sampling. Surface scum cell counts were only measured on highlighted sampling dates. Sampling dates of phytoplankton blooms are highlighted in the table.

Date	<i>D. circinale</i> (cells/mL)	<i>D. circinale</i> surface scum (cells/mL)	Date	<i>M. flos-aquae</i> (cells/mL)	<i>M. flos-aquae</i> surface scum (cells/mL)	<i>D. circinale</i> (cells/mL)	<i>D. circinale</i> surface scum (cells/mL)
21 Nov 2016	1,540	NA	2 Mar 2017	1,500	NA	162	NA
25 Nov 2016	4,490	NA	8 Mar 2017	2,100	9,123	680	2,226
28 Nov 2016	10,100	NA	9 Mar 2017	3,210	NA	346	NA
01 Dec 2016	12,700	NA	14 Mar 2017	93	NA	10	NA
05 Dec 2016	10,400	NA	16 Mar 2017	298	NA	31	NA
07 Dec 2016	8,390	169,833	20 Mar 2017	1,520	NA	14	NA
12 Dec 2016	32,800	NA	23 Mar 2017	1,800	NA	14	NA
15 Dec 2016	19,500	NA	27 Mar 2017	8,350	NA	13	NA

4.3.2 Disinfection By-product Formation

Total DBP formation including HAA, THM and DBP method 551 was determined across all locations and dates by analysing for AOX. Concentrations from each species were subtracted from AOX measurements to determine total unknown total organic halogens (UTOX) formed.

High winter flows corresponded with high DBP formation within the inflow and creek as indicated by AOX of 4972 µg/L and 4496 µg/L respectively (Figure 11A). UTOX of 2367 µg/L accounted for the bulk of the DBP formation within the inflow, whereas HAA formation of 2270 µg/L was the major species formed within the creek. Significantly lower chloroform and bromodichloromethane concentrations were measured within the inflow than the creek, likely due to non-preferential reaction rate kinetics resulting in the observed reduction in total THM formation and a spike in UTOX within the inflow (Figure 11A and Figure 12). Differences in DBP formation between the two inflows were likely caused by variation in allochthonous organic matter composition due to differences in catchment characteristics and the infrequency in which the creek is inundated. Total DBP formation was significantly lower within the intake and surface in June compared to inflow samples, presumably due to dilution or the riverine intrusion flowing lower in the reservoir (Hobson *et al.* 2010). However, brominated THM and HAA formation was higher within the reservoir (intake and surface) (Figure 12 and Figure 13) due to higher bromide concentrations than inflows (Table 5). Minimal differences in organic matter characterisation and DBP formation were observed between the intake and surface locations as they were geographically similar and thermal mixing allowed for increased homogeneity of the water column (Figure 10).

Summer inflows in December led to a reduction in allochthonous organic matter load; however, this did not coincide with a reduction in DBP formation (Figure 11B). AOX concentration of 5960 µg/L within the December inflow indicated that base flow allochthonous organic matter was more reactive with chlorine towards total DBP formation than fresh winter flows. Furthermore, December base flows had increased reactivity of allochthonous organic matter in the formation of all DBP species analysed (Table 7). Low allochthonous carbon loading in March resulted in lower AOX concentration of 4972 µg/L within the inflow compared with winter flows; however,

higher yields of all DBP species were formed per milligram of DOC reacted (Table 7). Higher formation of brominated THMs and HAAs were recorded within the inflows in December and March due to higher bromide concentrations compared with reservoir samples (Figure 12 and Figure 13).

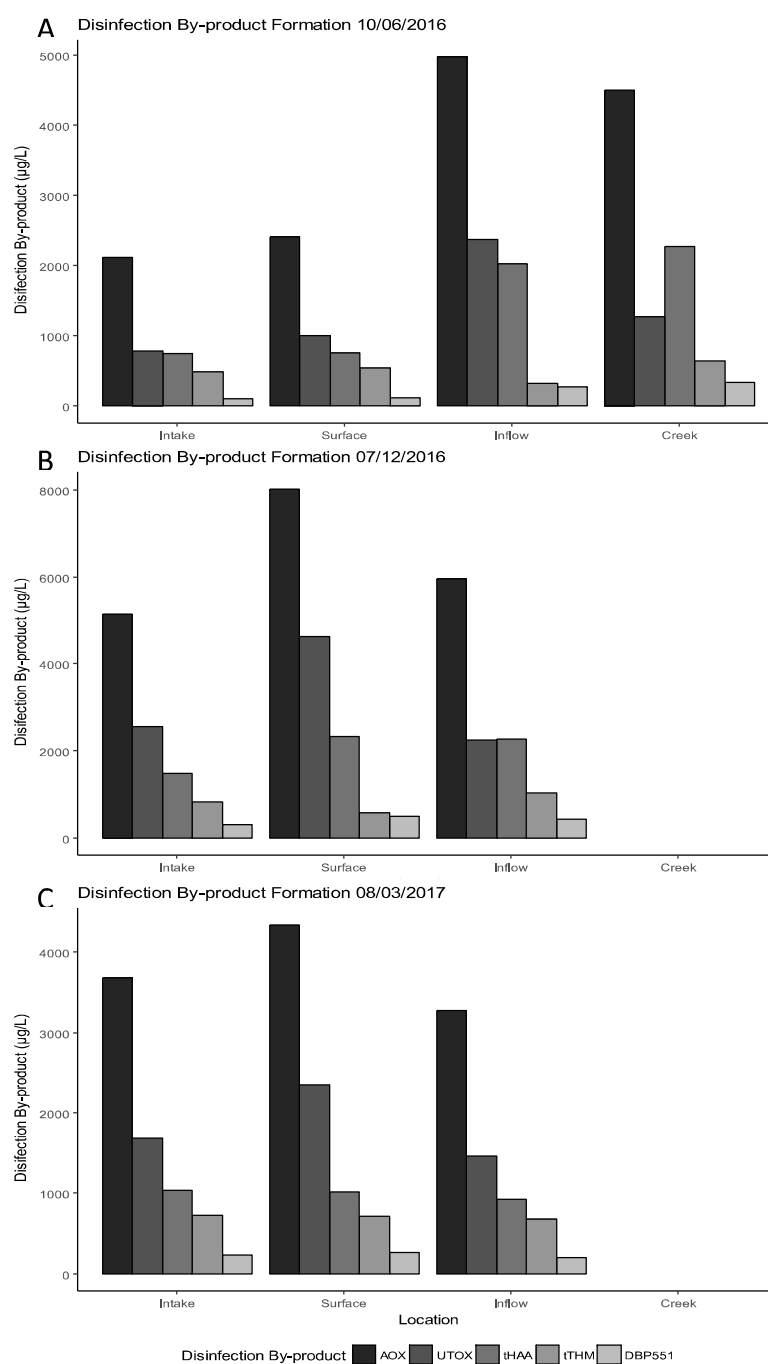


Figure 11. Disinfection By-Product (DBP) formation potential at Myponga Reservoir on 10/06/2016 (A), 07/12/2016 (B) and 08/03/2017 (C)

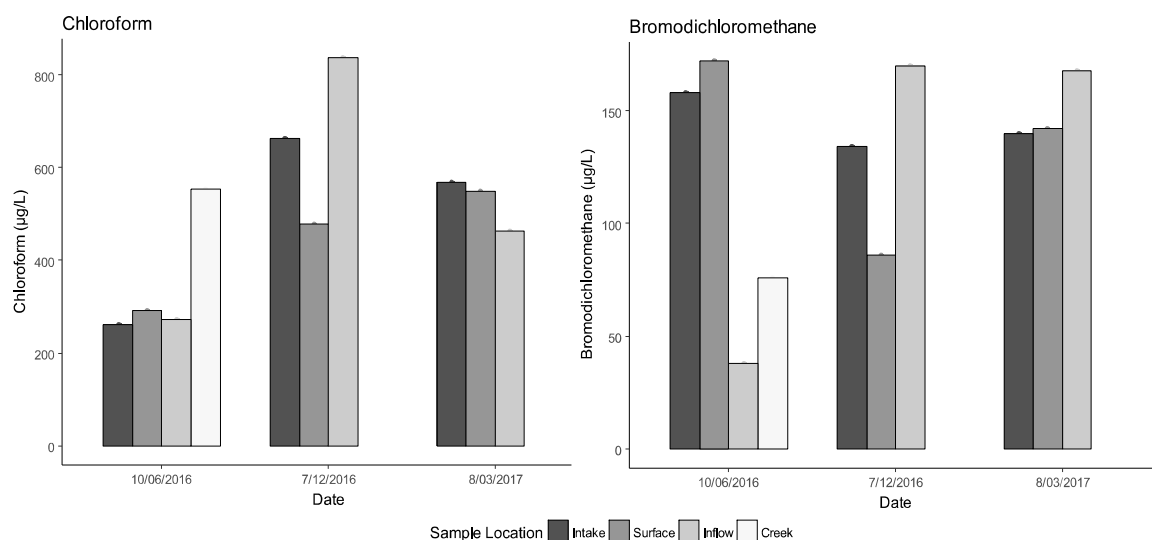


Figure 12. Formation of major trihalomethane (THM) species formed during analysis across all locations and dates.

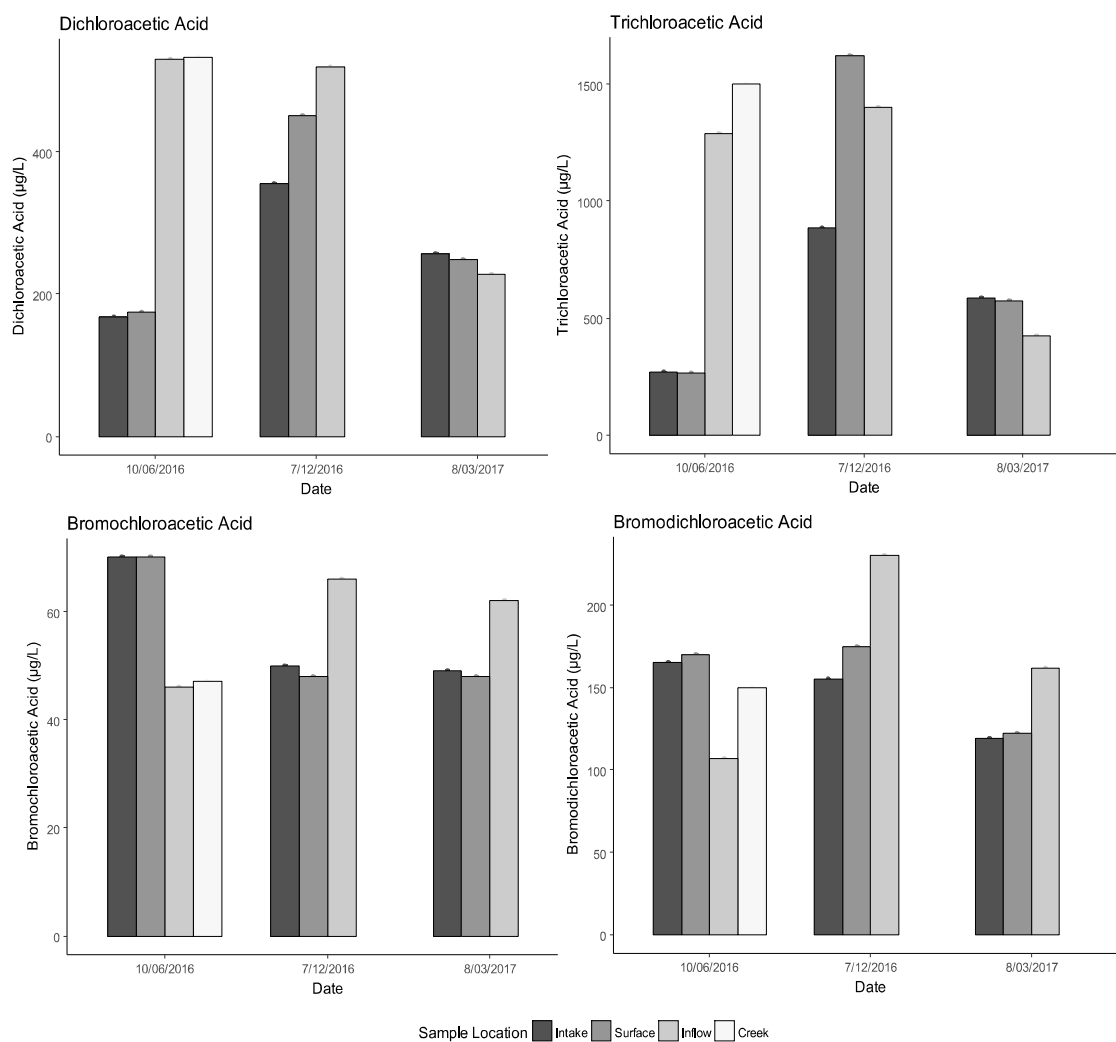


Figure 13. A comparison of major haloacetic acid (HAA) species formed across all locations and dates

Table 7. Reactivity of dissolved organic carbon (DOC) towards the formation of disinfection by-products (DBPs). The DBP formation is displayed per milligram of DOC.

Date	Location	DOC (mg L ⁻¹)	AOX:DOC (µg/mg)	UTOX:DOC (µg/mg)	HAA:DOC (µg/mg)	THM:DOC (µg/mg)	DBP 551:DOC (µg/mg)
10 Jun 2016	Intake	11.80	178.5	66.2	63.0	41.0	8.3
	Surface	11.89	202.0	84.0	63.3	45.2	9.4
	Inflow	25.08	198.2	94.4	80.5	12.6	10.8
	Creek	18.48	243.3	68.5	122.8	34.5	17.5
7 Dec 2016	Intake	16.63	309.7	153.4	89.0	49.0	18.3
	Surface	17.53	457.5	264.0	132.9	32.9	27.6
	Inflow	15.84	375.3	141.5	142.7	65.0	27.1
	Creek	NA	NA	NA	NA	NA	NA
8 Mar 2017	Intake	15.31	240.4	110.0	67.9	47.6	14.8
	Surface	15.80	274.7	148.4	64.6	45.1	16.6
	Inflow	9.85	332.5	148.9	94.3	68.9	20.3
	Creek	NA	NA	NA	NA	NA	NA

An established bloom of *D. circinale* increased relative contribution of autochthonous organic matter at the surface of the reservoir in December (Table 6), coinciding with the highest formation of DBPs recorded during this experiment (Figure 11B). AOX yield of 8020 µg/L during the phytoplankton bloom was predominantly comprised of 4628.7 µg/L of UTOX, accounting for 57.7 % of total formation. Furthermore, the presence of the dense *D. circinale* bloom coincided with the highest formation of UTOX, HAA and DBP 551 species due to higher reactivity of the DOC with chlorine (Table 7). The bloom also corresponded with the highest formation of N-DBP species producing 17.8 µg/L of bromochloroacetonitrile and 138 µg/L of dichloroacetonitrile (Figure 14). Chlorophyll *a* measurements of 2.07 µg/L at the intake indicated that the bulk of the phytoplankton bloom was not present at a depth of 15 m as a result of strong thermal stratification of the reservoir (Table 5). AOX formation of 5150 µg/L at the intake was still substantial, considering the bloom did not directly influence the organic matter characterisation and DBP formation at that location. High DOC, UV₂₅₄ and colour₄₅₆ measurements at the intake and surface would indicate that allochthonous humic and fulvic acids were present, contributing towards DBP formation (Table 5). Water entering the treatment plant in December had the highest reactivity with chlorine in the formation of AOX, UTOX and all measured species compared to both June and March (Table 7).

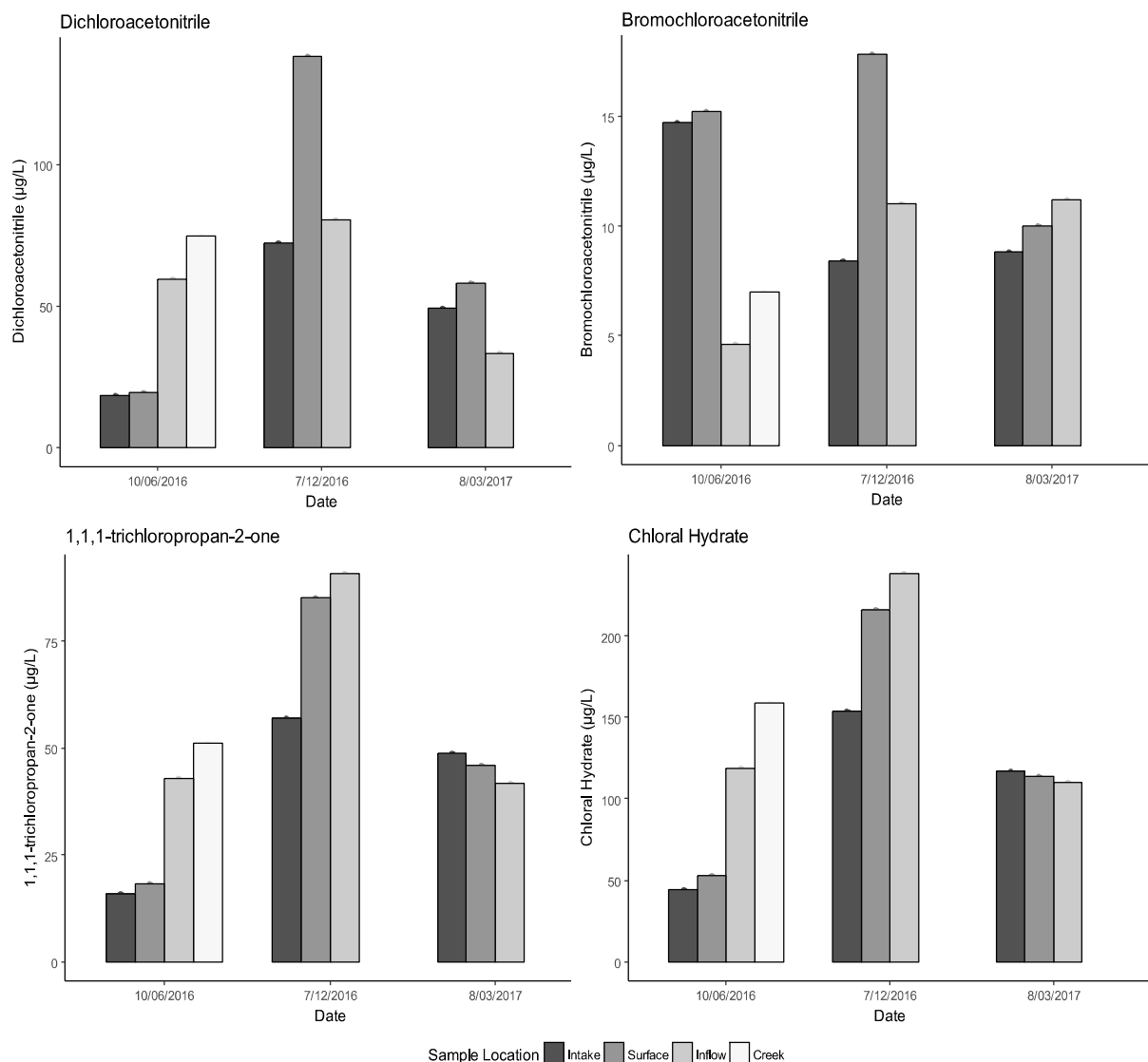


Figure 14. Comparison of major disinfection by-product (DBP) 551 species formed.

The chlorination of the smaller phytoplankton bloom on 8 March also resulted in considerable DBP formation within the reservoir. This smaller phytoplankton bloom was comprised of a mixture of *D. circinale* and *M. flos-aquae*. Upon chlorination of this bloom the formation of AOX, UTOX, HAA, THM and DBP 551 at the surface and intake locations were all significantly higher than reservoir measurements from June (Figure 11C). Furthermore, chlorinated surface samples were more reactive with chlorine per milligram of DOC in the formation of AOX, UTOX, THMs and DBP 551 species compared with high winter inflows (Table 7). N-DBP concentrations were also slightly elevated at the surface due to higher concentrations of dichloroacetonitrile compared with intake and inflow concentrations in March (Figure 14). Intake and surface DBP formation in March was lower compared with the much larger phytoplankton bloom in December.

A lack of thermal stratification in March influenced DBP formation within the intake due to stronger vertical mixing of the water column. This resulted in an elevated presence of phytoplankton was identified by high chlorophyll *a* measurements (Table 5). Inflows in March did not result in higher DBP concentrations in comparison to intake and surface locations, however the reactivity of the DOC was higher for AOX, HAAs, THMs and DBP 551 (Table 7).

4.4 Discussion

Seasonal variation impacted the character of NOM and resulting DBP formation upon chlorination. The winter rainfall event and summer phytoplankton blooms highlighted how variation in the relative contribution of allochthonous and autochthonous sources can influence DBP formation. Winter rainfalls increased the relative contribution of catchment NOM entering the system, indicated by high DOC, UV₂₅₄, turbidity and colour₄₅₆ measurements. In contrast, summer phytoplankton blooms increased the relative contribution of internally produced NOM within the reservoir which is indicated by higher chlorophyll *a* concentrations and high phytoplankton population. Observations from both events identified that an increase in the relative contribution allochthonous or autochthonous organic matter resulted in significantly higher formation of DBPs; however, the reactivity of organic matter with chlorine varied substantially temporally and between sources.

4.4.1 Winter DBP Formation

Winter inflows succeeding the rainfall event in June resulted in the highest DOC concentrations recorded. Significantly lower DOC concentrations were measured within the reservoir location as a result of cold riverine intrusion. Colder and hence denser water entering the system sinks and flows along the bottom of the reservoir towards the dam wall (Hobson *et al.* 2010). The intake into the treatment plant is closer towards the surface during winter to minimise the intake of high allochthonous organic matter loads and higher pathogens concentrations associated with dense riverine intrusions (Brookes *et al.* 2004). Therefore, high winter flows bringing in elevated concentrations of NOM does

not always directly or immediately impact the treatability of the water at Myponga Reservoir due to the adjustable height of the intake (Hobson *et al.* 2010).

DOC is often used as an explanatory parameter of DBP formation alongside pH, temperature, disinfectant dose, contact time and type of disinfectant (Sadiq and Rodriguez 2004; Chowdhury *et al.* 2009). It was therefore expected that there would be higher DBP formation within the inflows due to higher concentrations of DOC. Although AOX was significantly higher within the inflows compared with reservoir measurements in winter, the relationship between DOC and DBP formation did not correlate for each DBP species. This was highlighted by significantly lower THM formation within the inflow, even although DOC concentrations were considerably higher (Figure 11A). The strength of the correlation between DOC and DBP formation was further weakened when temporally analysing the contribution of inflow NOM towards DBP formation. Inflow DOC in December and March was significantly more reactive with chlorine per milligram of DOC present (Table 7). The increased reactivity of the allochthonous DOC could be due to variations in the composition/ source of the organics or because the organic matter had broken down over time into more labile and reactive compounds. Gang *et al.* (2003) identified that THM formation per milligram of chlorine consumed was higher for organics with lower molecular weights.

