MICROBIALLY-MEDIATED METHYL IODIDE CYCLING IN A PARTICLE-RICH ESTUARY

BY

NOBLE KWAME ASARE

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Abstract

The dynamics of aquatic systems (e.g. estuarine systems) are known to facilitate the formation of particle aggregates. These nutrient-rich particulate matter provide suitable substrate for bacteria colonization. Although bacteria-aggregate association is known to result in the degradation of particulate organic matter (POM) in aquatic systems, it has never been attributed to the production of methyl iodide (CH_3I) (an environmentally important biogas that has the potential to impact on atmospheric chemistry). From literature, there are evidences which suggest that, certain bacteria (methylotrophs) are capable of oxidizing methyl halides including CH_3I. Therefore this study investigates microbial production and removal of CH_3I in estuarine water through their association with aggregates and assesses the effect of physicochemical variables on bacterial-mediated production and removal of CH_3I. From the study, bacteria-aggregate processes were found to elevate the concentration of CH_3I between 15-22% of the total observed CH_3I concentration over the study period. Aggregate-attached bacteria which were estimated to represent about 17% of the total bacteria population were responsible for about 37% of the overall bacterial activity. To investigate bacterial-mediated removal of CH_3I in estuarine systems, a reliable and reproducible method through adaptations and modifications of existing methods was developed. This method involved the use of [^{14}C] radiolabelled CH_3I to estimate bacterial utilization of CH_3I. The application of the method confirmed the removal of CH_3I by methylotrophs in estuarine water with the total recorded amount in bacterial cells and oxidized CO_2 ranging between 9.3 – 24.5% (depending on the amount of the added substrate). However, this could only represent the potential microbial CH_3I removal in the natural aquatic environment. An investigation into spatial and temporal trends in bacterial-mediated removal of CH_3I in the Tamar estuary revealed no significant spatial variation but rather a strong seasonality in methylotrophic bacterial CH_3I removal. Spatial trends in CH_3I removal was found to be mostly influenced by temperature, bacterial abundance and dissolved oxygen concentration whilst the seasonality in the estuary was influenced by temperature, bacterial abundance, suspended particulate matter (SPM) and CH_3I concentration. Temperature was identified to be the single most influential physicochemical variable on both spatial and seasonal variation in bacterial CH_3I removal in the Tamar estuary. CH_3I concentration along the Tamar estuary was also investigated and using this data the total water to air flux of CH_3I over the estuary was estimated to be 0.31×10^3 g y^{-1}. From this study, it was apparent that bacteria activity in estuarine systems is potentially an important source of CH_3I in the aquatic environment when associated with aggregates or as sink of CH_3I through methylotrophic activity in estuaries.

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Author's Declaration

This is to declare that the following thesis was entirely based on field and laboratory work conducted personally and at no time during the registration for the degree of Doctor of Philosophy at the University of Plymouth has this thesis either whole or in part been presented for any other University award. This study was funded by and carried out at the Plymouth Marine Laboratory with the supervision of Drs. Carol Turley, Philip Nightingale and Malcolm Nimmo. Any assistance obtained during the course of the study has been duly acknowledged. Relevant scientific conferences were attended at which parts of the work were presented.

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Noble Kwame Asare

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CHAPTER ONE

Review: The role of physico-chemical properties and bacterial activity in the cycling of methyl iodide in particle-rich estuarine environments

1. Review: The role of physico-chemical properties and bacterial activity in the cycling of methyl iodide in particle-rich estuarine environment

1.1. Rationale

Physical and biogeochemical processes that regulate the global climate include microbially-mediated activities and the transfer of biogenic gases (biogases) between the aquatic environment and the atmosphere. Particulate organic matter (POM), enriched in nutrients provides a suitable energy source for microbial communities in the estuarine environment. This association results in the transformation of POM into by-products (including biogases). Other microbial processes lead to the transformation of these biogases which result in the cycling of the gases in estuarine and coastal waters. Methyl iodide (CH_3I), a halocarbon is one such biogas with the potential to impact on tropospheric chemistry (and human health). Understanding the role microbial communities play in the cycling of CH_3I in coastal and estuarine waters (especially with respect to their association with POM in estuaries) and the influence of environmental physico-chemical variables on such microbial processes is therefore important. This chapter will therefore review the current knowledge in the literature of (i) estuarine systems (characteristics and geochemistry), in particular the particle-rich Tamar estuary (ii) bacterial activity and the impact of physico-chemical properties in estuaries (iii) estuarine aggregates and associated bacterial communities (iv) the importance of the CH_3I global cycle (v) the effect of estuarine bacterial activity on the CH_3I cycle and present (vi) the aims and objectives of present study.