4.4.2 Summer DBP Formation

The relative contribution of autochthonous organic matter to the carbon budget was highest in December at the surface during a dense bloom of *D. circinale*. Allochthonous NOM load was still considered the primary source of NOM indicated by high DOC, UV₂₅₄ and colour₂₅₄, although phytoplankton population was significantly highest during this period. The phytoplankton bloom was well established, having been identified through routine monitoring 16 days prior to collection (Table 6). The bloom coincided with the highest DOC concentration recorded at the surface. It was hypothesised that summer phytoplankton blooms would be more reactive with chlorine in the formation of DBPs and in particular N-DBPs. The hypothesis was supported as chlorination of the December bloom produced the highest concentrations of AOX, UTOX, HAA and DBP 551 in comparison to all other locations and dates (Figure 11). Higher DBP formation at the surface in December occurred because the NOM proved to be more reactive per

milligram of DOC with chlorine in the formation of AOX, UTOX and DBP 551 (Table 7). Further, the December phytoplankton bloom also produced the highest concentration of N-DBPs identified within the DBP 551 assay (Figure 14). The significant increase in UTOX and N-DBPs increases the relative risk to human health (Richardson *et al.* 2007; Hua and Reckhow 2008; Plewa *et al.* 2008; Bull *et al.* 2011). Comparatively, the bloom in March had only established 6 days prior to sampling, with significantly lower cell concentrations (Table 6). The contribution of autochthonous organic matter was therefore lower. Resulting DBP formation was still relatively high, with a high yield of UTOX; however, March inflows proved to be the highest yielding DBP source per milligram of DOC.

Although water within the intake was not directly affected by the phytoplankton bloom in December, cell lysis and continuous cellular exudation would have indirectly contributed to the organic matter load in the surrounding environment (Franklin *et al.* 2006; Ye *et al.* 2011; Tomlinson *et al.* 2016). Increased concentration of extracellular organic matter has been associated with higher formation of THMs and NDMA and therefore poses a higher potential health risk (Li *et al.* 2012). Furthermore, extracellular organic matter has a high proportion of hydrophilic organic matter which is more recalcitrant to conventional treatment methods (Singer and Harrington 1993; Li *et al.* 2012; Goslan *et al.* 2017). No significant thermal stratification resulting in elevated phytoplankton population within the intake might explain why intake and surface DBP formations are more similar in March compared to December when the bloom was not prevalent within the intake (Figure 11). During periods of weaker or negligible thermal stratification it is critical to understand the relative contribution of phytoplankton towards DBP formation due to increased risk of live cells entering the treatment plant.

4.4.3 Winter Flows vs Summer Blooms

DBP formation related to the December phytoplankton bloom supports the hypothesis that summer phytoplankton blooms are more reactive per milligram of DOC in the formation of DBPs and N-DBPs. However, it is evident that duration, cell concentration and speciation of the bloom have significant effects on the reactivity of the autochthonous organic matter with chlorine in the formation of DBPs. The smaller bloom captured in March resulted in lower reactivity in the formation of DBPs compared to the bloom in

December; however, the bloom was still significantly more reactive in the formation of AOX, UTOX and HAA's in comparison to winter inflows (Table 7).

The hypothesis that winter flows would correlate with higher DBP concentrations was not supported, as the significantly higher contribution of DOC from winter flows did not result in the higher DBP formation (Figure 11). The reactivity of winter inflows was 1.8-2.3 times lower than the summer surface phytoplankton bloom in December. Furthermore, lower DOC concentrations within the inflow in December also resulted in higher DBP formation and higher reactivity. Inflows in December and March were both more reactive in the formation of AOX, THM and DBP 551.

4.5 Conclusion

Comparison of the relative contribution of NOM from winter flows and summer blooms to DBPs formation concluded that both sources contribute significantly (Figure 11). However, the reactivity of the NOM from each source varied significantly (Table 7).

Investigation into the relative contribution of winter NOM towards DBP formation has highlighted that fresh flows contribute significantly towards DBP formation due to the considerable increase in DOC. However, inflows during periods of lower rainfall were significantly more reactive, producing higher yields of DBPs per milligram of DOC (Table 7). Furthermore, NOM captured during low flows produced significantly more UTOX per milligram of DOC, increasing the relative risk of exposure to DBPs that are not regulated (Table 7).

The presence of a substantial phytoplankton bloom resulted in increased reactivity in the formation of AOX, UTOX and N-DBPs. Phytoplankton blooms can impact the treatability of intake water since they contribute significantly to the carbon budget of the surrounding environment due to continuous cellular exudation, high cell turnover rates and lysis upon cell death (Tomlinson *et al.* 2016). Intracellular and extracellular autochthonous organic matter has been identified to contain high proportions of hydrophilic content that is recalcitrant to conventional treatment (Singer and Harrington 1993; Bond *et al.* 2011a; Li *et al.* 2012). The difficulty in treatability of phytoplankton organic matter, and significantly higher formation of UTOX and N-DBPs not regulated by the Australian Drinking Water Guidelines increases the relative risk to human health

(Plewa *et al.* 2008; Bull *et al.* 2011). As highlighted by Chowdhury *et al.* (2009), an evaluation of models for predicting DBP formation is required to better account for reaction rate kinetics that are representative of real water supply systems. The improved management of phytoplankton populations within lakes and reservoirs is required to minimise DBP exposure upon treatment. Implementation of online fluorometric probes will allow for the rapid detection of the early onset of cyanobacterial blooms, resulting in improved response times to reduce cell biomass or alter the treatment process (Zamyadi *et al.* 2016). Limiting essential nutrients via improved catchment management will aid in reducing phytoplankton population and occurrences of blooms (Brookes and Carey 2011). Nutrient limitation of phytoplankton will reduce the relative contribution of intracellular and extracellular autochthonous organic matter exposed to chlorine during treatment thereby reducing the relative risk of DBP formation.

4.6 Acknowledgements

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Publication to be Associated with this Thesis

Chapter 5 – To be submitted for publication

Hydrophobic & hydrophilic – dissolved organic matter characterisation and disinfection by-product formation

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Overall Percentage: 55%

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Signed....

Date

27/7/2018

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Supervised development of work, helped in data interpretation and manuscript evaluation

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.....Date..27 July 2018

Chapter 5

Hydrophobic & hydrophilic – dissolved organic matter characterisation and disinfection by-product formation

The risk associated with disinfection by-products (DBPs) needs to be continuously evaluated given the necessity for continuous use of chemical disinfection in the foreseeable future. Critical insight into the reactivity of natural organic matter (NOM) towards the formation of DBPs was established via the fractionation of the organic matter based on its hydrophobic and hydrophilic properties. However, the chlorination of individual fractions does not consider the competition between fractions that occurs during the chlorination of the bulk raw water. This limitation is overcome here by the use of a linear model. The relative abundance of hydrophobic and hydrophilic fractions was determined and the DBPs measured following chlorination of the bulk raw water. The Akaike Information Criterion (AIC) was used to determine a statistical model that best fits the data, identifying the significant NOM fractions. The model was then further simplified via a manual stepwise reduction to provide an output that is easily interpreted. Both the AIC and simplified statistical models identified that very hydrophobic acids were strongly positively correlated with total DBP, unknown DBP and DBP 551 formation. This relationship has implications for increased DBP formation during winter when high inflows of hydrophobic NOM enter the system during catchment runoff. However, as hydrophobic NOM is readily removed during conventional treatment the impact on DBP formation post-treatment will be significantly reduced. The AIC evaluation also identified that the hydrophilic neutral (NEU) fraction was strongly correlated with DBP formation and common to most of the significant interactions. Further simplification of the model highlighted that there was a strong positive correlation between NEU and the formation of total DBPs and unknown DBPs. The NEU fraction is of more concern regarding the formation of DBPs post-treatment due to its recalcitrance during coagulation. Higher concentrations of hydrophilic NOM were associated with high populations of phytoplankton suggesting that there is an increased potential risk of DBP formation during phytoplankton blooms.

5.1 Introduction

Chlorination of water is an essential barrier against the threat of pathogens (Centers for Disease Control and Prevention 2012; Howe et al. 2012). However, chlorine oxidises natural organic matter (NOM) to form halogenated organic compounds known as disinfection by-products (DBPs) (Richardson et al. 2007). Since their discovery by Bellar *et al.* (1974b) and Rook (1974), epidemiological studies have identified that ingestion, inhalation or dermal contact with some DBPs can increase the risk of congenital malformations and cancer (Richardson 2003; Villanueva et al. 2004; Chisholm et al. 2008; Cantor et al. 2010; Villanueva et al. 2015). Although adverse health effects have been identified, causality is still not conclusive (Hrudey et al. 2015; Villanueva et al. 2015). With less than 100 of over 600 identified DBP species having undergone quantitative toxicology studies, regulation is challenging and guideline values often vary significantly between regulatory authorities (Hebert et al. 2010; Tomlinson et al. 2016). Due to the continuous use of chemical disinfection in the foreseeable future, it is critical to investigate how DBPs are formed and what methods can be implemented to mitigate their formation.

DBP formation is influenced by the physiochemical conditions of the disinfection process, the concentration of inorganic DBP precursors, the disinfection agent used and the organic DBP precursors (Tomlinson et al. 2016). The physiochemical conditions such as disinfection contact time, pH and temperature are driven by cost, aesthetics and the requirement to deliver potable water free of pathogens (Chowdhury et al. 2009). The concentration of inorganic DBP precursors such as bromide, iodide and nitrate can have a significant effect on the genotoxicity of the DBP species formed (Plewa *et al.* 2004b; Hua *et al.* 2006; Bond *et al.* 2011b). Iodated and brominated DBPs are more genotoxic than chlorinated species, and nitrogenous disinfection by-products (N-DBPs) are more genotoxic than their carbonaceous counterparts (Plewa et al. 2004a; Richardson et al. 2007; Plewa et al. 2008; Chowdhury et al. 2009). The formation of DBPs is not restricted to the use of chlorine, as alternative chemical disinfectants result in the formation of unique suites of DBPs that can have potentially higher risks to human health (Hua and Reckhow 2007b; Richardson et al. 2007). The concentration and chemical structure of NOM causes major variability in the speciation and concentration of DBPs formed upon chlorination (Świetlik et al. 2004; Westerhoff et al. 2004).

NOM is a complex mixture of aromatic and aliphatic compounds with attached functional groups. The composition and quantity of NOM is highly variable, dependant on the source (allochthonous or autochthonous) and the biogeophysical conditions of the aquatic environment (Leenheer and Croué 2003). The highly variable nature of NOM makes the management of DBP formation challenging. As NOM is a precursor for DBP formation, the effective removal prior to disinfection is paramount to ensure a reduction in the concentration of DBPs formed (Jacangelo et al. 1995; Metcalfe et al. 2015). Numerous studies have investigated the effects of water quality and operational parameters in the attempt to produce a model that accurately correlates with the formation of DBPs (Chowdhury et al. 2009). The majority of these models use UV₂₅₄, specific ultraviolet absorbance (SUVA) or dissolved organic carbon (DOC) as surrogates of NOM to estimate the DBP formation of specific species under particular physical conditions. These models provide insight into the influences of DBP formation; however, their application is often limited as they are developed with specific source water quality and do not take into account varying NOM loads and sources. The source and hence composition of organic matter alters reaction rate kinetics with chlorine and causes significant differences in DBP formation per milligram of DOC present (Gallard and von Gunten 2002). Chapter 4 indicated that per milligram of carbon, autochthonous dominated NOM from phytoplankton organic matter formed significantly higher concentrations of DBPs and N-DBPs compared with allochthonous flows.

For a more comprehensive understanding of which organic compounds are responsible for DBP formation, DOC can be fractionated based on specific hydrophobic and hydrophilic characteristics (Leenheer 1981). Fractionated DOC can then be reacted with chlorine to determine the suite of DBPs formed from each component (Kitis et al. 2002; Świetlik et al. 2004; Soh et al. 2008; Lu et al. 2009; Hua et al. 2015). The relative contribution of each fraction towards DBP formation is highly dependent on source water characteristics (Chiang et al. 2002). However, most of the investigations noted that hydrophobic NOM was the major driver of DBP formation (Kitis et al. 2002; Soh et al. 2008; Hua et al. 2015). Catchment NOM is often characterised by higher proportions of hydrophobic NOM fractions, with humic substances typically representing ~50% of the DOC (Lee et al. 2006). On the other hand, hydrophilic NOM can account for up to 86% of autochthonous phytoplankton NOM in some species (Li et al. 2012). Understanding how each fraction relates to DBP formation can aid in rationalising the expense of

analysis to target the DBPs most likely to be formed based on the relative abundance of hydrophobic and hydrophilic organic matter. Further comprehension of the relationship between the hydrophilic neutral (NEU) fraction and DBP formation would be beneficial, given its recalcitrant nature to conventional treatment (Soh et al. 2008; Matilainen et al. 2010). High concentrations of hydrophilic organic matter are often linked with increased populations of phytoplankton, further increasing the risk of DBP formation potential post-treatment (Li et al. 2012).

DBP precursors are best understood in the context of the component biochemistry. The chlorination of individual fractions provides crucial insight into the reactivity of organic matter, but it does not consider the competition between NOM fractions during the chlorination of bulk water. Reacting bulk water with chlorine enables competition between the various organic carbon species; however, post-hoc analysis is required to tease apart which species are responsible for any resultant DBP formation. The novelty of this research is the ability to statistically identify the correlations between individual fractions and their subsequent interactions with DBP formation by chlorination of the bulk water. This was achieved here by initially analysing for the relative abundance of hydrophobic and hydrophilic fractions towards the total DOC pool. The bulk raw water was chlorinated and analysed for DBP formation. A linear model was then used to determine the relative contribution of hydrophobic and hydrophilic fractions towards DBP formation. It was hypothesised that hydrophobic organic matter would be the primary contributor towards DBP formation. Further, it was expected that interactions between each component would have a considerable impact on the speciation and concentration of DBPs formed. The utilisation of a linear model can improve the way utilities determine the risk of DBP formation without the need of costly analysis.

5.2 Methodology

5.2.1 Site Description

The relationship between hydrophobic and hydrophilic organic matter with DBP formation was investigated at Myponga Reservoir, South Australia. The reservoir is subjected to a Mediterranean climate conditions of hot, dry summers and cool, wet winters. Catchment organic matter is usually the dominant contributor towards the carbon

budget of the reservoir, although phytoplankton have previously contributed up to 50% of the total carbon budget during elongated periods of low annual rainfall (Linden et al. 2004). The impact on treatability from frequent cyanobacteria blooms in summer is minimised by the adjustable height of the intake (Hobson et al. 2010). Further, artificial destratification within the reservoir reduces the autochthonous phosphorus input which may reduce the proliferation of phytoplankton due to the reduction of this limiting nutrient (Lewis et al. 2004; Brookes et al. 2008).

5.2.2 Experimental approach

Sampling at Myponga Reservoir was programmed to investigate how organic matter fractions correlate with DBP formation. This experiment was conducted during a winter rainfall event on 10 June 2016 and during three separate phytoplankton blooms on 7 December 2016, 8 March 2016 and 11 January 2018. The samples were collected from four locations; the intake into the treatment plant (A), the surface at the dam wall (B), Barclay Rd. Creek (creek) (C) and Myponga River (inflow) (D) (Figure 1). Sample locations were chosen to capture allochthonous organic matter inflows, autochthonous organic matter at the surface in summer during phytoplankton blooms and a representation of water entering the water treatment plant. Samples were collected in capped 600 mL PET bottles and 10 L polypropylene containers. Analysis of pH, colour₄₅₆, UV₂₅₄, turbidity and DOC was performed on each sample for water quality monitoring (Rice et al. 2012). Samples were then fractionated based on hydrophobic and hydrophilic properties utilising a rapid fractionation technique developed by Chow et al. (2004). Unfractionated raw water samples were chlorinated for 72 hours, quenched and analysed for DBP formation.

5.2.3 Analytical Methods

5.2.3.1 Rapid Fractionation

Organic matter was fractionated into 4 distinct components based on hydrophobic and hydrophilic properties (Figure 2). Myponga raw water was mixed until homogeneous and filtered through a 0.45 µm PES filter to remove particulate organic matter. Prior to fractionation, a 30 mL aliquot was taken for DOC analysis and the remaining sample was

pH adjusted to less than 2 using 1M HCl. Samples were then filtered in sequence at a flow rate of 3 ml/min through columns containing DAX-8, XAD-4 resins, retaining very hydrophobic acids (VHA) and slightly hydrophobic acids (SHA) fractions respectively. A 30 mL aliquot was collected after each filtration process for DOC analysis. XAD filtrate was then pH adjusted to 8 with 1M NaOH and the remaining sample was filtered through a column containing IRA-958 resin retaining the hydrophilic charged (CHA) fraction. The remaining sample was representative of NEU compounds and was analysed for DOC. Contribution of each fraction towards the DOC pool was then determined by back calculating the amount of DOC removed after filtration process.

5.2.3.2 Chlorination

Unfractionated raw water samples were chlorinated for 72 hours to equal the maximum exposure time within the local distribution network. Residual chlorine demand was measured using the DPD colourimetric method to ensure that the concentration of chlorine dosed was adequate (Harp 2002; Rice et al. 2012). Chlorine residual was quenched with ammonium chloride in preparation for DBP analysis.

5.2.3.3 Adsorbable Organic Halogens

Total DBP formation was determined by measuring for adsorbable organic halogens (AOX) the Mitsubishi Chemical Analytech Total Organic Halogen Analyser Model AOX-200. A 50 mL aliquot of chlorinated sample was filtered through two packed carbon columns in sequence. Halogenated species present within the sample were adsorbed onto carbon filter and the filtrate was disposed. The carbon columns were then primed with 10 mL of KNO₃ solution prior to ignition of the powdered activated carbon within a furnace at 950 °C. DBPs are identified by titrating against the potential difference caused by the presence of halogenated species.

5.2.3.4 Haloacetic Acids

Chlorinated samples were pH adjusted to 0.5 for acid extraction with methyl tert-butyl ether. Conversion of extracted acids to their methyl form was achieved by reacting with

diazomethane. Methyl esters were then analysed by gas chromatography after the removal of any excess diazomethane using the Varian GC/ECD with dual column configuration (Rice et al. 2012). Haloacetic acid (HAA) analysis covered 9 common species: bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid, dibromoacetic acid, dichloroacetic acid, bromoacetic acid, chloroacetic acid, tribromoacetic acid and trichloroacetic acid (APHA *et al.* 2005)

5.2.3.5 Trihalomethanes

Trihalomethane (THM) formation was determined by heating samples and extracting volatiles produced for analysis via gas chromatography using the Perkin Elmer head space/ECD single column (Rice et al. 2012). Trihalomethane analysis covered 4 commonly formed species: bromodichloromethane, bromoform, chloroform and dibromochloromethane (APHA *et al.* 2005).

5.2.3.6 DBP method 551

Haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones were analysed using the DBP 551 method. Chlorinated samples were extracted with methyl tert-butyl ether and analysed via gas chromatography with electron capture detection using the Varian GC/ECD with dual column configuration (US EPA 1990).

5.2.4 Statistical Analysis

The normality of the data was investigated and it was determined via Shapiro Wilk test that normality was lacking for multiple variables. The data set was pre-processed by application of the Yeo-Johnson power transformation to improve the normality of the data. Improved normality was confirmed by investigation with the Shapiro Wilk test. Correlations between variables were then investigated to ensure that each variable was independent (Table 8). Strong correlations between VHA, colour₄₅₆ and UV₂₅₄ were identified; however, these correlations with hydrophobic NOM have previously been well documented (Bolto *et al.* 2002; Leenheer and Croué 2003; Hongve *et al.* 2004)

Table 8. Correlations identified between variables used within the Akaike Information Criterion (AIC) evaluation. Higher values up to a maximum of 1.00 indicate a strong correlation between variables. Negative values down to a minimum value of -1.00 represent an inverse correlation between the two variables.

	VHA	SHA	CHA	NEU	Temperature	pH	Colour ₄₅₆	UV ₂₅₄
VHA	1.00	0.54	0.59	0.56	-0.01	-0.29	0.92	0.95
SHA	0.54	1.00	0.56	0.69	-0.16	0.13	0.43	0.44
CHA	0.59	0.56	1.00	0.39	-0.12	0.03	0.37	0.45
NEU	0.56	0.69	0.39	1.00	-0.14	-0.11	0.56	0.50
Temperature	-0.01	-0.16	-0.12	-0.14	1.00	-0.18	0	-0.01
pH	-0.29	0.13	0.03	-0.11	-0.18	1.00	-0.33	-0.28
Colour	0.92	0.43	0.37	0.56	0	-0.33	1.00	0.97
UV	0.95	0.44	0.45	0.50	-0.01	-0.28	0.97	1.00

A statistical approach using a linear model was chosen to aid in the identification of which fractions of DOC were the major contributors to particular suites of DBPs. Statistical modelling provides a method for predicting the unknown parameters of a model within a specified dimension and structure. The Akaike Information Criterion (AIC) method considers that the framework of the model dimension is also unknown and therefore uses the data to determine the framework of the model. The AIC simultaneously determines model estimation and selection, where the likelihood function indicates the conformity of the model to the observed data (Akaike 2011; Cavanaugh and Neath 2011). The stepwise AIC method was used to determine which model provided the best fit to the data by adding or subtracting variables and their interactions of varying degrees of complexity in a stepwise manner. This is achieved using three strategies to find the model with the lowest AIC value to ensure exploration of full range of interaction complexity (Figure 15):

- Beginning with the most complex interactions and stepwise removing interactions one at a time
- Beginning with the individual fractions and adding more complicated interactions one at a time
- Beginning with intermediate complex reactions and both adding and subtracting interactions of varying complexity one at a time

The model with the lowest AIC output is considered to be the best fit to the data and is used to evaluate the significance of the observed interactions. As well as DOC fractions, location was used as a variable to account for spatial differences between samples. The influence of temperature, pH, UV₂₅₄ and colour₄₅₆ were also investigated within the model. Upon determining the model of best fit, temporal differences were also accounted for and the stepwise model was rerun and summarised to determine the relationships between DOC fractions and DBP formation. Temperature, pH, UV₂₅₄ and colour₄₅₆ were investigated because these variables are known to significantly influence DBP formation potential (Tomlinson *et al.* 2016). Therefore, addition of these variables into the model strengthens the outcome, and if the DOC fractions survive the model reduction process, then their significance is better represented.

The initial AIC reduction was used to determine the model of best fit and to determine the correlations between NOM fractions and their respective interactions with DBP formation. Not all variables identified in the AIC output are significantly correlated with DBP formation but their presence in the reduced model increases the strength of the output. The output highlights all significant correlations between variables and response variables; however, interpretation can be difficult. Further model reduction was manually completed after the utilisation of the stepwise AIC reduction method to produce a more simplistic output. This was achieved by manually removing insignificant variables from the AIC model in a stepwise order. Fractions were removed from highest complexity to lowest ensuring that variables with the lowest significance were removed first. This manual reduction was performed until all remaining variables had a P value of <0.01. All statistical analysis for this project was completed using R studio (R Core Team 2017).

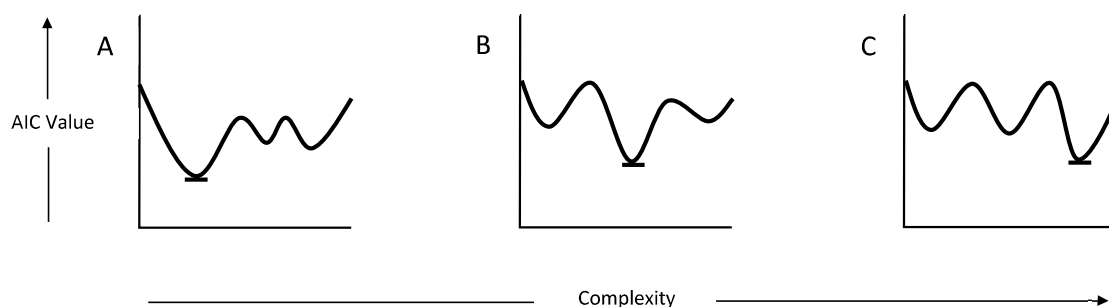


Figure 15. Use of the stepwise Akaike Information Criterion (AIC) to determine the model that has the highest conformity to the data. The model with the lowest AIC value is the model determined to have the best fit. To determine the model with the lowest AIC value, the stepwise AIC method is used in three different scenarios represented by the above arbitrary graphs: (A) beginning with lowest model complexity and adding more complex interactions, (B) starting with interactions of intermediate complexity and adding and subtracting interactions of varying complexity and (C) using the most complex interaction and subtracting less complicated interactions.

5.3 Results

5.3.1 Organic Matter Characterisation

The quantity and source of NOM varied substantially, both temporally and spatially. Changes in season influenced the relative contribution of allochthonous and autochthonous NOM (Grey et al. 2001; Hein et al. 2003; Rautio et al. 2011), and in turn, alter its reactivity with chlorine (Owen et al. 1995; Fabris et al. 2008). On 10 June, the carbon loading of Myponga Reservoir was driven by inflows that were predominately comprised of catchment NOM. The reservoir was subject to high rainfalls coinciding with low chlorophyll *a* concentrations across all locations. Myponga River was characterised by high DOC (24.5 mg/L), colour₄₅₆ (186 HU) and UV₂₅₄ (0.97 cm⁻¹) concentrations (Table 9). The sampling regime in June was the only date where significant flows were observed at Barclay Rd. Creek, contributing a further 18.51 mg/L of characteristically allochthonous NOM (Table 9). The other three sampling dates all coincided with low inflows and surface phytoplankton blooms associated with high DOC and chlorophyll *a* concentrations near the dam wall (Table 9). On 7 December, a cyanobacteria bloom of *D. circinale* (169,833 cells/mL) was observed, and high populations had prevailed since 12 November. A phytoplankton bloom was observed in its infancy on 8 March which was comprised of a mixture of cyanobacterial species *Microcystis flos-aquae* and *D. circinale*, at 9123 cells/ mL and 2226 cells/mL respectively. Finally, the bloom observed

on 11 January had prevailed since late November, transitioning from a *D. circinale* bloom in November/December to a mixed *Microcystis aeruginosa* and *M. flos-aquae* dominated bloom in early to mid-January. Cell concentrations of the bloom on 11 January were significantly higher than both previous blooms, with surface scum samples recording a combined cell count of 60-70,000,000 cells/mL. Thermal stratification of the reservoir during December, March and January restricted the depth at which the bloom was observed within the water column, indicated by significantly lower chlorophyll *a* reading within the intake (Table 9). The influence of the surface phytoplankton bloom on the treatability of the water at Myponga Water Treatment Plant was minimised by the adjustable height of the offtake (Hobson et al. 2010)

Table 9. Water quality parameters spanning the sampling regime from 2016 - 2018

Date	Location	DOC (mg L ⁻¹)	pH	UV ₂₅₄ abs (cm ⁻¹)	Colour ₄₅₆ (HU)	Turbidity (NTU)	Chlorophyll a (µg/L)
10 June 2016	Intake	11.69	7.72	0.34	28	3.10	2.89
	Surface	11.74	7.59	0.34	28	2.46	3.04
	Inflow	24.54	6.98	0.97	186	9.64	3.88
	Creek	18.51	7.25	0.75	150	33.58	4.35
7 December 2016	Intake	16.68	7.43	0.67	116	5.93	2.07
	Surface	17.53	8.01	0.68	123	15.84	131.69
	Inflow	16.06	7.32	0.81	161	5.29	1.60
	Creek	NA	NA	NA	NA	NA	NA
8 March 2017	Intake	15.36	7.11	0.61	88	4.02	9.01
	Surface	15.89	7.37	0.61	92	12.49	85.33
	Inflow	9.96	7.10	0.40	52	2.98	1.60
	Creek	NA	NA	NA	NA	NA	NA
11 January 2018	Intake	14.30	7.5	0.540	76	20.9	0.37
	Surface	21.40	6.3	0.611	152	4244	7111
	Inflow	12.47	7.2	0.544	72	3.6	2.13
	Creek	NA	NA	NA	NA	NA	NA

5.3.2 Organic Matter Fractionation

The concentration, chemical composition and source of the NOM is variable between location and temporally; evident by differences in the relative proportions of the hydrophobic and hydrophilic fractions (Figure 16). In June, the relative composition of VHA within the inflow and creek samples was significantly higher than reservoir samples, accounting for nearly 80% of total DOC during the rainfall event (Table 10). Consequently, winter inflows were comprised of lower relative compositions of SHA, CHA and NEU than reservoir samples. During the bloom in December, the relative composition of VHA was lower at the surface compared with inflow and intake samples; instead, a higher percent concentration of the SHA fraction was observed. The smaller bloom in March did not indicate any significant differences in the relative contribution of any fraction between the locations sampled. The significantly larger phytoplankton bloom in January resulted in a higher composition of the NEU fraction within surface samples, accounting for 22.2% of total DOC (Table 10). The higher composition of the NEU fraction correlated with lower percent composition of VHA, SHA and CHA fractions at the surface compared to the inflow and intake locations. For all samples collected, the hydrophobic fractions (VHA and SHA) accounted for > 72% of the total DOC content (Table 10). The composition of hydrophilic fractions (CHA and NEU) were always slightly higher at the intake and surface samples than within the inflows, especially in June and January during the larger phytoplankton blooms.

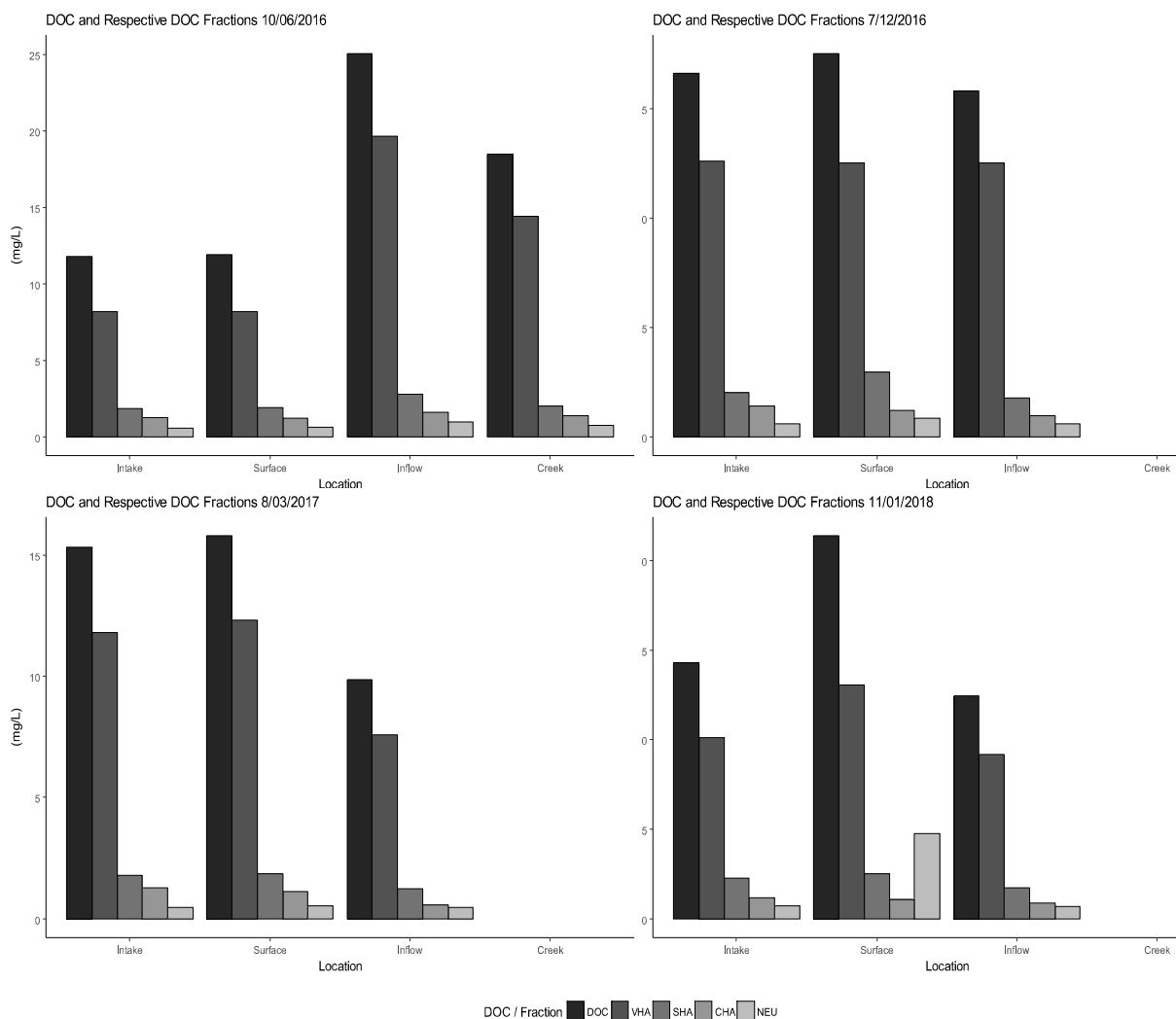


Figure 16. Dissolved organic carbon (DOC) concentrations and corresponding very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), charged hydrophilic acids (CHA), and hydrophilic neutrals (NEU) fractions.

Table 10. Relative contribution of hydrophobic and hydrophilic fractions towards total dissolved organic carbon (DOC) pool.

Date	Location	DOC (mg L ⁻¹)	Hydrophobic Fractions		Hydrophilic Fractions	
			VHA (%)	SHA (%)	CHA (%)	NEU (%)
10 June 2016	Intake	11.69	68.76	16.00	9.75	4.62
	Surface	11.74	69.51	15.67	9.80	5.03
	Inflow	24.54	79.79	10.23	6.44	3.50
	Creek	18.51	78.55	10.59	7.13	3.73
7 December 2016	Intake	16.68	76.38	12.35	7.49	3.84
	Surface	17.53	73.13	14.55	7.30	4.96
	Inflow	16.06	79.27	10.52	1.43	3.92
	Creek	NA	NA	NA	NA	NA
8 March 2017	Intake	15.36	77.07	11.92	7.69	3.39
	Surface	15.89	77.39	12.22	6.80	3.59
	Inflow	9.96	77.41	12.35	10.14	4.32
	Creek	NA	NA	NA	NA	NA
11 January 2018	Intake	14.30	70.91	15.87	8.18	4.97
	Surface	21.40	61.03	11.64	5.09	22.24
	Inflow	12.47	73.70	13.71	6.98	5.53
	Creek	NA	NA	NA	NA	NA

5.3.3 Disinfection By-product formation

DBP formation varied considerably both temporally and spatially, coinciding with alterations in the concentration and chemical composition of the NOM precursors (Figure 17). Higher DBP formation was observed within the inflows in June compared with reservoir samples (Figure 17). Elevated DBP formation within winter inflows correlated with a significant increase in allochthonous DOC that was washed in from the catchment during the captured rainfall event (Figure 16). All three phytoplankton blooms observed during December, March and January coincided with higher DBP formation at the surface location compared with intake and inflow samples (Figure 17). It was evident that as phytoplankton cell concentrations increased, the formation of DBPs also increased. UTOX usually accounted for the majority of all DBPs formed, especially during the phytoplankton bloom in January (Figure 17). HAA's were commonly the largest known class of DBPs formed, followed by THM's and DBP 551 species.

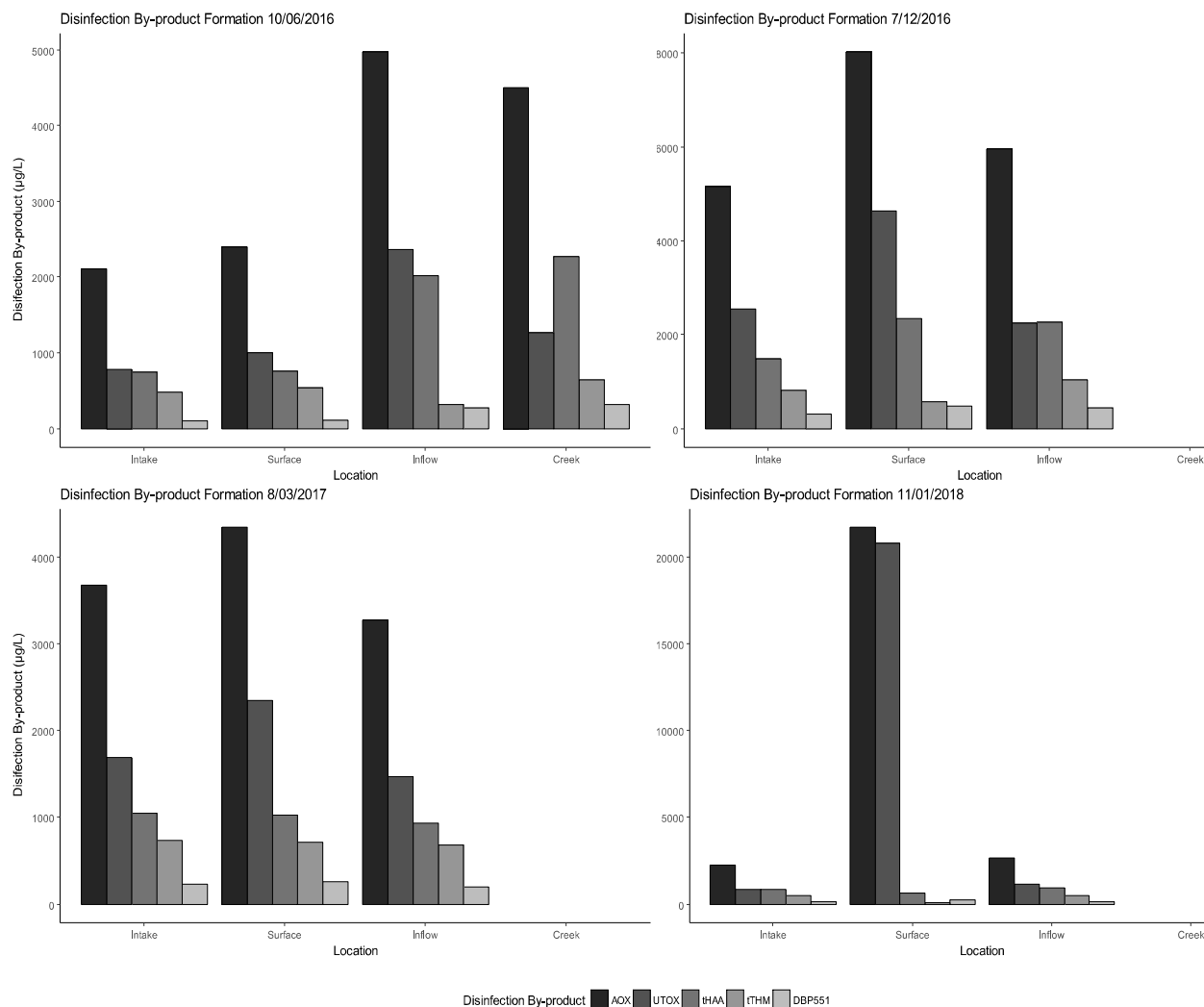


Figure 17. Disinfection by-product (DBP) formation representing the formation of adsorbable organic halogens (AOX), unknown total organic halogens (UTOX), total haloacetic acids (tHAA), total trihalomethanes (tTHM) and DBP 551.

5.3.4 Akaike Information Criterion

The results of the AIC evaluation indicated that both individual fractions of NOM and their subsequent interactions significantly influenced the formation of DBPs (Table 11). The identified correlations indicate that the variables survived the model reduction via the AIC method. Location, pH, UV₂₅₄ and colour₄₅₆ were also determined to significantly influence DBP formation (Table 11). Temperature was not identified as a significant influencer of DBP formation; however, this is because the temperature at which the samples were chlorinated was kept stable at approximately room temperature (~20°C)

Table 11. Relationship summary established via the Akaike Information Criterion method. The strength of the relationship between dissolved organic carbon (DOC) fractions and disinfection by-product (DBP) formation is denoted as strongest to weakest by the following symbols; ***, **, and * which correspond with P Values of $< 10^{-4}$, $< 10^{-3}$ and < 0.01 respectively. No symbol represents a negligible relationship between DOC fraction and DBP formation.

Fractions and interactions	AOX			UTOX			THMs			HAAs			DBP551		
	F value	P value		F value	P value		F value	P value		F value	P value		F value	P value	
VHA	271	***		180	***					150	***			***	
SHA															
CHA	8.08	*													
NEU	49.2	***		46.2	***		69.7	***							
VHA : SHA															
VHA : CHA	14.3	**		9.05	*										
VHA : NEU							50.3	***		5.58	*				
SHA : CHA															
SHA : NEU	7.80	*													
CHA : NEU				12.4	**										
VHA : SHA : CHA															
VHA : SHA : NEU															
VHA : CHA : NEU				17.1	**										
SHA : CHA : NEU															
VHA : SHA : CHA : NEU															
Location	9.79	**		5.92	*										
pH							6.96	*							
Temperature															
UV ₂₅₄	63.3	***		49.9	***					14.2	**				
Colour ₄₅₆							26.9	***		151	***		22.1	***	

5.3.4.1 Individual NOM Fractions and DBP Formation

The AIC investigation highlighted that the VHA fraction significantly influenced the formation of AOX and UTOX and the DBP 551 (Table 11). The recalcitrant NEU fraction significantly correlated with the formation of AOX, UTOX and THMs (Table 11). To a slightly lesser degree, the CHA fraction also correlated with AOX formation (Table 11). The SHA fraction did not significantly correlate with DBP formation. Further, no individual fractions were found to correlate with the formation of HAAs.

5.3.4.2 NOM Interactions and DBP Formation

The AIC model also identified that two and three-way interactions between hydrophobic and hydrophilic fractions correlated significantly with DBP formation. The VHA and NEU fractions were common to most of these interactions; representing strong correlations with AOX, UTOX and DBP 551 formation via multiple two-way interactions; and towards UTOX via a three-way interaction (Table 11). The CHA fraction was also involved in two-way interactions correlating with AOX and UTOX and involved in a three-way interaction correlating with UTOX (Table 11). Finally, the SHA fraction was identified to correlate with AOX formation via a two-way interaction with NEU.

5.3.4.3 Simplified Model

To simplify the interpretation of the AIC output the model was further reduced manually by removing variables that were not significantly correlated with DBP formation in a stepwise manner (Table 12). The simplified model identified that AOX formation was positively correlated with the individual VHA and NEU fractions and negatively correlated with UV₂₅₄ (Table 12). The location also significantly impacted the formation of total DBP formation, likely due to the stark differences in AOX concentration between surface samples and inflow samples (Figure 17). Similarly, UTOX formation was positively correlated with both VHA and NEU fractions and negatively correlated with UV₂₅₄ (Table 12). The simplified model identified that the NEU fraction was the only fraction that significantly correlated with THM formation; however, this relationship was

negative. Colour₄₅₆ and pH correlated positively with THM formation (Table 12). This model did not identify any organic matter fractions that correlated with HAA formation; instead, pH and colour₄₅₆ were found to positively correlate with HAA formation. There were no further simplifications made to the AIC model correlating NOM fractions with DBP 551 formation

AOX			UTOX			THMs			HAAs			
Coefficient	F value	P value	Coefficient	F value	P value	Coefficient	F value	P value	Coefficient	F value	P value	Coefficient
+	107	***	+	54.8	***							+
+	17.9	***	+	15.8	***	-	25.01	***				
												-
NEU												
	3.94	*										
							6.25	*	+	6.92	*	
-	26.4	***	-	14.6	**							+
							21.4	***	+	150	***	+

(Table 12).

5.4 Discussion

The regulation and management of DBP formation is critical to ensure the provision of potable water and minimising the associated adverse health effects. The formation of DBPs via the oxidation reaction between the disinfectant and NOM is simple. However, the variation in the chemical structure of NOM, the concentration of organic precursors, the influence of inorganic precursors and the effect of physical conditions of the reaction increases its complexity by altering the reaction rate kinetics and allowing for the

formation of over 600 individual DBPs (Hebert et al. 2010; Tomlinson et al. 2016). The complexity of this reaction is driven by variable meteorological conditions that influence the relative contribution of allochthonous and autochthonous organic matter loads to rivers or reservoirs, altering the concentration and chemical composition of NOM reacted with chlorine. Fractionation of NOM has been critical in advancing knowledge of the composition and reactivity of NOM, especially with regards to DBP formation (Soh et al. 2008; Lu et al. 2009; Hua et al. 2015). Expanding on this, a linear model can statistically determine correlations between NOM fractions and DBP formations via the chlorination of the bulk raw water. This method provides further insight into NOM reactivity by accounting for the competition between fractions during chlorination.

Table 12. Simplified model correlating natural organic matter (NOM) fractions with disinfection by-product (DBP) formation whilst also accounting for location, pH, temperature, UV₂₅₄ and colour₄₅₆.