1.2. Estuaries and their biogeochemistry

1.2.1. Background

An estuary is a semi enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage (Pritchard, 1952). Due to their position, estuaries serve as transition zones between land and water serving as the final recipient of catchment input and tidal inflows from the sea. They therefore provide a major pathway for the transfer of dissolved and particulate material from the continent to the marine environment through rivers (Frankignoulle & Middelburg, 2002).

Estuaries are highly dynamic systems with complex mixing zones that display physical, chemical and biological interfaces (Relexans, *et al.*, 1988; Fichez *et al.*, 1993). Due to the presence of high suspended particles (e.g. Uncles *et al.*, 1994; Tattersall *et al.*, 2003), estuaries are characterized by high heterotrophic microbial activity resulting in high turn-over rates of carbon, nitrogen and other biogeochemical components. This makes estuaries important in nutrient fluxes, organic carbon and nitrogen cycling in the environment. During the transition from freshwater to marine environments, estuaries undergo sharp changes in physical and chemical conditions (Miller, 1999) such as suspended particles, salinity, temperature, dissolved oxygen, pH and light intensity. There is also the presence of mixed assemblages of flora and fauna along the estuary due to the prevalence of the wide range of physical and chemical conditions. A major part of the mixed microbial assemblages comprise of bacteria, algae, fungi and protozoa (Azam *et al.*, 1993).

There is water movement in estuaries which is influenced by two primary forcing mechanisms (1) freshwater inflows attempting to drain to sea and (2) the regular tidal movement of seawater into and out of the estuary. These movements are regulated by the ebb and flood flows of tides and differences in the density of water with their propagation

affected by the geometry of the estuarine bed. Strong winds that blow across the surface waters of estuaries may also cause nontidal circulation. The movement of the water therefore facilitate mixing of water parcels which is importance to the distribution of salinity and water quality levels throughout the water mass of the estuary.

Estuaries experience high detrital organic matter and nutrient levels making them highly productive systems (Jackson *et al.*, 1987). In most aquatic ecosystems (including estuarine systems), dissolved organic matter (DOM) serves as the dominant pool of reduced carbon (Del Giorgio & Cole, 1998). In addition to local production (Herman & Heip, 1999), a major fraction of estuarine DOM is riverine-derived (Langenheder *et al.*, 2004; Sandberg *et al.*, 2004). This riverine DOM is chiefly made up of allochthonous humic substances (Ivarsson & Jansson, 1994). A component of the organic matter (also allochthonous) is however marine derived (Soetaert & Herman, 1995).

Economically, estuaries are used for anthropogenic purposes such as ports and harbours vital for shipping, transportation, industrial activities (Canuel & Zimmerman, 1999; Latimer *et al.*, 1999, Manning & Dyer, 1999) as well as serving the local commerce (e.g. tourism and recreation). Through these anthropogenic activities, estuaries are subjected to intense ecological disturbances in the form of introduced waste materials that result in high loading of many contaminants such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls. These disturbances also include elevated amount of heavy metals (e.g. Cu, Zn, Cd, Fe, Al, and Mn) which have a strong affinity for suspended particulates (Ko & Baker, 1995). These resulting situations thereby increase the potential for biogenic gas production within estuaries (Frankignoulle & Middelburg, 2002). This makes estuarine environment important in the investigation of environmental biogeochemical processes that facilitate the cycling of ecologically important atmospheric gases.

1.2.2. Physico-chemical properties of estuarine systems with an emphasis on the Tamar estuary

This section discuss some key biogeochemical processes in estuarine environment including the formation of turbidity maximum zone (TMZ), freshwater-saltwater interface (FSI) as well as the major physico-chemical properties which exhibit large gradients in estuarine systems and help describe the estuarine biogeochemical processes. Since the Tamar estuary is the subject of this study (see aims and objectives in section 1.8), it has been used in the illustration of these physical and chemical properties.