Fractions and interactions	AOX			UTOX			THMs			HAAs			DBP551		
	Coefficient	F value	P value	Coefficient	F value	P value	Coefficient	F value	P value	Coefficient	F value	P value	Coefficient	F value	P value
VHA	+	107	***	+	54.8	***				+	150	***			
SHA															
CHA															
NEU	+	17.9	***	+	15.8	***	-	25.01	***						
VHA : SHA															
VHA : CHA															
VHA : NEU										-	5.58	*			
SHA : CHA															
SHA : NEU															
CHA : NEU															
VHA : SHA : CHA															
VHA : SHA : NEU															
VHA : CHA : NEU															
SHA : CHA : NEU															
VHA : SHA : CHA : NEU															
Location		3.94	*												
pH								6.25	*	+	6.92	*			
Temperature															
UV ₂₅₄	-	26.4	***	-	14.6	**							+	14.2	**
Colour ₄₅₆								21.4	***	+	150	***	+	22.1	***

5.4.1 AIC Statistical Approach to Determine Contributors to DBP Suite

The aim of this statistical approach was to determine the significance of the correlations between each NOM fraction and their subsequent interactions with DBP formation at Myponga Reservoir. The initial AIC evaluation recognised that the individual hydrophobic and hydrophilic fractions were not the only identified relationships correlated with DBP formation (Table 11). Rather, two and three-way interactions between fractions and DBP formation were also significant. This supports the hypothesis that interactions between NOM fractions should be considered to further understand the correlation between NOM and the speciation and concentration of DBP formation. The AIC evaluation did not recognise a significant relationship between the interaction of all four fractions and DBP formation, implying that the bulk DOC was not as strongly correlated with DBP formation when compared with the other interactions evaluated. A weaker relationship between the interaction of all four DOC fractions and DBP formation was expected as numerous other studies have highlighted that DOC alone is not a good indicator/ predictor of DBP formation as investigated in Chapter 4. These relationships between DOC fractions and DBP formation highlight the significance of the composition, reactivity, and in turn, the source of the NOM on the quantity and speciation of DBPs formed.

5.4.2 Simplified Model to Determine Contributors to DBP Suite

The AIC evaluation identified the model that best fits the data and therefore provides the best understanding as to how organic matter fractions and their interactions correlate with DBP formation. However, the complexity of the model chosen makes the interpretation of the output difficult. Further simplification of the model was achieved by manually removing any variables that had no significant correlation with DBP formation in a stepwise manner. The significance of two and three-way interactions identified by the AIC evaluation did not survive the manual model reduction for AOX, UTOX, THM and HAA formation (Table 12). This does not indicate that two and three-way interactions are not significant as they were determined significant via the AIC best fit model. In this instance removal of the two and three-way interactions allowed for easier interpretation of individual fractions towards DBP formation. The simplified model for DBP 551

formation did not show any differences in comparison with the AIC evaluation (Table 12).

5.4.3 Hydrophobic Fractions and interactions

The AIC evaluation identified that a significant determinant of DBP formation at Myponga Reservoir was the VHA fraction and its interactions (Table 11). Elevated concentrations of the VHA fraction were measured during the rainfall event in June, increasing the risk of associated DBPs during periods of increased inflows (Table 10). Further, the VHA fraction was always present in significantly higher concentrations in comparison to all other fractions (Table 10). The correlation between VHA and DBP formation supports the hypothesis and coincides with other studies that have highlighted the significance of bulk hydrophobic fractions in the formation of both AOX and UTOX (Singer 1993; Kitis et al. 2002; Hua and Reckhow 2007a; Soh et al. 2008; Hua et al. 2015). The individual VHA fraction did not correlate strongly with THMs, contradicting the findings from Soh et al. (2008), which is likely due to competition of reaction kinetics between various NOM fractions when the bulk raw water is chlorinated. Instead, both VHA and NEU fractions correlate strongly with THMs via a two-way interaction (Table 11). In contrast with the research of Liang and Singer (2003), the VHA and SHA fractions did not strongly correlate with HAA formation, highlighting the significant contribution from hydrophobic fractions towards THM and HAA formation (Table 11). The VHA and SHA fractions are readily removed during conventional treatment via coagulation. Therefore, although the VHA fraction strongly correlated with DBP formation, their impact will be reduced post-treatment (Nilson and DiGiano 1996; Bolto et al. 2002; Chow et al. 2004; Metcalfe et al. 2015).

Further simplification of the model provided insight into how individual fractions of NOM correlate with DBP formation (Table 12). This process confirmed that the VHA fraction was positively correlated with AOX, UTOX and DBP 551 formation. Manual model reduction also indicated that the individual VHA fraction was not significantly correlated with THM formation upon removal of the VHA: NEU interaction. There was no evidence that the SHA fraction was strongly correlated with DBP formation (Table 12). Although the individual VHA fraction positively correlated with DBP 551 formation, the interaction between VHA and NEU had an inverse effect (Table 12).

5.4.4 Hydrophilic Fractions and interactions

High concentrations of phytoplankton are generally associated with increased concentrations of recalcitrant hydrophilic NOM (Lui et al. 2011). This is evident by the higher NEU composition of surface samples during the largest bloom in January (Figure 16). Higher concentrations of the recalcitrant NEU fraction may correlate with higher DBP formation within the product water, increasing the relative risk to human health upon exposure. Further, the eutrophication of freshwater systems through climatic variability as well as urban, agricultural and industrial development has increased the prevalence of phytoplankton blooms (Paerl and Huisman 2008; Paerl and Paul 2012); raising the potential risk of DBP formation associated with hydrophilic NOM.. The NEU fraction was strongly associated with the formation of AOX, UTOX and THMs (Table 11). Interactions with the NEU fraction strengthened the observed relationship with AOX, UTOX and THM formation. Further, an interaction between VHA and NEU identified a strong correlation with DBP 551 formation (Table 11). The CHA fraction was also strongly correlated with AOX and UTOX formation, both individually and via two and three-way interactions with other fractions. The CHA fraction is also deemed partially recalcitrant to conventional treatment, increasing the probability of escalated DBP formation within the product water.

Manual model reduction highlighted that the NEU fraction was positively correlated with the formation of AOX and UTOX (Table 12). The correlation between the NEU fraction and THM formation was identified as an inverse relationship indicated by the negative coefficient (Table 12). The reduced model also identified that the CHA fraction was no longer significantly correlated with DBP formation (Table 12). The interaction between NEU and VHA was also defined as an inverse relationship with the formation of DBP 551.

5.4.5 Location and Water Quality Parameters

The AIC evaluation identified that location, pH, colour₄₅₆ and UV₂₅₄ were all significantly correlated with DBP formation (Table 11). Temperature was not identified

as a significant variable relating to DBP formation; however, this is because the experiments were all run at room temperature ($\sim 20\text{ }^{\circ}\text{C}$) nullifying its effect. For further insight into how the location and water quality variables correlated with DBP formation the model was further simplified. Upon model simplification, it was evident that location was only identified to significantly influence the formation of AOX (Table 12). This is likely due to the significant disparity in AOX concentration between surface and inflow samples (Figure 17). UV_{254} positively correlated with the formation of AOX, UTOX and DBP 551 formation, coinciding with observations of correlations between VHA and DBP formation (Figure 17). It was expected that VHA and UV_{254} would have similar relationships with DBP formation due to the initial investigations identifying that UV_{254} and VHA variables correlated strongly; however, colour_{456} did not conform to this expectation (Table 8). Colour_{456} was deemed positively correlated with THM, HAA and DBP 551 formation and pH was positively correlated with THM and HAA formation.

5.4.6 Implications

The utilisation of a linear model provides a comprehensive method to determine how NOM fractions are correlated with DBP formation. This method gives insight into how fluctuations in NOM character driven by meteorological conditions influence the formation of DBPs. In winter, allochthonous NOM is the dominant source of carbon within the reservoir, often comprised of higher concentrations of hydrophobic NOM. However, as the VHA fraction is efficiently removed via the coagulation and sedimentation processes of conventional treatment, the impact on DBP formation within product water will be minimised

During summer, there is a higher prevalence of phytoplankton blooms, increasing the autochthonous organic matter load within the reservoir. With this increase, the relative composition of hydrophilic organic matter can increase (Table 10). The hydrophilic fraction of NOM is of particular concern due to its recalcitrant nature towards the conventional treatment processes. Higher concentrations of the hydrophilic NOM observed in the larger phytoplankton blooms (December 2016 and January 2018) positively correlated with AOX and UTOX in accordance with both the AIC evaluation and the simplified model. This correlation was evident by the obvious increase in both AOX and UTOX formation during these phytoplankton blooms (Figure 17). It is

noteworthy that the DOC was characterised by significantly higher proportions of hydrophobic NOM across all locations and all dates sampled. However, the hydrophobic fraction is readily removed during treatment (Singer and Harrington 1993).

The relative contribution of hydrophobic and hydrophilic fractions towards DBP formation is informative; however, the chemical composition of that NOM should still be considered. Higher risk of N-DBP formation associated with algal organic precursors is due to the lower mean C:N ratio of autochthonous NOM than allochthonous NOM (Elser et al. 2000) and due to elevated cellular exudation of algal fixed nitrogen as dissolved organic nitrogen (Nguyen *et al.* 2005a). However, comprehension of the relative contribution of hydrophobic and hydrophilic NOM fractions towards DBP formation will enable water treatment plants to tailor treatment processes to reduce DBP formation within the product water. Understanding how allochthonous and autochthonous sources of NOM differ with regards to their hydrophilic and hydrophobic properties is critical to operating a water treatment plant that minimises the formation of DBPs rather than reacting to increases in their detection. Further, the identification of relationships between DOC fractions and DBP formation determined using a linear model, removes the need to perform costly DBP analysis on each fraction.

5.5 Conclusion

The relative risk to human health from DBP exposure is difficult to manage due to fluctuations in the concentration and chemical composition of NOM precursors, which in turn dramatically impacts the abundance and type of DBPs formed. The variability of NOM reactivity with chlorine increases the difficulty to accurately correlate DBP formation with basic surrogates of NOM such as DOC and UV₂₅₄. These NOM surrogates do not account for reaction rate kinetics or variability of NOM source. Therefore, fractionation of NOM into discrete categories based on hydrophobic and hydrophilic properties allows for improved understanding of the reactivity of the organic matter. Separation of NOM into smaller fractions also provides insight into the origin of the organics due to an understanding of the relative composition of fractions present. However, the chlorination of individual fractions does not account for the competition between fractions that occurs when bulk raw water samples are chlorinated. This limitation is overcome with the use of a linear model, allowing for correlations to be

determined between NOM fractions and their interactions towards the DBP formation. This highlighted the significance of the VHA and NEU fractions towards DBP formation via individual, two-way and three-way interactions. The VHA fraction is readily removed via coagulation during conventional treatment reducing the relative risk of DBP formation; however, the NEU fraction is recalcitrant, often persisting in product water (Soh et al. 2008; Matilainen et al. 2010).

High concentrations of hydrophilic NOM are often associated with high concentrations of intracellular and extracellular phytoplankton organic matter (Li et al. 2012) (Figure 16). This has implications for the treatability of water during summer phytoplankton blooms with regards to the management of DBP formation. The NEU fraction correlated with AOX and UTOX formation and strongly correlated with THM formation (Table 11). Further, the NEU fraction also strongly correlated with DBP formation via two and three-way interactions with other fractions; however, the impact of these correlations will be reduced due to the effective removal of VHA and SHA fractions via conventional treatment.

The use of a linear model provided a tool to further enhance our understanding as to how NOM fractions correlate towards DBP formation within Myponga Reservoir. This provided further insight into how NOM from allochthonous and autochthonous sources react with chlorine to form DBPs.

5.6 Acknowledgements

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Publication to be Associated with this Thesis

Chapter 6 – To be submitted for publication

Simulated phytoplankton blooms and disinfection by-product formation potential

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A. Tomlinson

Performed analysis, interpreted data, wrote manuscript and acted as corresponding author.

Overall Percentage: 60%

I hereby certify that the statement of contribution is accurate

Signed..... Date, 27/7/2018

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Supervised development of work, helped in data interpretation and manuscript evaluation

Overall Percentage: 20%

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Supervised development of work, helped in data interpretation and manuscript evaluation

Overall Percentage: 20%

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed.....

.....Date, 27 July 2018

Chapter 6

Simulated phytoplankton blooms and disinfection by-product formation potential

The significant contribution of phytoplankton towards disinfection by-product (DBP) formation is not limited to cyanobacteria that are prone to forming dense blooms. Rather, the entire phytoplankton community continuously contributes highly reactive organic matter that forms DBPs upon chlorination. Phytoplankton organic matter is often comprised of a high proportion of intracellular and extracellular hydrophilic organic matter in comparison to allochthonous carbon sources. Therefore, these organics are more recalcitrant to conventional treatment practices, posing a higher risk of increased DBP formation post-treatment. Further, phytoplankton organic matter is a known source of organic nitrogen, which can increase the concentration of genotoxic nitrogenous disinfection by-product (N-DBP) upon chlorination. This study investigated the contribution of green algae *Ankistrodesmus sp.* and cyanobacteria *Dolichospermum circinale* towards DBP formation when added to raw water in controlled concentrations. This experiment was designed to allow for the complex reaction competition imposed by the organic matter present within the raw water samples during winter when natural phytoplankton populations were low. The addition of simulated phytoplankton blooms did not significantly increase the concentration of the dissolved organic carbon compared with raw water concentrations. However, a significant impact on DBP formation was measured. Both species of phytoplankton significantly increased the concentration of total DBP formation and unknown DBP formation, posing a potential increased health risk upon exposure. In relation to cell biovolume, *Ankistrodesmus sp.* correlated with a higher concentration of DBPs compared to *D. circinale*. However, *D. circinale* was associated with the higher formation of N-DBP and is more prone to forming dense blooms. Therefore, *D. circinale* is more likely to pose a risk of increased DBP formation. This research demonstrates the significant contribution of *D. circinale* and *Ankistrodesmus sp.* species towards DBP formation; highlighting the need to consider the entire contribution of the phytoplankton population to DBP formation.

6.1 Introduction

Chlorination of water is a critical component of conventional treatment that provides an effective barrier against a range of bacterial, viral and protozoan pathogens (Centers for Disease Control and Prevention 2012; Howe *et al.* 2012). However, chlorine also oxidises natural organic matter (NOM) to form halogenated organic compounds known as disinfection by-products (DBPs). Epidemiological studies have acknowledged a positive correlation between several DBPs and an increased risk of congenital birth defects and occurrence of cancers, namely of the colorectum and bladder (Lee *et al.* 2004; Villanueva *et al.* 2004; Richardson *et al.* 2007; Villanueva *et al.* 2007; Hwang *et al.* 2008; Cantor *et al.* 2010; Freeman *et al.* 2017). Although positive correlations between DBP exposure and adverse health effects have been identified, some epidemiological studies provide contradicting evidence (Savitz *et al.* 2006; Nieuwenhuijsen *et al.* 2010; Hrudey and Fawell 2015) and the causal link between DBPs and health issues is still unknown (Hrudey *et al.* 2015). Nonetheless, there is a need to accurately identify the risk of exposure and to further our understanding of DBP formation for improved management and regulation.

The formation of DBPs is best understood by considering the chemical composition and concentration of the natural organic matter (NOM) precursors. The biochemistry of NOM is significantly influenced by the biogeophysical conditions of the catchment, affecting the relative composition of allochthonous and autochthonous organic matter within the aquatic environment (Leenheer and Croué 2003; Finlay and Kendall 2007). Catchment (allochthonous) organic matter is characteristically hydrophobic comprising relatively high concentrations of aromatic molecules and humic and fulvic acids derived from soils and the breakdown of vegetation (Hwang *et al.* 2001; Leenheer and Croué 2003; Bond *et al.* 2011a). On the other hand, internally produced (autochthonous) organic matter is characteristically hydrophilic and is comprised of higher concentrations of carboxylic acids, polyuronic acids, amino acids, peptides, proteins and carbohydrates that are derived from phytoplankton and macrophytes (Leenheer and Croué 2003; Bond *et al.* 2011a). High turnover rates and continuous cellular exudation throughout the phytoplankton life cycle can significantly contribute towards the total carbon budget of the local aquatic environment (Tomlinson *et al.* 2016).

The contribution of phytoplankton precursors can have a significant impact on the concentration and genotoxicity of DBPs formed due to higher concentration of

hydrophilic organic matter and higher concentration of organic nitrogen (Tomlinson *et al.* 2016). For example, the cellular composition of the cyanobacteria species *Microcystis aeruginosa* is comprised of 86% hydrophilic organic matter and the cells continuously exuded organic matter that was 63% hydrophilic (Li *et al.* 2012). The high composition of hydrophilic organic matter within some species of phytoplankton is problematic due to the recalcitrant nature of hydrophilic NOM during conventional treatment (Singer and Harrington 1993; Matilainen *et al.* 2010). Specifically, the hydrophilic neutral fraction of organic matter is often higher in some species of phytoplankton and has been associated with increased formation of DBPs. High populations of phytoplankton can therefore increase the concentration of hydrophilic NOM reacting with chlorine to form DBPs during disinfection post-coagulation and filtration. Epidemiological research has also identified that nitrogenous disinfection by-products (N-DBPs) are significantly more genotoxic and cytotoxic than their carbonaceous counterparts (Plewa *et al.* 2004a; Plewa *et al.* 2004b; Richardson *et al.* 2007; Plewa *et al.* 2008). Phytoplankton can contribute significantly towards the total dissolved organic nitrogen (DON) concentration of an aqueous environment by exuding up to 45% of their fixed nitrogen as DON (Nguyen *et al.* 2005b). Further, phytoplankton organic matter is characterised with lower carbon to nitrogen ratios than allochthonous organic matter (Meyers 1994; Elser *et al.* 2000). The chlorination of phytoplankton organic precursors has been linked with increased formation of N-DBPs (Mitch 2009; Fang *et al.* 2010). Eutrophic lakes and reservoirs with high phytoplankton productivity correlated with higher DON concentrations than mesotrophic and oligotrophic systems (Wetzel 1983; Westerhoff and Mash 2002). Therefore, the degree of eutrophication, phytoplankton productivity and phytoplankton speciation will have significant impact on the concentration of DON and hence, N-DBP formation potential.

The DBP and N-DBP formation potential of phytoplankton precursors depends on the biochemical composition of the species. Hong *et al.* (2008) investigated the correlation between protein, carbohydrate and lipid concentrations with the formation of the common trihalomethane (THM) chloroform and two haloacetic acids (HAA); dichloroacetic acid and trichloroacetic acid. This highlighted the differences in DBP formation potential from numerous phytoplankton species due to variations in their chemical composition. However, the reactivity of a raw water samples containing those species would vary due to reaction competition of other organic matter compounds present. The aim of this paper

is to further explore the influence that monocultures of phytoplankton have on DBP formation when reacted with chlorine in Myponga raw water. The novelty of this research is the addition of each culture back into raw water to determine how these simulated blooms will react with chlorine within real water samples. This was achieved by dosing known biovolumes of cultured *D. circinale* and *Ankistrodesmus sp.* into Myponga raw water samples to create simulated blooms. The impact of the simulated blooms on DBP formation potential was determined by comparing organic matter characterisation and DBP concentration of a control raw water sample with dosed samples. Furthering the critical work of Hong *et al.* (2008), this paper also aims to investigate correlations with a broader array of DBPs as well as total DBP formation and unknown DBP formation. It is hypothesised that the introduction of phytoplankton organic matter will increase DBP, and in particular, N-DBP formation. It is also hypothesised that cyanobacterial species *D. circinale* would result in higher formation of haloacetic acids (HAAs), whereas green algal species *Ankistrodesmus sp.* would produce higher concentrations of trihalomethanes (THMs), aligning with predictions based on biochemical composition (Hong *et al.* 2008). Finally, it is hypothesised that there will be a significant increase in unknown DBP formation within simulated bloom samples as identified by the chlorination of naturally occurring phytoplankton blooms in Chapter 4.

6.2 Methodology

6.2.1 Experimental Approach and Logic

Cyanobacteria *D. circinale* and green algae *Ankistrodesmus sp.* were cultured to simulate phytoplankton blooms within Myponga Reservoir raw water. These two species of phytoplankton were originally cultured from a South Australian reservoir sample and are prevalent species within Myponga Reservoir. Further, *D. circinale* commonly forms dense blooms within Myponga Reservoir in summer and is therefore important to investigate the relationship between the presence of these phytoplankton species and DBP formation. Raw water samples were collected using capped 600 mL PET bottles or 10 L polypropylene containers from the intake into the Myponga Water Treatment Plant on the 26/06/2017. Sample collection was undertaken during winter when natural phytoplankton populations were low and allochthonous organic matter was the dominant

source of carbon. The intake was situated 10 m below the surface of the reservoir at the Myponga dam wall.

The organic matter was characterised for raw water and simulated phytoplankton bloom samples by turbidity, pH, temperature, UV₂₅₄, colour₄₅₆ and dissolved organic carbon (DOC) assays (APHA *et al.* 2005). Samples were then chlorinated for a 72 hour contact time that is representative of the average maximum contact time of the local distribution network. Residual chlorine was quenched prior to DBP analysis.

6.2.2 Phytoplankton Culture and Maintenance

6.2.2.1 Media Preparation

D. circinale was grown in ASM-1 culture media (Gorham *et al.* 1964) and *Ankistrodesmus* *sp.* was grown in WC culture media (Guillard and Lorenzen 1972). Cultures were prepared and grown in 10 L Nalgene containers. The culture media was prepared using autoclaved equipment to minimise the risk of contamination. All experiments concerning the use of algal media or cultured phytoplankton were performed within a laminar flow cabinet to further minimise the risk of contamination.

6.2.2.2 Cell Culture Maintenance

Phytoplankton cultures were routinely subcultured to ensure populations remained within the exponential growth phase. Subculturing was performed when both media and culture solutions were at room temperature (~20°C) to ensure thermal shock did not affect phytoplankton populations. Spare subcultures of each species were kept as a backup in case of contamination.

Phytoplankton cultures were grown in a Thermocline Scientific illuminated refrigerated incubator at 20°C with a 16-hour day and 8-hour night cycle. Gentle aeration through a 0.22 µm filter ensured that the culture remained well mixed to allow for even light exposure.

6.2.2.3 Phytoplankton Microscopy and Enumeration

Aliquots of well mixed, homogeneous cultures were prepared with Lugol's iodine in a Sedgewick Rafter Counting Chamber. Prepared samples were allowed to settle for approximately 20 minutes prior to enumeration. Phytoplankton cultures were enumerated using a Nikon Eclipse 50i microscope and the procedure for randomly selected fields described by Chorus and Bartram (1999). Cell enumeration was performed regularly to determine the phase of the culture, risk of contamination, cell health and population densities.

6.2.3 Sample Preparation

Cell cultures were initially mixed to ensure cell dispersal was homogeneous. Phytoplankton cells were enumerated and the volume required to achieve a dose of 2×10^5 cells per mL was calculated. The culture was mixed again to ensure the culture was homogeneously dispersed prior to dosing into raw water samples. Separate Myponga raw water samples were then dosed with known concentrations of *D. circinale* and *Ankistrodesmus* sp. The simulated bloom samples were made up to 750 mL with Myponga Raw water. The organic matter characterisation and DBP formation potential of each simulated bloom was compared with a 750 mL control sample of Myponga raw water (Figure 18).

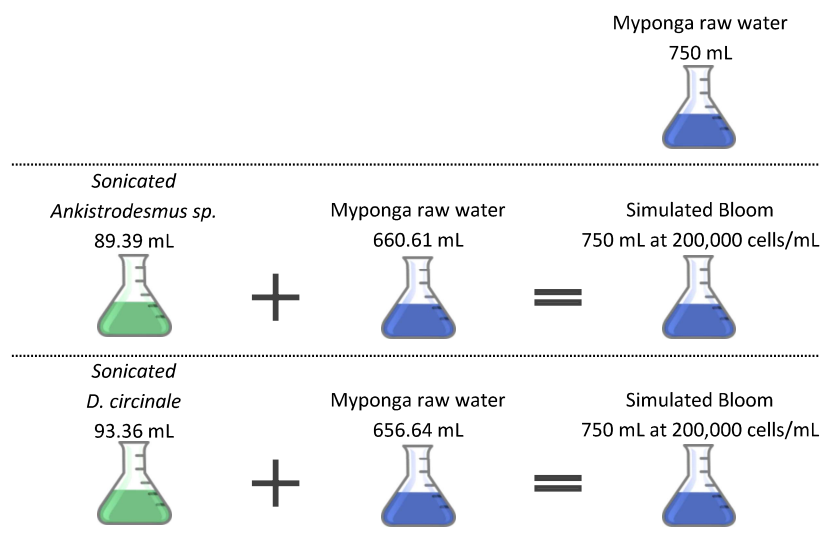


Figure 18. Myponga raw water control is compared with two simulated phytoplankton blooms of *Ankistrodesmus* sp. and *D. circinale*. Simulated phytoplankton blooms were prepared to achieve equivalent cell concentrations of 200,000 cells/mL for both species. Samples were sonicated prior to addition to Myponga Raw water to ensure release of intracellular organic matter.

6.2.4 Analytical Methods

6.2.4.1 Sonication

Probe sonication was performed using the Branson Digital Sonifier. Samples were pulse sonicated for 10-second intervals for a total duration of 120 seconds based on a preliminary investigation (Figure 19). The samples were kept on ice to prevent over heating of the probe and sample during sonication.

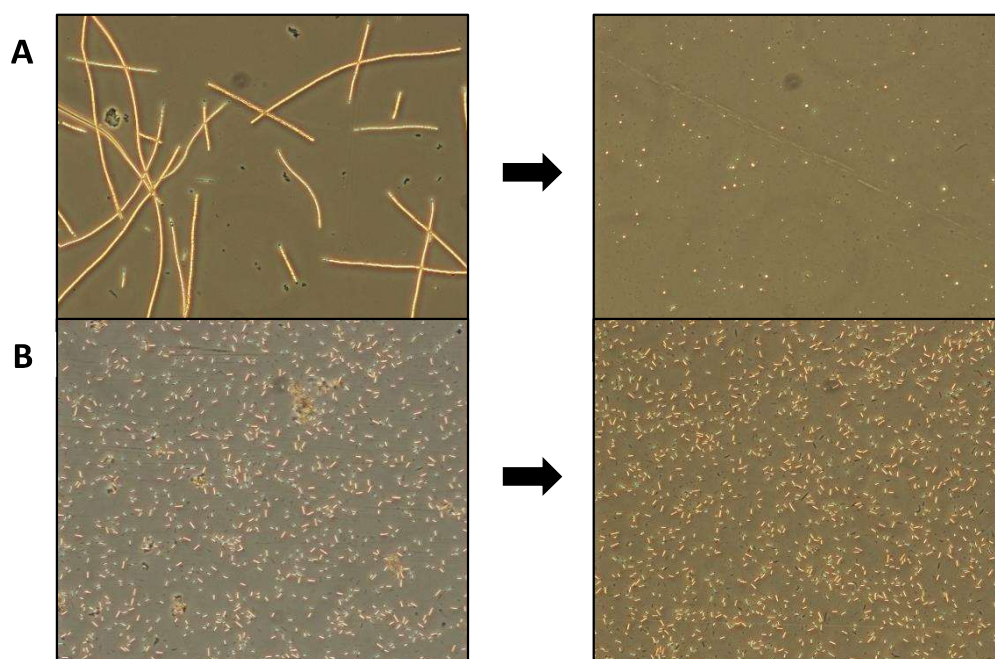


Figure 19. Visual analysis of phytoplankton cell integrity before (left) and after (right) 120 seconds of sonication of *D. circinale* (A) and *Ankistrodesmus* (B).

6.2.4.2 Chlorination

The chlorine demand was calculated by dosing each sample with known concentrations of chlorine stock solution at increments of 5 mg L^{-1} . Samples were stored in a dark location for 72 hours to resemble the maximum contact time of the local distribution network. The resulting chlorine concentrations were measured using the DPD colourimetric titration method (Harp 1995; APHA *et al.* 2005). Samples that produced a chlorine residual between 0 mg/L and 5 mg/L were used to calculate chlorine demand. Raw water and simulated bloom samples were then dosed with the calculated chlorine concentration and stored for 72 hours in the absence of light to minimise UV decay of chlorine. Chlorine residuals were then checked to ensure accurate dosing was successful and residual chlorine was then quenched with ammonium chloride. Samples were then analysed for DBP formation.

6.2.4.3 Disinfection By-product Analysis

DBP formation potential was determined by analysing for total formation as well as specific, common classes of DBPs. Analysis of adsorbable organic halogens (AOX) provided a quantitative measurement of the total concentration of DBPs present. The three DBP classes investigated in this project were THMs, HAAs and DBP 551. Analysis of DBP 551 included the formation of haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones. The concentration of unknown total organic halides (UTOX) was then back calculated by subtracting the concentration of known DBP classes from AOX. Refer to Chapter 4 for detailed methodology of DBP analysis.

6.3 Results

6.3.1 Preliminary Sonication Experiment

Effective sonication of phytoplankton cells was necessary to ensure complete cell lysis and enable accurate measurement of the DOC contribution from each species. Cells were pulse sonicated for 120 seconds and cell integrity was visually examined under microscope (Figure 19). It was evident that sonication of *D. circinale* samples was effective at breaking apart cell filaments and successfully lysing the majority of cells (Figure 19A). However, visual investigation did not support the successful cell lysis of *Ankistrodesmus* cells (Figure 19B). Cell integrity was further investigated by comparing the DOC concentration of each sample before and after sonication (Table 13). DOC results indicated that sonication at 120 seconds was successful for both species even although the cell integrity of *Ankistrodesmus* cells looked unaffected; evidently the cells had been perforated allowing DOC to be released (Table 13).

Table 13. Preliminary investigation into sonication efficiency

Sonication	<i>Ankistrodesmus</i> sp. DOC (mg/L)	<i>D. circinale</i> DOC (mg/L)
0 Seconds	3.86	4.93
120 Seconds	6.71	11.63

6.3.2 Organic Matter Characterisation

DBP formation is significantly impacted by the relative composition of allochthonous and autochthonous organic matter due to variations of the biochemistry of each source (Leenheer and Croué 2003; Lee *et al.* 2006). Myponga raw water samples were collected from the intake during winter when phytoplankton populations were naturally low and allochthonous NOM was considered to be the dominant source of carbon within the reservoir. This allowed for the investigation into the contribution of simulated phytoplankton blooms towards the formation of DBPs with minimal influence of other sources of autochthonous carbon.

6.3.3 Myponga Raw Water

The intake raw water was characterised by a high DOC concentration of 14.67 mg/L, high colour₄₅₆ of 75 HU and high UV₂₅₄ of 0.540 cm⁻¹ (Table 14). The intake water was also characterised by low chlorophyll *a* measurement of 3.92 µg/L (Table 14). The high DOC, colour₄₅₆ and UV₂₅₄ measurements coinciding with low chlorophyll *a* indicates that the intake water was characteristically allochthonous. Further, surface phytoplankton populations were comprised of low populations of *M. flos-aquae*, *Phormidium* sp., *Closterium* sp. and *Staurosira* sp. with a total concentration of 825 cells/mL. Low surface phytoplankton populations indicated that there was a minimal influence from surface water phytoplankton exudates on intake samples that were collected 10 m below the surface. The raw water sample was also characterised by a low turbidity measurement of 2.76 NTU (Table 14). Bromide concentrations were also measured to provide insight into the formation of brominated DBPs. Bromide concentrations were 0.36 mg/L for raw water samples and simulated phytoplankton blooms (Table 14).

Table 14. General organic matter character comparison between Myponga raw water samples and Myponga raw water samples with the addition simulated phytoplankton blooms of *Ankistrodesmus sp.* and *D. circinale*. Simulated blooms samples were dosed with 200,000 cells/mL of the respective species and were pulse sonicated for 120 seconds to release intracellular organic matter.

Sample	DOC (mg/L)	pH	UV ₂₅₄ abs (cm ⁻¹)	Colour ₄₃₆ (HU)	Turbidity (NTU)	Cell biovolume (mm ³ /L)	Chlorophyll <i>a</i> (µg/L)	Bromide (mg/L)
Raw Water	14.67	7.70	0.540	75	2.76	-	3.92	0.36
<i>Ankistrodesmus sp.</i>	13.07	7.53	0.471	64	4.22	13.2	-	0.36
<i>D. circinale</i>	13.72	7.31	0.488	69	7.80	50	-	0.36

6.3.4 Simulated Phytoplankton Blooms

The simulated *Ankistrodesmus sp.* and *D. circinale* phytoplankton blooms had lower measured total DOC concentrations than the Myponga raw water sample (Table 14). This was due to the phytoplankton cultures having lower initial DOC than the raw water prior to combining the solutions. The *Ankistrodesmus* bloom sample had a DOC concentration of 13.07 mg/L, UV₂₅₄ measurement of 0.471 cm⁻¹ and colour₄₅₆ of 64 HU (Table 14). The addition of the 200,000 cells/mL increased the turbidity of the sample substantially to 4.22 NTU. Addition of the *D. circinale* bloom resulted in DOC measurements of 13.72 mg/L, UV₂₅₄ of 0.488 cm⁻¹ and colour₄₅₆ of 69 HU (Table 14). The addition of 200,000 cells/mL also significantly increased the turbidity of to 7.80 NTU. It is notable that the cell biovolumes of each species were not identical even though equivalent cell concentrations of *Ankistrodesmus sp.* and *D. circinale* were added to Myponga raw water samples. Samples of *Ankistrodesmus sp.* had a total cell biovolume of 13.2 mm³/L and samples of *D. circinale* had a total cell biovolume of 50 mm³/L (Table 14).

6.3.5 Disinfection By-product Formation

DBPs were measured by analysing the total formation which is represented by the concentration of AOX. The concentration of THMs, HAAs, haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones were also measured to provide insight into the formation of specific DBPs categories. The concentration of each species formed was subtracted from AOX to determine the fraction of unknown by-products, UTOX.

Chlorination of Myponga raw water samples, without additional phytoplankton added, resulted in the formation of a high concentration of DBPs with AOX concentration 2710 µg/L (Figure 20). The largest category of DBPs formed was the HAAs at 1050 µg/L which was primarily comprised of trichloroacetic acid and dichloroacetic acid (Figure 21). The second largest category of DBPs formed was 658 µg/L of THMs. The bulk of the THMs were chloroform (Figure 22). The total concentration of DBPs categorised by the DBP 511 method was the lowest at 214.2 µg/L. N-DBP formation measured by the DBP 551 method identified that dichloroacetonitrile was primarily formed over bromochloroacetonitrile, most likely due to a limiting concentration of bromide (Figure

23). The remaining 788.4 µg/L of DBPs formed were defined as UTOX, equating to only 29.09% of DBPs formed (Figure 20). This contrasts sharply with samples where simulated phytoplankton blooms were added.

The simulated *Ankistrodesmus sp.* bloom (Myponga water + *Ankistrodesmus* culture) formed a substantially higher concentration of DBPs in comparison to chlorinated Myponga raw water without phytoplankton addition. The concentration of AOX formed was 4310 µg/L which was comprised of 1090 µg/L of HAAs, 617 µg/L of THMs and 202.6 µg/L of DBPs measured by the DBP 551 method (Figure 20). The concentration of measured THM, HAA and DBP 551 categories did not significantly vary from measurements from raw water samples without additional phytoplankton added. Therefore, the significant variation in DBP formation was evident by the much larger component of UTOX, accounting for 55.7% of total formation (Figure 20).

The simulated *D. circinale* bloom formed the highest concentration of DBPs with an AOX measurement of 5670 µg/L (Figure 20). The formation of 1220 µg/L of HAAs was significantly higher than the formation measured from the simulated *Ankistrodesmus sp.* bloom and raw water samples. The increase in HAA formation was due to higher concentrations of dichloroacetic acid and trichloroacetic acid (Figure 21). THM formation of 634 µg/L did not significantly differ from the *Ankistrodesmus sp.* or raw water samples (Figure 22). The formation of 324.8 µg/L of DBP 551 was significantly higher than the simulated *Ankistrodesmus sp.* bloom and raw water samples (Figure 20). Elevation of DBP 551 concentrations was primarily caused by the increased concentration of the N-DBP dichloroacetonitrile, and higher concentrations of the regulated DBP chloral hydrate (Figure 23 and Figure 24). Finally, the chlorination of the simulated *D. circinale* bloom resulted in the highest concentration of UTOX at 3491.2 µg/L, accounting for 61.6% of total formation.

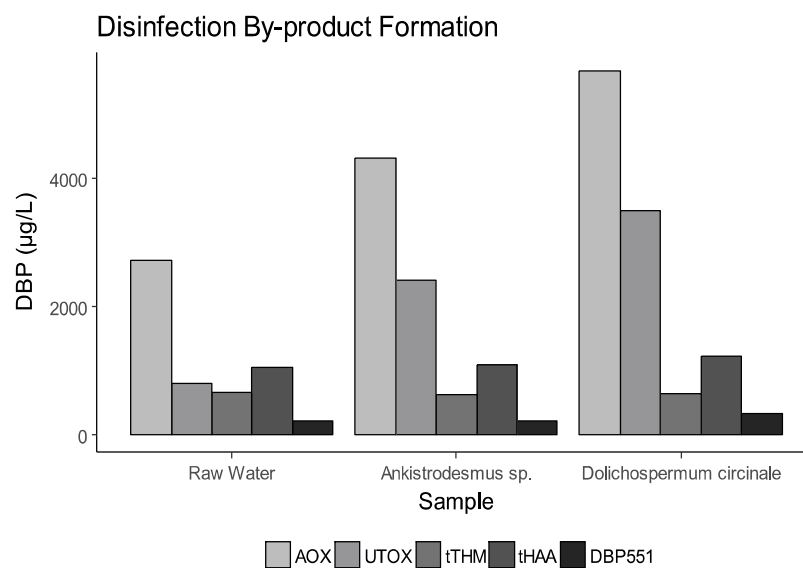


Figure 20. Collated disinfection by-product (DBP) formation from the chlorination of raw water samples, the *Ankistrodesmus sp.* simulated bloom and the *D. circinale* simulated bloom. DBP formation was investigated for the formation of total adsorbable organic halogens (AOX), unknown total organic halogens (UTOX), total trihalomethane (tTHM), total haloacetic acid (tHAA) and DBPs within the DBP 551 class.

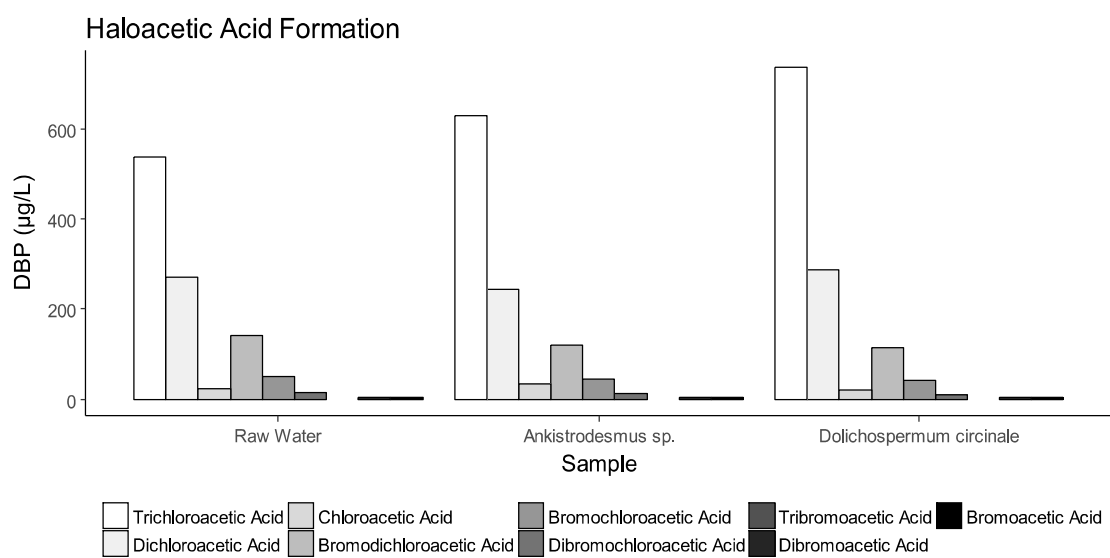


Figure 21. A comparison of haloacetic acid species formed between each sample.

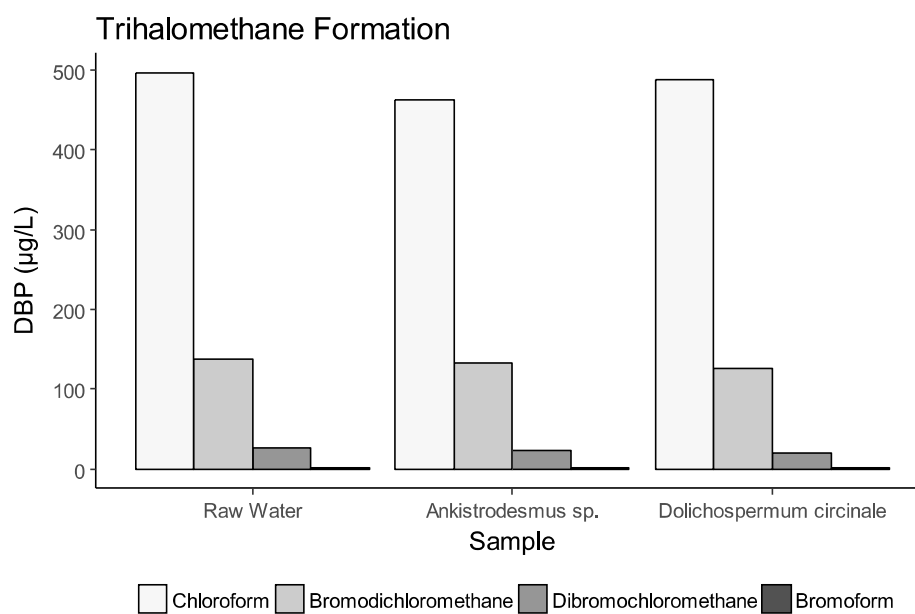


Figure 22. A comparison of trihalomethane species formed between each sample.

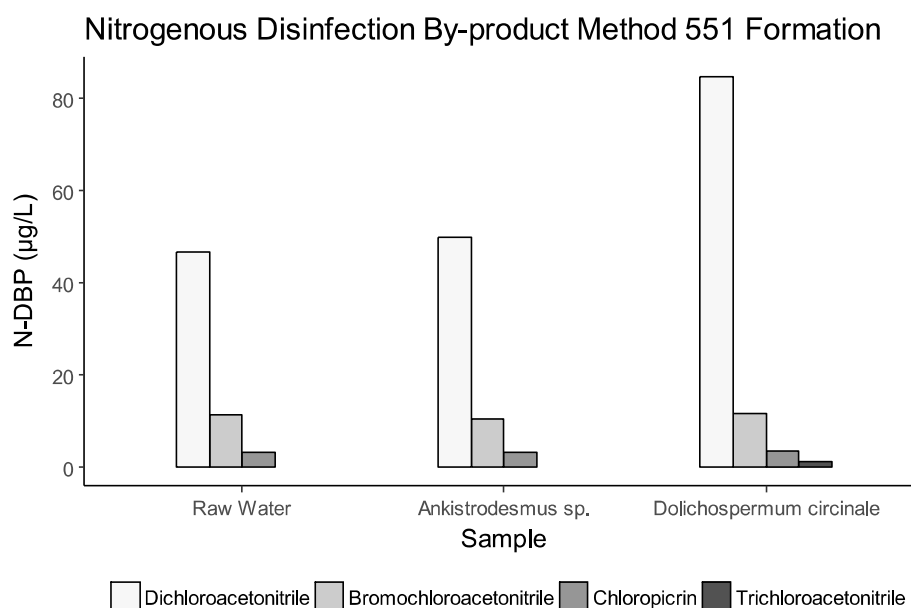


Figure 23. Nitrogenous disinfection by-product (N-DBP) formation from the chlorination of raw water samples, the *Ankistrodesmus sp.* simulated bloom and the *D. circinale* simulated bloom. Dichloroacetonitrile, bromochloroacetonitrile, chloropicrin and trichloroacetonitrile were measured using the DBP 551 method.

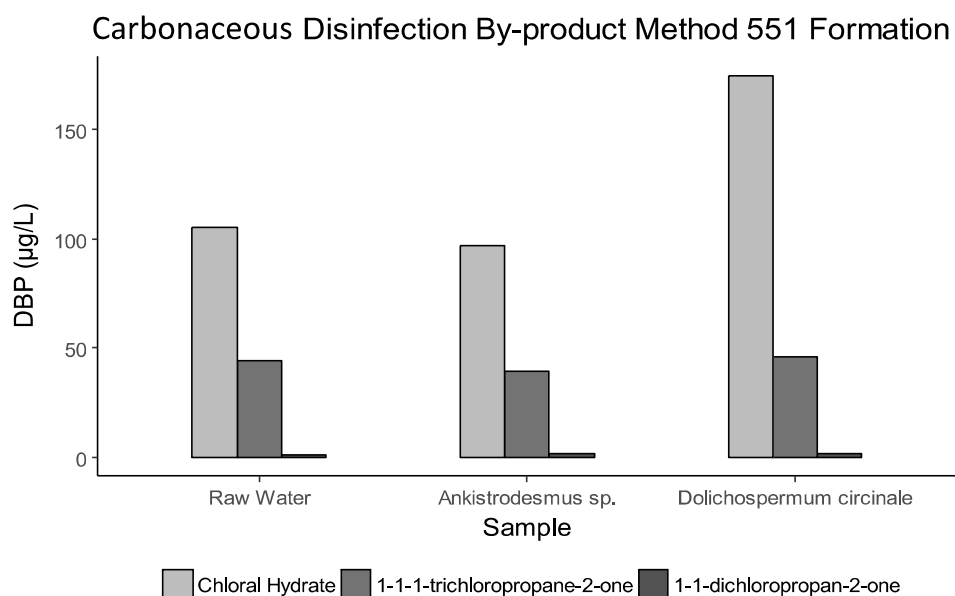


Figure 24. Carbonaceous disinfection by-product (DBP) formation measured via the DBP 551 method. Comparison between raw water samples, the *Ankistrodesmus sp.* simulated bloom and the *D. circinale* simulated bloom.

6.4 Discussion

The formation of DBPs is influenced by the chemical composition and concentration of reactive allochthonous and autochthonous NOM precursors. A growing pool of literature has identified the significance of phytoplankton towards the formation of DBPs by investigating the associated genotoxicity, DBP speciation and treatment efficiency (Hong *et al.* 2008; Fang *et al.* 2010; Lui *et al.* 2011; Li *et al.* 2012; Liao *et al.* 2015; Goslan *et al.* 2017). DBP formation is not limited to bloom-forming cyanobacteria, but green algae and diatoms also contribute effective DBP precursors (Nguyen *et al.* 2005b; El-Aty *et al.* 2009; Zhang *et al.* 2014). When considering the relative contribution of phytoplankton towards DBP formation the accumulation of all phytoplankton species present must be taken into account.

Research by Hong *et al.* (2008) examined the relative contribution of the major biomolecules (proteins, lipids and carbohydrates) towards DBP formation. Hong *et al.* (2008) identified that chlorination of cyanobacteria species resulted in higher formation of HAAs whereas the chlorination of green algal species formed higher concentrations of THMs. This finding was based on the relative abundance of the three major biomolecules, with cyanobacteria containing higher concentrations of proteins, and green algae containing higher concentrations of lipids (Hong *et al.* 2008). In this chapter, the relationship of DBP formation and simulated *D. circinale* and *Ankistrodesmus sp.* blooms in Myponga raw water samples is further investigated by measuring a larger suite of DBPs.

6.4.1 Influence of Simulated Blooms

The simulated phytoplankton blooms did not significantly alter the organic matter characterisation of the Myponga raw water samples (Table 14). The concentration of DOC decreased slightly after the addition of phytoplankton to raw water samples indicating a minor diluting effect. The UV₂₅₄ concentration also decreased upon the addition of simulated phytoplankton blooms due to a reduction of UV₂₅₄ absorbing compounds, aligning with observations by Henderson *et al.* (2008).

6.4.2 Regulated Disinfection By-products

The addition of *Ankistrodesmus sp.* and *D. circinale* cultures into Myponga raw water samples had a minor effect on the formation of regulated DBPs (NHMRC 2011). The Australian Drinking Water Guidelines (ADWGs) regulates the formation of a small suite of DBPs including HAAs, THMs and chloral hydrate (NHMRC, 2011). The addition of *Ankistrodesmus sp.* did not have a significant effect on the formation of routinely monitored HAAs or THMs, which does not support the hypothesis of a predicted increase in THM formation (Figure 21 and Figure 22). Chlorination of the simulated *D. circinale* bloom did not significantly influence the formation of THMs (Figure 22). However, an observed increase in HAA formation supports the hypothesis that the *D. circinale* bloom will increase HAA formation. Chlorination of the *D. circinale* bloom also significantly increased the concentration of chloral hydrate. Higher concentrations of HAAs and chloral hydrate associated with the increased population of *D. circinale* cells could have implications for human health if the organic matter is not effectively removed during treatment. Higher concentrations of hydrophilic organic matter are often associated with higher populations of phytoplankton which increases the likelihood that the organic matter will be retained in the product water and exposed to chlorine during treatment (Singer and Harrington 1993; Li *et al.* 2012).

Elevated population of *D. circinale* can increase regulated HAA and chloral hydrate concentrations which will be identified during routine monitoring for the regulated DBPs. However, it is evident that by only monitoring for regulated DBPs, a large proportion of the DBPs formed during chlorination of *Ankistrodesmus sp.* will go undetected. Consequently, monitoring of regulated DBPs does not provide an accurate insight into the formation of DBPs. Ideally, a wider spectrum of DBPs should be measured and compared to total DBP formation which can then be used to determine the unknown formation potential.

6.4.3 Unregulated Disinfection By-products

Measurement of total DBP formation and a broader range of DBPs identified by the DBP 551 method provided insight into the formation of some of the DBPs that are not regulated and are therefore not routinely monitored. The unregulated DBP formation was significantly affected by the addition of *Ankistrodesmus sp.* or *D. circinale* relative to

Myponga raw water samples where no additional phytoplankton cells were added. A significant increase in the concentration of unregulated DBPs poses an increased potential health risk upon exposure.

The total DBP formation of each sample was represented by the concentration of AOX which significantly increased with the addition of either phytoplankton species (Figure 20). An increase in AOX and minimal variation in the concentration of regulated DBPs resulted in a significant increase in the formation of the unknown fraction of DBPs (Figure 20). Higher concentrations of UTOX, coinciding with an increased concentration of autochthonous NOM, supports observations made previously in Chapter 4 and again in Chapter 7. Higher formation of UTOX signifies a higher concentration of DBPs which are not routinely monitored that may potentially have a higher risk to human health (Hua and Reckhow 2008; Bull *et al.* 2011).

The concentration of measured unregulated carbonaceous DBPs did not vary significantly between Myponga raw water samples and simulated phytoplankton bloom samples (Figure 24). The addition of *Ankistrodesmus sp.* did not significantly increase the concentration of measured N-DBPs (Figure 23). However, the addition of *D. circinale* cells significantly increased the formation of the N-DBPs; dichloroacetonitrile and trichloroacetonitrile (Figure 23). Correlated higher N-DBP formation associated with increased populations of cyanobacteria cells has been observed in previous studies due to high concentrations of organic nitrogen (Fang *et al.* 2010; Li *et al.* 2012). Higher formation of N-DBPs influences the potential genotoxicity of DBPs formed (Plewa *et al.* 2004a; Richardson *et al.* 2007; Plewa *et al.* 2008). Interestingly, there was no variation in the concentration of chloropicrin formed between any of the samples, indicating that a recognised increase in N-DBP formation does not imply that all N-DBPs increase by similar proportions. There was no notable difference in concentration of bromochloroacetonitrile between raw water and bloom samples as the formation was limited by the bromide concentration of each sample (Table 14).

These experiments were performed using equivalent cell counts of *Ankistrodesmus sp.* and *D. circinale*. Although cell counts were equivalent, the cell biovolumes of each species were different (Table 14). Therefore, the green algal *Ankistrodesmus sp.* had a significantly higher impact on AOX and UTOX formation in relation to cell biovolume, supporting the findings of Zhang *et al.* (2014). However, significant increases in N-DBP

formation were still associated with the addition of *D. circinale* cyanobacteria cells (Figure 23). Although *Ankistrodesmus* contributed more towards DBP formation with respect to cell biomass, *D. circinale* is more prone to form dense surface blooms within reservoirs which can relate to a significant increase in UTOX and N-DBP formation. This research highlights the importance of considering the contribution of the bulk phytoplankton community towards DBP formation to improve our understanding of the potential risk to human health.

6.5 Conclusion and Industry Relevance

Both *D. circinale* and *Ankistrodesmus* sp. contributed significantly towards the formation of total and unknown DBPs. Although cyanobacteria species are prone to dense blooms, this research highlights the importance of considering the contribution of the entire phytoplankton community towards DBP formation. The increased DBP formation associated with phytoplankton NOM was primarily related to increased concentrations of unregulated DBPs including UTOX and N-DBPs. Therefore, DBP formation derived from phytoplankton NOM precursors is likely to be underestimated by routine DBP monitoring. A higher concentration of unregulated DBPs and DBPs with known increased genotoxicity heightens the potential risk to human health upon exposure. Although it is unrealistic to monitor for every potential DBP formed during routine monitoring, this research highlights the need to at least monitor for AOX to understand how the organic matter influences total DBP formation.

Improved catchment management can aid in limiting essential nutrients and hence reduce the frequency of phytoplankton blooms. Alternatively, phytoplankton organic matter is readily biodegradable, particularly the associated autochthonous organic nitrogen component (Westerhoff and Mash 2002; Wert and Rosario-Ortiz 2013). Alteration in the treatment process and retention times may reduce the impact of DBP formation from phytoplankton precursors. However, if the intracellular phytoplankton organic matter is released via cell lysis during treatment, then advanced treatment processes such as biofiltration, activated carbon or enhanced coagulation should be considered (Wert and Rosario-Ortiz 2013).

6.6 Acknowledgements

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Publication to be Associated with this Thesis

Chapter 7 – To be submitted for publication

Removal efficiency of disinfection by-product precursors during a cyanobacteria bloom

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Chapter 7

Removal efficiency of disinfection by-product precursors during a cyanobacteria bloom

Disinfection is a necessary process in water treatment to ensure that pathogenic organisms are successfully removed. However, chemical disinfection results in the formation of disinfection by-products (DBPs) via an oxidative reaction between the chemical disinfectant and natural organic matter (NOM). The concentration and speciation of DBPs formed are significantly impacted by the chemical structure, and hence, the source of NOM precursors. Hydrophilic NOM is partially recalcitrant to conventional treatment which has implications for the treatability and DBP formation potential. Increased concentration of phytoplankton NOM has repercussions for both the increased formation of more genotoxic nitrogenous DBPs and higher chemical composition of hydrophilic NOM. This research investigated the NOM characterisation and DBP formation potential at multiple locations within Myponga Reservoir and throughout the Myponga Water Treatment Plant during a significant phytoplankton bloom. Conventional treatment effectively removed the hydrophobic and charged hydrophilic fractions; however, the majority of the hydrophilic neutral (NEU) fraction was retained within the product water. The phytoplankton bloom was associated with higher concentrations of the NEU fraction, which would have increased the risk of higher DBP formation post-treatment had the bloom entered the treatment plant. The product water contained higher concentrations of regulated DBPs during the bloom compared with historic values during periods of lower surface chlorophyll *a* values. Further, total DBP formation within the product water was high, comprising of 44.8 % unknown DBPs which poses an unknown potential health risk.

7.1 Introduction

Since the discovery of disinfection by-products (DBPs) by Rook (1974) and Bellar *et al.* (1974a), research has focused on improving the understanding of DBP formation mechanisms, human health impacts and methods to mitigate exposure (Singer 1994; Chowdhury *et al.* 2009; Hebert *et al.* 2010; Xie 2016). Epidemiological studies have investigated the association of DBP and the relative risk towards human health (Cantor *et al.* 1998; Villanueva *et al.* 2004; Villanueva *et al.* 2007). These studies have identified a positive correlation with the lifetime exposure to DBPs and an increased risk of cancer, particularly of the bladder and colorectum (Richardson *et al.* (2007). Exposure to DBPs has also been linked to congenital malformations (Wright *et al.* 2004; Hinckley *et al.* 2005; Hwang *et al.* 2008). The causal association of DBPs with several types of cancers and congenital malformations is still unknown (Hrudey 2009; Nieuwenhuijsen *et al.* 2010; Hrudey *et al.* 2015).

Regulation of DBPs is difficult, with less than 100 of the 600+ identified DBPs having undergone extensive epidemiological studies (Hebert *et al.* 2010). Further, the causal connection between DBP exposure and human health outcomes is not definitively understood (Hrudey *et al.* 2015). Consequently, the list of individual DBPs and their corresponding guideline values vary considerably between regulatory bodies (Tomlinson *et al.* 2016). Trihalomethanes (THMs) and haloacetic acids (HAAs) are the predominant focus of regulatory guidelines, as these classes are commonly formed in higher concentrations upon chlorination (Gopal *et al.* 2007). Nonetheless, recent literature suggests that intermediate DBPs, other than THMs and HAAs, are associated with increased genotoxicity (Zhang *et al.* 2014). Iodine and bromine halogenated DBPs are more genotoxic than chlorinated species (Richardson *et al.* 2007), and nitrogenous disinfection by-products (N-DBPs) are more toxic than their carbonaceous counterparts (Plewa *et al.* 2004a; Plewa *et al.* 2004b; Richardson *et al.* 2007; Plewa *et al.* 2008). Although genotoxicity indicates a higher risk upon exposure of emerging and N-DBPs, they are not strongly represented by authoritative guideline values (Richardson *et al.* 2007; Tomlinson *et al.* 2016).

The heightened health risk associated with N-DBP exposure has implications on the management of phytoplankton within reservoirs. Phytoplankton natural organic matter (NOM) has a lower C:N ratio than allochthonous organic matter (Meyers 1994) and is capable of exuding up to 45 % of its fixed nitrogen as dissolved organic nitrogen (Nguyen

et al. 2005b). This results in an increased risk of N-DBP formation from both intracellular and extracellular phytoplankton NOM (Westerhoff and Mash 2002; Nguyen *et al.* 2005b; Zhang *et al.* 2014; Tomlinson *et al.* 2016). As well as an increase in N-DBP formation, chlorination of phytoplankton NOM has also been associated with an increase in unknown total organic halogens (UTOX) (Fang *et al.* 2010). Exposure to higher concentrations of UTOX creates an additional unknown risk towards human health upon exposure. Furthermore, the reactivity of chlorine with phytoplankton NOM can be significantly higher than the reactivity with catchment NOM towards the formation of DBPs (See Chapter 4). The risk of DBP formation from phytoplankton NOM sources is dependent upon the efficiency of its removal during conventional treatment. Phytoplankton NOM is often characterised by high concentrations of hydrophilic organic matter (Bond *et al.* 2011a; Lui *et al.* 2011; Li *et al.* 2012) that is more recalcitrant to the coagulation process (Singer and Harrington 1993). The recalcitrant nature of phytoplankton organic matter can increase the risk of forming potentially hazardous DBPs upon disinfection.

The aim of this paper is to investigate the formation of DBPs at numerous stages of the conventional treatment process during a substantial phytoplankton bloom. This will help understand the efficiency of DBP precursor removal during conventional treatment. Samples were collected from the inflow, surface scum, raw water entering the treatment plant, post-coagulation and rapid mix, post dissolved air flotation (DAF) from the underflow and post-filtration. It is hypothesised that DBP formation will substantially decrease post coagulation and rapid mix since dissolved organic carbon (DOC) is coagulated into flocs and removed during DAF. It is also hypothesised that an increase in hydrophilic phytoplankton organic matter will result in elevated DBP formation post-treatment compared with historic values.

7.2 Methods

7.2.1 Site Description

Myponga Reservoir is influenced by a Mediterranean climate with hot dry summers and cool wet winters. The Myponga catchment is approximately 124 km² and predominantly used for dairy pastures, beef and hay farming with patchy native vegetation.

Allochthonous organic matter sources contribute significantly towards the carbon load of the reservoir, with cyanobacterial blooms increasing the relative proportion of autochthonous organic matter throughout the summer and during elongated periods of low rainfall (Linden *et al.* 2004). The reservoir is generally characterised by high DOC (~14 mg/L), colour₄₅₆ (~60 HU) and low turbidity (<5 NTU) (Lewis *et al.* 2004; Linden 2008).

Myponga Water Treatment Plant (WTP) provides potable water via a stepwise conventional treatment method. The conventional treatment processes includes: coagulation, flocculation, DAF, filtration, disinfection, fluoridation, storage and distribution. Chlorine is used as the sole disinfectant at Myponga WTP, and pre-chlorination does not occur at this WTP. Potassium permanganate is used occasionally for pre-oxidation when manganese concentrations in the influent are high, but was not used during this trial. An adjustable offtake is in operation to minimise the risk of a surface phytoplankton blooms entering the plant and elevated concentrations of iron and manganese which can be higher in the hypolimnion during summer thermal stratification (Hobson *et al.* 2010). The adjustable height of the intake also minimises the effects of cold riverine intrusions such as high turbidity and the intake of pathogens (Brookes *et al.* 2005; Hipsey *et al.* 2006).

7.2.2 Experimental Approach and Logic

The sampling regime was designed to analyse water quality parameters and DBP formation potential within Myponga Reservoir and at multiple stages throughout the treatment plant during a phytoplankton bloom. Sample locations were: Myponga River inflow (A), the surface at the dam wall (location 2) (B), the intake pipe into the treatment plant (C), after coagulation, flocculation and post-coagulation rapid mix (D), after clarification with DAF from the underfloat (E) and post-filtration (F) (Figure 25). Samples were collected on 11 January during a substantial bloom of *Microcystis aeruginosa* and *Microcystis flos-aquae* with pre-rinsed PET bottles or sterilised 10 L polypropylene containers. The intake into the treatment plant was positioned at an approximate depth of 30 metres in an attempt to avoid the intake of live cells into the treatment plant. The hydraulic residence time was calculated from flow rates entering the

WTP to ensure that the same batch of water was collected from locations C-F (Figure 24).

Upon collection, samples were transported back to the laboratory and stored at 4°C. All experiments were conducted at room temperature (~20°C) and processed within 48 hours of collection. Samples were characterised by water quality parameters pH, temperature, turbidity, UV₂₅₄, colour₄₅₆ and DOC (APHA *et al.* 2005). The samples were then chlorinated for 72 hours to represent the maximum contact time of the local distribution system. Residual chlorine was then quenched with ammonium chloride in preparation of DBP analysis.

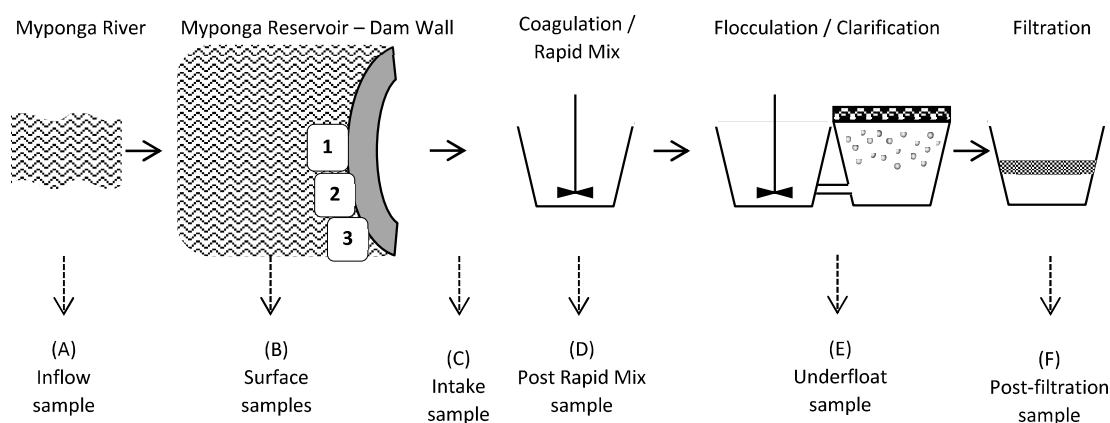


Figure 25. Sampling Regime and simplified schematic of Myponga Treatment plant. Samples were collected from Myponga River (A), surface at the dam wall (B), raw water entering the treatment plant (C), post-coagulation and rapid mix (D), after clarification in the underfloat below the sludge collection (E) and post-filtration (F). Phytoplankton enumeration was performed for all three locations for surface samples (B), however only location 2 was used for organic matter characterisation and disinfection by-product analysis of raw water and sonicated surface samples.

To provide further insight into phytoplankton's contribution towards the NOM pool and DBP formation, surface samples were exposed to two separate treatments. The first treatment involved series filtration of surface samples down to 0.45 µm to retain any intact cells, leaving a filtrate comprised of raw water and phytoplankton exudates. The second treatment involved 120 seconds of pulse sonication to lyse the cyanobacteria cells with the aim of releasing intracellular organic matter. The contribution of phytoplankton intracellular organic matter towards the NOM pool and DBP formation is then calculated by subtracting the measurements from the second treatment from the first treatment.

7.2.3 Analytical Methods

7.2.3.1 Rapid Fractionation

Organic matter was rapidly fractionated into four components based on hydrophobic and hydrophilic properties: very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), charged hydrophilic acids (CHA) and hydrophilic neutrals (NEU) (Chow *et al.* 2004). This was achieved by sequential filtration through DAX-8, XAD-4 and IRA-958 resins. An aliquot of filtrate was collected after filtration through each resin for DOC analysis. Back calculation of DOC results determined the relative proportions of each fraction towards total DOC.

7.2.3.2 Chlorination

All samples were collected prior to the addition of any chlorine during the treatment process. Samples were dosed with chlorine concentrations at increasing increments of 5 mg/L for a contact time of 72 hours to determine the chlorine demand. Residual chlorine was then measured using the DPD colourimetric titration method (Harp 1995; APHA *et al.* 2005). The samples that had a chlorine residual of less than 5 mg/L were selected to back calculate the concentration of chlorine required to successfully satisfy the largest DBP formation potential, whilst, retaining a chlorine residual of less than 0.5 mg/L. Each of these samples were then chlorinated with the calculated dose for a 72 hour contact time and stored away from light sources. Residual chlorine was then measured and quenched prior to DBP analysis.

7.2.3.3 Measurement of Disinfection By-Products

Total DBP formation was determined by measuring total adsorbable organic halogens (AOX) using the Mitsubishi Chemical Analytech Total Organic Halogen Analyser. The formation of specific DBP classes were also investigated by measuring for THMs, HAAs the DBP 551 category which includes haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones (US EPA 1990; APHA *et al.* 2005). UTOX were then calculated by

subtracting the concentrations of measured species from AOX. Refer to Chapter 4 for a more detailed methodology of DBP analysis.

7.3 Results

7.3.1 Organic Matter Characterisation

NOM is a complex, heterogeneous mixture of aromatic and aliphatic carbon based compounds that are derived from allochthonous and autochthonous sources (Leenheer and Croué 2003). The relative proportion of allochthonous and autochthonous organic matter is influenced by the immediate meteorological conditions and long term climatic conditions (Tranvik *et al.* 2009). The characterisation of organic matter is critical in understanding how the chemical and physical properties of water correlate with DBP formation (Kitis *et al.* 2002; Yang *et al.* 2008).

7.3.1.1 Myponga River Water Characterisation

Myponga Reservoir had been exposed to warm dry summer conditions that resulted in a prolonged period of low flow within Myponga River, averaging 3.14 ML/day since the beginning of November. The organic matter within the inflow was predominantly derived from catchment sources indicated by a low chlorophyll *a* reading of 2.13 µg/L (Table 15). DOC concentrations and UV₂₅₄ of measurements of 12.47mg/L and 0.544 (cm⁻¹) were relatively low within the inflow compared with periods of high flow described in Chapter 4. Low inflows would have had a reduced influence on the character of the organic matter at the dam wall during this sampling regime.

Table 15. Organic matter characterisation

Location	DOC (mg L ⁻¹)	pH	UV ₂₅₄ abs (cm ⁻¹)	Colour ₄₅₆ (HU)	Turbidity (NTU)	Chl <i>a</i> (µg/L)
Inflow	12.47	7.2	0.544	72	3.6	2.13
Surface	21.40	6.3	0.611	152	4244	7111
Surface Sonicated	21.37	6.2	0.526	151	578	-
Intake	14.30	7.5	0.540	76	20.9	0.37
Post-Coagulation and Rapid Mix	6.17	6.7	0.140	9	15.5	0.50
Underfloat	5.64	6.8	0.130	8	0.79	0.22
Post-filtration	5.44	6.8	0.120	7	0.16	<0.1

7.3.1.2 Phytoplankton Bloom

The character of the organic matter is closely monitored near the dam wall where the intake into the treatment plant is located. Changes in the water's organic properties require minor alterations in the treatment process to enable the provision of high quality product water. Prior to sampling, Myponga Reservoir had been inundated by a surface cyanobacterial bloom of *Dolichospermum circinale* from late November until late December (Table 16). As the *D. circinale* population decreased, the succession of the bloom transformed becoming dominated by *M. aeruginosa* before changing again late January into a predominately *M. flos-aquae* cyanobacteria bloom (Table 16). During sampling on 11 January, the phytoplankton bloom was primarily comprised of *M. aeruginosa* and a smaller component of *M. flos-aquae* with five metre integrated cell counts of 334,000 cells/mL and 10,200 cells/mL respectively (Table 17). Thermal stratification of the reservoir indicated a limited depth of surface mixing, resulting in a reduced vertical intrusion of the phytoplankton bloom down to a depth of approximately 10 metres (Table 17). Phytoplankton cell enumeration of the surface scum at Location 2 and Location 3 were 60-70,000,000 cells/mL and 904,700,000 cells/mL respectively (Table 17), implying that wind driven surface currents were concentrating the bloom towards surface Location 3. NOM and DBP formation potential of the surface samples was analysed at Location 2 (Figure 25).

7.3.1.3 Surface Characterisation

The dense surface phytoplankton bloom increases the relative contribution of autochthonous NOM towards the total carbon budget of the localised aquatic environment. High phytoplankton cell concentrations were responsible for the elevated turbidity of 4244 NTU and the high chlorophyll *a* measurements of 7111 µg/L. Surface samples were subjected to two treatments to investigate the contribution of phytoplankton towards the organic matter pool. Filtered surface samples correlated with a high DOC concentration of 21.40 mg/L and UV₂₅₄ measurement of 0.611 cm⁻¹ (Table 15). Sonicated surface samples were characterised by a DOC of 21.37 mg/L and UV₂₅₄ of 0.526 (Table 15). Minimal variation in DOC concentrations pre and post-sonication indicates that sonication for 120 seconds was not substantial enough to successfully lyse

cells, although a preliminary investigation on cultured cells indicated otherwise. Both filtered and sonicated surface samples had significantly higher concentrations of the NEU fraction (Table 18).

Table 16. Phytoplankton cell counts during a *D. circinale* bloom in “Event 1” and a combined *M. aeruginosa* and *M. flos-aquae* bloom in “Event 2”. All cell counts given below are from Location 1 in Myponga Reservoir at the surface near the dam wall (see **Figure 25** for location details). Cell counts determined are from a 5-metre integrated sample.

Event 1		Event 2		
Date	<i>D. circinale</i> (cells/mL)	Date	<i>M. aeruginosa</i> (cells/mL)	<i>M. flos-aquae</i> (cells/mL)
20 Nov 2017	34,700	9 Jan 2018	146,000	-
22 Nov 2017 *	29,300	11 Jan 2018 *	334,000	10,200
27 Nov 2017	58,600	15 Jan 2018	27,900	57
30 Dec 2017	27,400	18 Jan 2018 *	114,000	19,800
4 Dec 2017	946	22 Jan 2018 *	110,000	194,000
7 Dec 2017	2,240	24 Jan 2018 *	63,500	100,500
11 Dec 2017	8,140	29 Jan 2018 *	52,000	229,000
13 Dec 2017	4,910	1 Feb 2018	3,500	83
18 Dec 2017	68,100	5 Feb 2018 *	16,100	134,000
20 Dec 2017	36,500	8 Feb 2018	3,100	66,000
27 Dec 2017	1,640	12 Feb 2018	85	32,300
2 Jan 2018	373	15 Feb 2018	3,500	37,700
5 Jan 2018	300	19 Feb 2018	61	747

* Signifies when cells were detected within the intake into the treatment plant

Table 17. Phytoplankton cell enumeration at Myponga Reservoir on 11 January 2018. Location 1 is directly above the intake into the treatment plant, Location 2 and 3 are further east along the dam wall.

Location	<i>M. flos-aquae</i> (cells/mL)	<i>M. aeruginosa</i> (cells/mL)	Combined (cells/mL)
Location 1 at 0 m depth	10,200	334,000	344,200
Location 1 at 10 m depth	1,320	186,000	187,320
Location 1 at 20 m depth	121	153	274
Location 1 at 30 m depth (depth of intake)	254	0	254
Location 2 Surface Scum Sample *	-	-	60-70,000,000
Location 3 Surface Scum Sample	814,000,000	90,700,000	904,700,000

* Location 2 was used for organic matter characterisation and DBP formation analysis of raw water and sonicated surface samples.

Table 18. Disinfection by-product (DBP) formation of product water after conventional treatment at Myponga Reservoir.

Date	THMs (µg/L)	HAA5 (µg/L)	DBP 551 (µg/L)	DOC (mg/L)	Chl <i>a</i> at Surface (µg/L)
14 June 2017	153	121	29.5	5.2	3.92
10 July 2017	127	97	20.9	5.3	2.80
07 August 2017	152	124	29.2	5.0	2.19
04 September 2017	160	124	29.0	4.9	0.77
16 October 2017	167	129	29.5	5.1	1.60
13 November 2017	154	104	22.8	4.9	6.54
11 December 2017	140	95	22.7	5.2	5.30
11 January 2018	216	201	50.2	5.4	7111
15 January 2018	146	112	26.1	5.3	5.62

Myponga Water Treatment Plant Characterisation

Although the dense cyanobacterial bloom was present between a depth of 0 and 10 metres, only 254 cells/mL of *M. aeruginosa* were detected at a depth of 30 metres near the intake (Table 17). The source of the organic matter could be classified as primarily allochthonous, based on chlorophyll *a* concentration of 0.37 µg/L (Table 15) and similar DOC and UV₂₅₄ concentrations comparative to the inflow. However, the persistence of the bloom prior to sampling would have altered the characterisation of the organics due to continuous phytoplankton exudation and sunken cell detritus. The origin of the organic matter could be further identified through isotope analysis as previously completed in studies such as Grey *et al.* (2001); however, this was outside of the scope of this project. The water quality entering the Myponga WTP was characterised by high DOC and UV₂₅₄ measurements of 14.30 mg/L and 0.540 cm⁻¹ associated with high turbidity readings of 20.9 NTU (Table 15). Conventional treatment was successful at significantly reducing the organic matter concentration and improving the aesthetics of the water. After the addition of the coagulant alum and the rapid mix process, DOC concentration was reduced to 6.17 mg/L (Table 15). The colour₄₅₆ of the water had also decreased to 9 HU due to the removal of large proportions of VHA and SHA fractions that are known to contain colour inducing humic and fulvic acids (Figure 26). Although slightly reduced, a high turbidity measurement of 15.5 NTU remained due to the presence of colloidal material. Application of DAF further reduced the DOC component and significantly reduced turbidity to 0.79 NTU (Table 15). The resulting product water after filtration was characterised by a DOC concentration of 5.44 mg/L, UV₂₅₄ absorbance of 0.120 cm⁻¹, colour₄₅₆ of 7 HU, turbidity of 0.16 NTU and a negligible chlorophyll *a* measurement (Table 15). Although VHA, SHA and CHA components were efficiently removed during conventional treatment, concentrations of NEU remained stable (Figure 26).

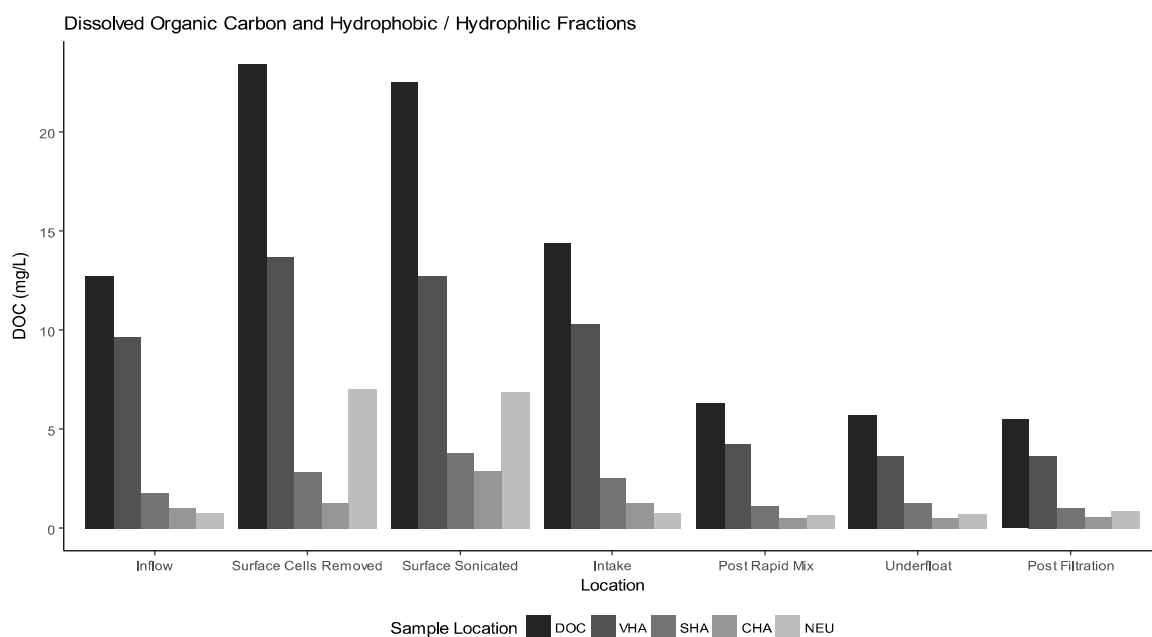


Figure 26. Dissolved organic carbon (DOC) concentrations alongside four fractions separated by hydrophobic and hydrophilic properties. The four fractions are defined as; very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), charged hydrophilic acids (CHA) and hydrophilic neutrals (NEU).

7.3.2 Disinfection by-product Formation

The concentration and speciation of DBPs formed upon chlorination are heavily dependent on the chemical composition of the organic matter and resulting reaction rate kinetics (Barrett *et al.* 2000; Hua and Reckhow 2007a). The reactivity of chlorine and organic matter precursors were therefore expected to have considerable variation between each location.

The influence of the cyanobacterial bloom on the formation of DBPs was determined by subjecting surface samples to two treatments prior to analysis. The removal of phytoplankton cells upon the first treatment allowed for isolation of the DOC component containing phytoplankton exudates. Upon chlorination, the filtered surface samples produced 1360 µg/L AOX, which was predominately comprised of 835.1 µg/L of UTOX (Figure 27). Sonication of surface water upon the second treatment allowed for the reaction of chlorine and unfiltered NOM, including both the intracellular and extracellular components of phytoplankton. Sonicated surface samples produced 21,700 of AOX, which was significantly higher than filtered surface samples (Figure 27). Although sonication did not prove to increase the DOC component of the surface sample,

it is evident that chlorination successfully lysed the cells which enabled the reaction with the released intracellular organic matter. The majority of DBPs formed from the sonicated surface scum were not identified via the HAA, THM and DBP 551 assays, with 95.8 % of total formation being identified as UTOX (Figure 27). Both surface treatments formed higher concentrations of N-DBPs than any other locations (Figure 28). Filtered surface samples formed higher concentrations of bromochloroacetonitrile, whereas sonicated surface samples formed higher concentrations of dichloroacetonitrile (Figure 28).

The DOC within the intake was lower than surface samples due to the lower concentration of live cells present at this location. The resulting DBP formation was therefore noticeably lower at only 2240 µg/L (Figure 27). A lower proportion of DBPs were characterised as UTOX at only 37.1 %. The largest known DBP class formed were HAAs at 821 µg/L, followed by THMs at 471 µg/L and DBP 551 at 116.5 µg/L (Figure 27). Hydraulic retention times were calculated to ensure that the same batch of intake water was then analysed at the following locations through the treatment plant.

DBP formation after coagulation and rapid mix was only slightly lower than intake results. Although there was a significant reduction in DOC, AOX formation had only reduced to 1990 µg/L which was comprised of 44.5 % UTOX (Figure 26 and Figure 27). The minimal decrease in DBP formation between intake and post-coagulation and rapid mix samples was most likely because the flocs had not been filtered out prior to DBP analysis. This allowed for chlorine to react with the organic matter bound up in the flocs. A significant decrease in DBP formation occurred after the flocs were removed during the DAF process. AOX concentrations were reduced to 994 µg/L; however, the relative composition of UTOX had increased to 47.31 % (Figure 27). Removal of flocs during DAF also reduced the formation of known DBP classes to 231 µg/L of HAAs, 239 µg/L of THMs and 50.2 µg/L of DBP 551 (Figure 27). There was a minor reduction of AOX and concentrations of all DBP classes post-filtration. There was also a notable reduction in UTOX composition, accounting for 44.8 % of AOX after the conventional treatment process.

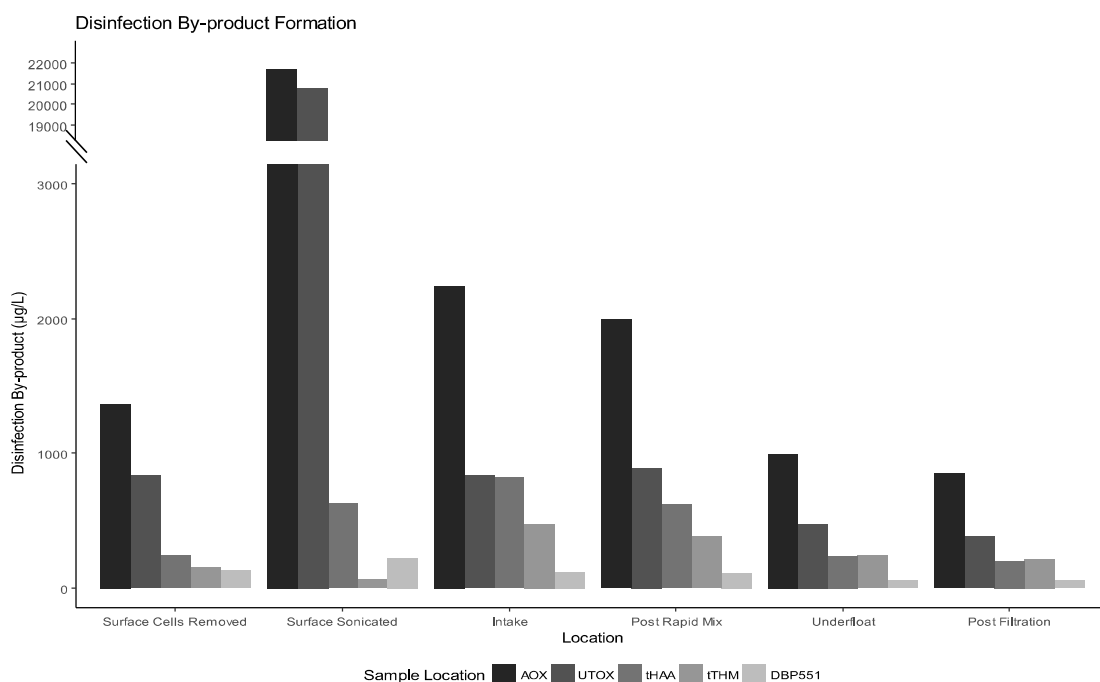


Figure 27. Total disinfection by-product (DBP) formation was measured alongside DBP classes of trihalomethanes (THMs), haloacetic acids (HAAs) and haloacetonitriles, chloral hydrate, chloropicrin and chloropropanones categorised as DBP 551. The unknown total organic halogen (UTOX) component was back calculated by subtracting known DBP class formation from adsorbable organic halogens (AOX) concentrations. The surface sample was exposed to two treatments, series filtration down to 0.45 µm to remove whole cells, and sonication to lyse cells and release their intracellular organic content.

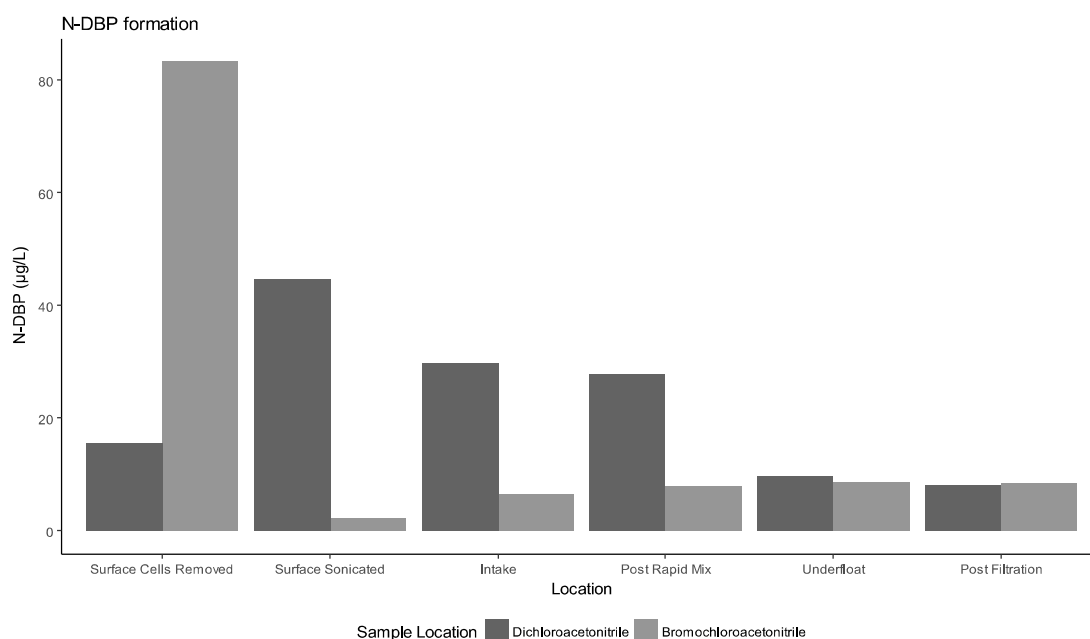


Figure 28. Nitrogenous disinfection by-product (N-DBP) formation as analysed by the DBP 551 assay.

7.4 Discussion

Disinfection is a critical step in water treatment to ensure potable water is free from pathogenic organisms. In Australia, it is necessary for WTPs to meet the Australian Drinking Water Guidelines (ADWG) for regulated DBPs, as long as achieving these guidelines does not compromise disinfection (NHMRC 2011). Improving our understanding of DBP formation will allow for the development of strategies to minimise formation through enhanced treatment mechanisms or improved catchment management. The contribution of phytoplankton organic matter as a significant precursor towards DBP formation has become a key focus of recent literature (Hong *et al.* 2008; Fang *et al.* 2010; Li *et al.* 2012; Tomlinson *et al.* 2016). Phytoplankton organic matter often contains increased proportions of hydrophilic compounds that are partially recalcitrant to conventional treatment practices, and hence, are more likely to react with chlorine to form DBPs (Singer and Harrington 1993). Furthermore, phytoplankton organic matter is characterised by lower C:N ratios compared with allochthonous organic matter (Meyers 1994; Vreca and Muri 2006). The phytoplankton cells are also capable of exuding high concentrations of dissolved organic nitrogen (Nguyen *et al.* 2005b; Zhang *et al.* 2014). An increased concentration of intracellular and extracellular nitrogen can result in increased formation of N-DBPs that are often considered to be of higher risk than their carbonaceous counterparts (Richardson *et al.* 2007; Plewa *et al.* 2008).

7.4.1 Impact of Phytoplankton Bloom on DBP Formation

The succession of the cyanobacterial bloom prior to analysis in January highlighted the extent of the rapid turnover rates of phytoplankton communities (Table 16). Phytoplankton influence the NOM content in the surrounding aquatic environment as cells continuously exude organic matter (Fogg 1983; Malinsky-Rushansky and Legrand 1996; Tomlinson *et al.* 2016). Furthermore, phytoplankton either lyse upon cell death releasing intracellular organic matter, or sink during sedimentation (Crompton and Wetzel 1982; South and Whittick 1987).

The formation of a dense cyanobacteria bloom had major implications on DBP formation at the surface location near the dam wall. It was evident that intracellular organic matter was the primary contributor towards DBP formation from phytoplankton precursors, given the substantially higher concentrations of AOX upon chlorination of sonicated

surface samples (Figure 27). Interestingly, DBP formation from sonicated surface samples was overwhelmingly categorised as UTOX, accounting for 95.8 % of total formation. As the majority of the DBPs monitored by the ADWG were analysed under HAA, THM and DBP 551 assays; the formation of UTOX comes with an unknown potential human health risk.

The meteorological conditions that favoured the formation of the cyanobacterial bloom also reduced the likelihood of vertical mixing at the time of sampling due to thermal stratification, with most of the bloom remaining near the surface. Dense concentrations of phytoplankton cells were only recorded down to a depth of 10 metres, with only 254 cells/mL of *M. aeruginosa* counted at the intake a further 20 metres deeper (Table 17). Although the bulk of the bloom was concentrated between 0-10 metres, destratification or an increase in surface wind driven currents could have increased the depth at which the bloom intruded the vertical profile, causing significant treatment issues. High cell concentrations were not detected on 11 January; however, destratification of the reservoir caused an increase in cell counts entering the WTP later in January (Table 16)

7.4.2 Removal of DBP Precursors and Treatment Efficiency

Comparison of the organic matter characterisation of intake water and product water post-filtration highlighted a significant decrease in organic matter concentration and Chlorophyll *a* measurements (Table 15). The aesthetics of the product water were also considerably improved, with significantly lower colour₄₅₆ and turbidity measurements (Table 15). The largest reduction in DOC occurred post rapid mix after dense flocs had formed during coagulation and flocculation (Figure 26). The treatment process was ineffective at removing the NEU component of DOC, which would have been problematic had higher cell concentrations of the bloom entered the treatment plant (Figure 26). The formation of DBPs post-coagulation and rapid mix was quantitatively similar to the intake sample as chlorine was able to react with the flocs still present in the solution. The largest reduction in DBP formation occurred after DAF, when the flocs were removed from the solution. The product water contained concentrations of regulated DBPs that were all lower than ADWG values; however, the formation of THMs was at the upper bounds of their recommended limits. The formation of 82 µg/L of bromodichloromethane was of particular concern as the World Health Organisation

recommends that formation should not exceed 60 µg/L (World Health Organisation 2011). It is important to note that the formation of bromodichloromethane did not exceed the ADWG concentrations. This highlights how discrepancies between multiple regulatory bodies can become confusing, misleading and increase the challenge of regulation (Tomlinson *et al.* 2016). The conventional treatment process was successful at significantly removing precursors of the N-DBP, dichloroacetonitrile, with significantly lower concentrations measured after DAF; however, the formation of bromochloroacetonitrile remained stable (Figure 28).

DBP concentrations within the product water are monitored regularly to ensure treatment processes are conforming to the ADWGs. The DBP formation and DOC concentrations of the product water during the *Microcystis* bloom were compared with concentrations from historic routine monitoring at Myponga WTP (Table 18). During the bloom, the DOC concentration of product water was marginally elevated compared with the historic average; however, significantly higher DBP formation was recorded for HAAs, THMs and DBP 551 assays. It is evident that Myponga WTP is efficient at reducing DOC concentrations to approximately 5.0 mg/L (Table 18); however, the organics within the product water during the bloom were more reactive with chlorine in the formation of DBPs.

Although the product water contained low concentrations of organics, improved aesthetics, and complied with ADWG DBP guideline values; AOX formation was still high at 846 µg/L (Figure 27). The majority of DBPs formed were accounted for via HAA, THM and DBP 551 assays; however, 44.8 % of AOX was classified as UTOX (Figure 27). The formation of UTOX poses a potential, unintentional health risk due to unknown toxicity of the chemicals formed and because they are not regulated or routinely monitored. The three common classes of DBPs investigated in this paper only account for 24 of the 600+ DBPs identified, making it difficult to determine which chemicals are contributing towards UTOX. It is unrealistic and costly to routinely monitor for all known DBPs; however, it would be advisable to routinely monitor for AOX concentrations to provide insight into total DBP formation. Regulated DBPs should still be monitored to meet regulatory guidelines; however, aiming to reduce total DBP formation from improved catchment management or advanced treatment practices will reduce the potential increased health risk posed by high concentrations of UTOX.

Improved management of DBP formation during phytoplankton blooms is necessary, as increased concentration of autochthonous organic matter has correlated with higher reactivity with chlorine towards the formation of DBP (Table 18). Phytoplankton blooms significantly alter the surrounding aquatic environment due to continuous cellular exudation, lysis upon cell death and sedimentation (Tomlinson *et al.* 2016). Historic comparison of DBP formation within product water has identified substantially higher formation of HAAs, THMs and DBP 551 classes during the January bloom (Table 18). To ensure compliance with regulatory guideline values, drinking water utilities are switching to alternative disinfectants (eg. Chloramine, ozone and chlorine dioxide) as they have been shown to reduce the formation of regulated THMs and HAAs (Hebert *et al.* 2010). However, the use of alternative disinfectants often results in the formation of higher concentrations of UTOX, other potentially harmful intermediate DBPs and N-DBPs (Hua and Reckhow 2007b; Richardson *et al.* 2007; Hebert *et al.* 2010). To ensure total DBP formation is minimised, improved treatment practices such as the addition of powdered activated carbon, enhanced coagulation or point of use activated carbon filters could be implemented (Xie 2016). Early detection of phytoplankton blooms with fluorometric probes will allow WTPs to respond rapidly and effectively (Zamyadi *et al.* 2016). Alternatively, improved catchment management can reduce the extent to which autochthonous organic matter reacts with chlorine. Reducing the autochthonous organic matter load can be achieved by limiting nutrients to reduce productivity (Brookes and Carey 2011).

7.5 Conclusion

Fractions of VHA, SHA and CHA were effectively removed during the conventional treatment process at Myponga WTP; however, the NEU fraction was recalcitrant to conventional treatment (Figure 26). DBP formation within the product water was higher during the January bloom in comparison to historic data where chlorophyll *a* measurements were lower (Table 18). Although treatment resulted in reduced organics, improved aesthetics and compliance with DBP ADWGs, the formation of AOX was still relatively high. The majority of DBPs formed within the product water were identified via HAA, THM and DBP 551 assays; however, 44.8 % of AOX was unaccounted for, and hence, classified as UTOX. The significant formation of UTOX coincides with an unknown potential human health risk upon exposure to these DBPs.

Routine DBP monitoring should be altered to include AOX formation as well as the formation of regulated DBPs. This will allow for a better understanding of the extent of UTOX formation, whilst allowing utilities to strive towards reducing the concentration of AOX via improved treatment processes and catchment management. Higher DBP formation in product water during a dense phytoplankton bloom highlights the need to better manage DBPs during a bloom event, even if cells are not entering the treatment plant. Reducing the occurrence of phytoplankton blooms can be achieved by limiting nutrients through improved catchment management (Brookes and Carey 2011). Early detection of the onset of a phytoplankton bloom with fluorometric probes will allow for WTPs to quickly respond and alter treatment processes accordingly (Zamyadi *et al.* 2016). Alterations in treatment practices including the use of powdered activated carbon, enhanced coagulation or point of use carbon filters will also reduce DBP formation; however, these alternatives come with a significant increase to the cost of treatment.

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Chapter 8

General Discussion

The formation of disinfection by-products (DBPs) is best understood in the context of the biochemistry of the natural organic matters (NOM) precursors. The chemical composition and concentration of NOM is significantly influenced by the relative composition of catchment (allochthonous) and internally produced (autochthonous) carbon. This thesis highlights the significant contribution of autochthonous phytoplankton organic matter towards the formation of DBPs. The necessity to investigate the relationship between phytoplankton organic matter and DBP formation was driven by two fundamental reasons. Primarily, phytoplankton intracellular and extracellular organic matter are associated with a high proportion of hydrophilic NOM (Li *et al.* 2012). An increased concentration of hydrophilic NOM has the potential to result in higher DBP formation as it is more recalcitrant to coagulation during conventional treatment (Singer and Harrington 1993). Secondly, the formation of more genotoxic nitrogenous DBPs (N-DBPs) from phytoplankton organic matter is likely to be higher than from catchment NOM because the average C:N ratio of autochthonous organic matter is substantially lower than allochthonous organic matter (Meyers 1994; Elser *et al.* 2000; Vreca and Muri 2006).

The structure of this thesis was designed to investigate four key concepts to support the overarching hypothesis that phytoplankton organic matter is a significant contributor towards the formation of DBPs. Firstly, Chapter 4 compared the reactivity of chlorine with catchment and phytoplankton NOM to determine the relative contribution of each towards DBP and N-DBP formation. Secondly, Chapter 5 investigated the correlations between hydrophobic and hydrophilic NOM fractions and DBP formation to provide insight into why different organic carbon loads reacted differently with chlorine. Thirdly, Chapter 6 compared DBP formation before and after cultured phytoplankton blooms were added to raw water as well as DBP formation between phytoplankton species. Finally, Chapter 7 investigated the efficiency of a conventional water treatment plant to remove DBP precursors during a substantial surface phytoplankton bloom.

The major findings of this thesis are conceptualised to aid future risk assessment of DBP formation potential (Figure 29). This model represents the major processes that influence the relative contribution of allochthonous and autochthonous NOM loads towards the total carbon budget of the reservoir. The major findings of this thesis are then conceptualised to inform risk based decision making regarding the concentration and speciation of DBPs formed as a result of chlorinating allochthonous and autochthonous NOM sources of carbon. The endpoints of this model are numerous decision making outputs aimed to reduce the relative risk of DBP exposure.

The relative contribution of allochthonous and autochthonous organic matter is driven by the local meteorological conditions and the catchments biogeophysical characteristics (Figure 29). In winter, the frequency and intensity of rainfall impacts the relative allochthonous NOM load within the reservoir. The concentration and chemical composition of organic matter is defined by the catchment characteristics including soil type, vegetation, topography and land use (Frimmel 1998; Findlay and Sinsabaugh 2003). In summer, higher temperatures and increased light exposure correlate with higher phytoplankton populations, and hence, a higher contribution of autochthonous NOM to the total carbon budget of the reservoir (Paerl and Huisman 2009). Elevated temperatures, longer days and extended periods of vertical thermal stratification favour the proliferation of some cyanobacteria species of phytoplankton which is attributed to their gas vesicles that provide buoyancy and reduce sedimentation loss (Fong and Zedler 1993; Brookes and Ganf 2001). The formation of dense cyanobacteria blooms is enhanced by the eutrophication of freshwater systems through urban, agricultural and industrial development (Paerl and Huisman 2008; Paerl and Paul 2012). The relative contribution of the autochthonous NOM is further influenced by the duration of the phytoplankton bloom, speciation, growth rates, cellular exudation rates and turnover rates (Tomlinson *et al.* 2016). The large proportion of future climate change model scenarios predict an increased contribution of autochthonous NOM towards the carbon budget of lakes and reservoirs due to higher estimated lake temperatures and occurrences of thermal stratification (Brookes and Carey 2011; O'Neil *et al.* 2012; O'Reilly *et al.* 2015).

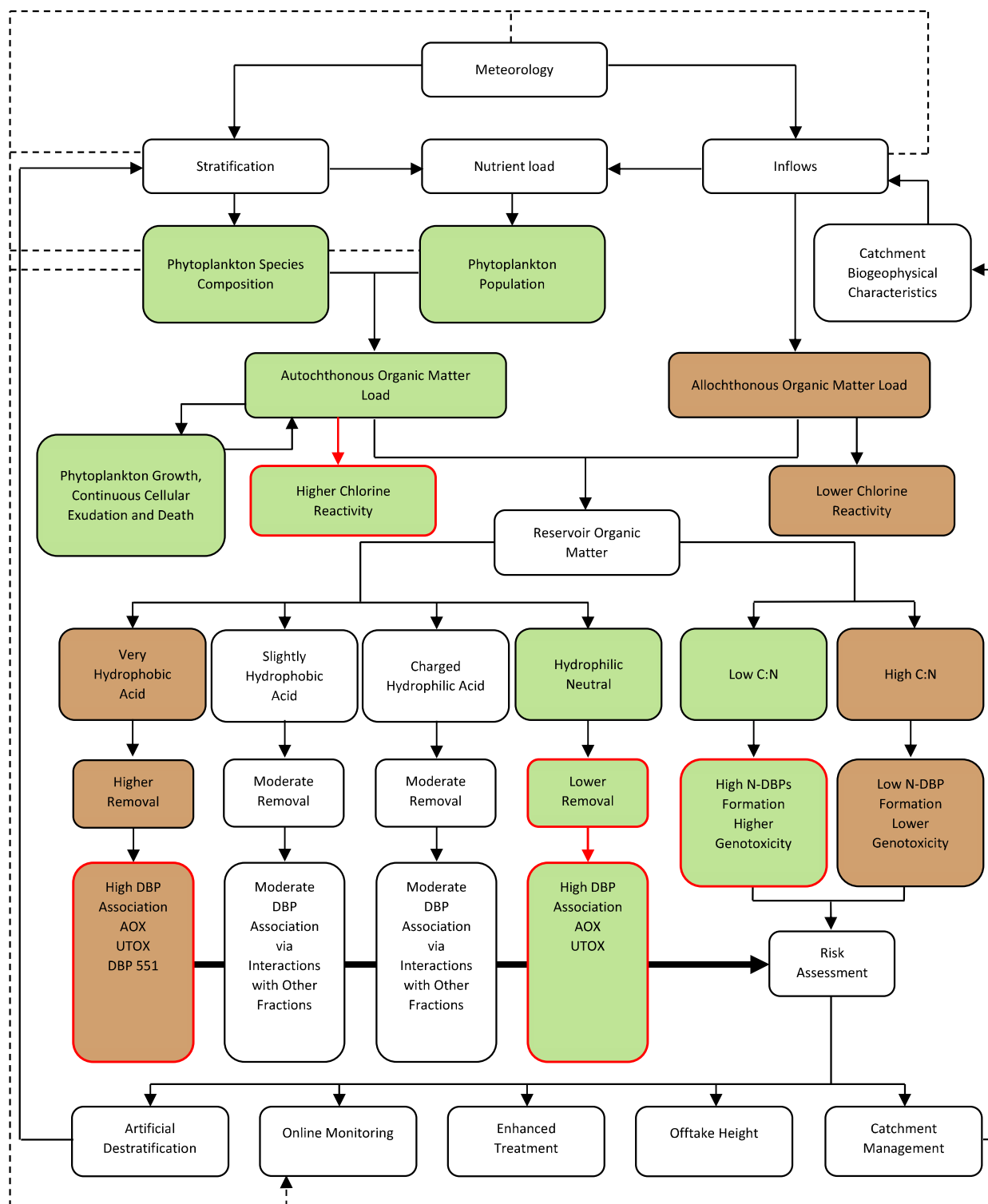


Figure 29. Conceptual model of relative contribution of allochthonous and autochthonous natural organic matter (NOM) sources towards disinfection by-product (DBP) formation. The green boxes highlight concepts that are highly associated with autochthonous NOM and the brown boxes highlight concepts that are highly associated with allochthonous NOM. Concept boxes that are outlined in red signify outputs that may increase DBP formation or increase risk of exposure.

8.1 Conceptual Model

The estimated increase in the relative contribution of autochthonous NOM can have major implication on the formation of DBPs within drinking water systems. The conceptual model highlights a stark difference in the reactivity of allochthonous and autochthonous NOM with chlorine (Figure 29). To explore this difference, Chapter 4 compared the relative contribution of winter flows and summer phytoplankton blooms towards DBP formation. Phytoplankton blooms had a significant impact on DBP formation potential due to the higher reactivity of autochthonous NOM with chlorine comparative to allochthonous carbon sources (Table 7). Chlorination of the significant December summer phytoplankton bloom produced higher concentrations of DBPs than chlorination of winter flows (Figure 11).

The increased reactivity associated with autochthonous NOM also influenced the genotoxicity of DBPs produced (Figure 29). Chlorination of summer phytoplankton blooms produced higher concentrations of N-DBPs resulting in a relative increase in genotoxicity (Figure 14) (Richardson *et al.* 2007; Hua and Reckhow 2008; Plewa *et al.* 2008; Bull *et al.* 2011). This was attributed to lower average C:N ratios of phytoplankton organic matter comparative to allochthonous organic matter (Meyers 1994; Elser *et al.* 2000). However, chlorination of summer phytoplankton bloom in March resulted in an overall lower concentration of DBPs formed per milligram of carbon compared with the December bloom (Table 7).

Variation in the reactivity of each carbon source is due to differences in the chemical composition of the organic carbon (Westerhoff *et al.* 2004; Golea *et al.* 2017). The increased potential risk of DBP formation associated with the chlorination of raw water containing a higher proportion of autochthonous phytoplankton NOM influences the risk assessment decision making process (Figure 29). The increase in potentially more genotoxic DBP formation and overall higher production of DBPs can have major implications on the perception of the potable water, treatment cost and the human health outcomes associated with a potential increased exposure.

To explain the observed differences in DBP formation between allochthonous and autochthonous NOM loads, Chapter 5 investigated the relative composition and reactivity of hydrophobic and hydrophilic NOM fractions. Reservoir samples containing high

populations of phytoplankton during summer phytoplankton blooms were associated with higher concentrations of hydrophilic NOM (Table 10). The model summarises that allochthonous winter flows were associated with higher concentrations of very hydrophobic acids (VHA) and dense phytoplankton blooms can contain higher concentrations of hydrophilic neutrals (NEU) (Figure 29). A linear model emphasised the importance of the interactions between each NOM fraction towards DBP formation (Table 11). Further model reduction determined that both VHA and NEU had a strong positive correlation with total DBP formation (AOX) and the formation of unknown DBPs (UTOX) (Table 12). This finding indicates an increased risk in DBP formation associated with major influxes of allochthonous catchment NOM after a rainfall event and from increased autochthonous phytoplankton NOM during substantial blooms (Figure 29). The association between the concentration of VHA and NEU fractions with DBP formation also impacts the treatability of the water and therefore significantly influences the necessity to reassess the risk analysis process (Figure 29).

As alluded to in Chapter 4, the model (Figure 29) also highlights that phytoplankton species composition plays a significant role in the contribution towards DBP formation (Hong *et al.* 2008). Variations of the chemical composition of each species also influences the treatability of the water and hence reactivity with chlorine, producing unique suites of DBPs (Figure 29). Expanding on the work by Hong *et al.* (2008), Chapter 6 furthered this research by investigating the contribution of a green algae and a cyanobacteria species towards the formation of a broader range of DBPs within Myponga Reservoir. This research concluded that green algae *Ankistrodesmus sp.* contributed more towards AOX and UTOX relative to cell biomass. However, cyanobacteria *Dolichospermum circinale* contributed more towards the formation of N-DBPs. Chapter 6 signified the importance of considering the contribution of the entire phytoplankton community when determining the relative risk of phytoplankton precursors forming DBPs upon chlorination. Further, investigation of total, unknown and N-DBP formation helped understand the impact each species can have towards DBP formation and potential adverse human health effects. The phytoplankton species composition significantly influences the population densities, exudation rates, growth rates and mortality rates, and hence, has a major impact on the relative contribution of autochthonous NOM (Figure 29).

Chapter 7 determined that the relative contribution of hydrophobic and hydrophilic NOM also had implications for the removal efficiency of the organic fractions during conventional treatment (Figure 29). Conventional treatment was efficient at removing the bulk of the hydrophobic NOM load; however, the concentration of the NEU fraction was not significantly reduced (Figure 26). The phytoplankton bloom at the surface of the reservoir during this investigation was characterised by a significantly higher proportion of the NEU fraction (Figure 26). If the bloom had entered the treatment plant during the sampling regime it would have had a significant impact on the treatability and DBP formation based on the removal inefficiency of the NEU fraction. Higher composition of the NEU fraction has an increased risk of forming AOX and UTOX (Figure 29). This was evident by increased DBP formation within the product water during this phytoplankton bloom in comparison to historic DBP values in the absence of high phytoplankton concentrations (Table 18).

The conceptual model summarises that NOM loads with a higher proportion of autochthonous carbon are correlated with an increased concentration of hydrophilic NOM, reduced removal efficiency during treatment and increased reactivity with chlorine to form AOX, UTOX and N-DBPs (Figure 29). Comparatively, NOM loads with higher proportions of allochthonous carbon correlated with higher hydrophobic NOM and high AOX, UTOX and DBP 551 formation (Figure 29). However, catchment carbon also correlated with lower N-DBP formation and higher removal rates during conventional treatment (Figure 29). An understanding of the differential impact of allochthonous and autochthonous NOM precursors upon DBP formation and treatment efficiencies highlights that risk assessment should be undertaken. Risk assessment may indicate a need for improved catchment management and treatment strategies.

Improved catchment management may prove to be an effective method to reduce the nutrient load of nitrogen and phosphorous within the system and hence reduce the severity and frequency of phytoplankton bloom formation. The reduction of nitrogen and phosphorus loading on reservoirs should minimise phytoplankton growth as they have previously been identified as limiting nutrients (Nagar et al. 1974; Hecky and Kilham 1988; Baker et al. 2000; Guildford and Hecky 2000). Catchment management has a direct impact on the biogeophysical characteristics of the land and hence influences the organic matter within the upstream inflows and the resultant nutrient load of the aquatic

environment (Figure 29). Catchment management projects that can reduce the autochthonous NOM load include riparian restoration by improving natural water purification and improved agricultural practices by reducing fertiliser application and enhancing the soils ability to retain limiting nutrients such as phosphorus (Loomis *et al.* 2000; Cox *et al.* 2005; Bussi *et al.* 2016; Halliday *et al.* 2016). Mechanical approaches such as artificial destratification can be implemented to manage the species composition and populations of phytoplankton. Hypolimnetic aeration can reduce the phytoplankton population by limiting the sediment release of phosphorus by creating oxic conditions (Lewis *et al.* 2004; Brookes *et al.* 2008; Bormans *et al.* 2016). Oxic conditions are created by increasing the oxygen content of the water and reducing the severity of the thermal stratification bands. Further, artificial destratification works by eliminating the advantage of buoyant cyanobacteria by keeping the cells entrained within the turbulent flow (Visser *et al.* 2016). This mixing limits the cyanobacteria cells light availability by vertically distributing cells which often results in a change in species dominance within the system. Implementation of artificial destratification can therefore reduce the proportion of autochthonous NOM and hence increase the treatability of the organic matter and reduce associated DBP formation (Figure 29). Enhanced treatment practices can also reduce the concentration of organic matter reacting with a disinfectant. There is a large and continually advancing suite of enhanced treatment methods that can be implemented to reduce the formation of DBPs. Some of these include: the use of alternative disinfectants, biofiltration, activated carbon and enhanced coagulation (Wert and Rosario-Ortiz 2013). The suitability of these methods is dependent on the characteristics of the water being treated. However, implementation of aeration and enhanced treatment practices has a significant capital and/ or operational cost.

Online monitoring is a useful tool to aid the decision-making process, ensuring that the water treatment process is as efficient and effective as possible. Online monitoring can measure numerous variables including: meteorology, thermal stratification, upstream inflow volume, phytoplankton species composition, phytoplankton population density and general water quality parameters (Figure 29). The data obtained by continuous online monitoring can help us understand the risk of DBP formation and can ultimately be used to justify alterations in the treatment process if the system is prone to particular adverse conditions such as phytoplankton blooms. This can then be used to potentially rationalise expenditure on catchment management projects and enhanced treatment practices. Online

monitoring can also be used to determine the optimum height of the reservoir offtake that will result in reduced cost of treatment or improved water quality (Hobson *et al.* 2010).

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