1.2.2.1. Suspended particulate matter and the turbidity maximum zone

Estuaries are fed by river discharge which results in high loads of suspended particles as compared to other natural water systems. Often, estuaries have an area of very high particulate concentration known as the turbidity maximum zone (TMZ) (Uncles & Stephens, 1989; Uncles & Stephens, 1993b). Processes that are known to facilitate the generation and maintenance of a TMZ include complex interactions between the tidal dynamics, erosion and deposition of fine sediment (Dyer, 1988). The formation of the TMZ originate mainly through tidally-induces resuspension (Uncles *et al.*, 1988; Uncles & Stephens, 1989) of bed sediment (Grabemann *et al.*, 1997).

Due to the behaviour of water movement, resuspension and transport of bed sediment occurs. The process of resuspension describes the lifting of bed sediment into the water column where consolidation has not taken place (Tattersall *et al.*, 2003). Tidal pumping, a dynamic trapping of water in the upper reaches of the estuary act to increase the seaward flow of water which exerts a shear stress on the bed of the estuary. At higher levels of bed shear stress, upward turbulent forces lift sediment particles from the bed and carry them into the water column (Uncles & Stephens, 1993b; Tattersall *et al.*, 2003). In addition, gravitational circulation (a longitudinal density gradient due to salinity difference)

enhances flood tide velocities near the bed and ebb tide velocities near the surface (Officer & Kester, 1991) thereby serving as an important mechanism for transport of sediment upstream.

The Tamar estuary exhibits a strong TMZ (Grabemann *et al.*, 1997). Although this TMZ is located in the upper reaches during the summer (Uncles & Stephens, 1989; Uncles & Stephens, 1993b), it is known to migrate down to the middle reaches during winter due to increased river discharge (Tattersall *et al.*, 2003). This migration is described as being longitudinal in response to changes in river flow (Uncles & Stephens, 1989; West *et al.*, 1990). During summer and early fall, the TMZ has a large mobile stock of bed-sourced fine sediment (Bale *et al.*, 1985; Uncles *et al.*, 1988) through resuspension. Dispersal of suspended particulate matter (SPM) from the TMZ to a new location down the estuary or even into the coastal zone could be caused by river spates (Grabemann *et al.*, 1997). Following a spate, the TMZ may require weeks or even months to re-establish (Grabemann *et al.*, 1997).

The TMZ acts as a reservoir within which physical and chemical reconstitution of SPM takes place (Morris *et al.*, 1987). The composition of the SPM within the Tamar TMZ is controlled by the composition of resuspendable sediment with a mean sediment grain size of 20-30μm (Uncles *et al.*, 1992) associated with tidal influence (Morris *et al.*, 1987) and is dominated by mud (Uncles *et al.*, 1994). Hence increases in tidal range cause resuspension of bed sediments leading to enhanced SPM levels (Uncles *et al.*, 1994).

Estuarine turbidity varies with seasonal river discharge and that of any associated tributaries, as well as the tidal pattern experienced within the estuary. The combined effect results in a variation in the load of suspended sediments and associated materials, especially in the TMZ. The TMZ in the Tamar estuary has been known to reach up to 800

mg L^{-1} of suspended solids (Jackson *et al.*, 1987) during periods of high rates of sediment resuspension. In the seaward reaches of the estuary however, the concentrations of SPM are comparatively very low and are attributed to the flushing of fresh water from the estuary (Uncles & Stephens, 1993b). According to Bale *et al.* (1985), seasonal changes in the pattern of sediment transport to and from the upper estuary correlate with river flow.

1.2.2.2. Salinity

Due to the intrusion of freshwater into seawater in the Tamar estuary, there exists a freshwater-saltwater interface (FSI). This FSI is a region of steep salinity and density gradients often associated with the TMZ (Uncles et al., 1985; Uncles & Stephens, 1993a). The position of the FSI which could be influenced by runoff may determine the extent of preferential flood or ebb SPM transport by tidal and residual current present in the estuary (Uncles & Stephens, 1993b). The FSI takes the form of a tidal intrusion front under low runoff conditions (Simpson & Nunes, 1981) and a near-frontal FSI under strong freshwater runoff and neap-tide conditions (Uncles & Stephens, 1993a). At the location of the FSI, the water column is highly stratified with fresh water flowing seawards over the more dense seawater (Schumann *et al.*, 1999).

During periods of heavy rain, the Tamar estuary experiences a maximal riverine input which leads to the displacement of the FSI down the estuary (Miller, 1999). Whenever the FSI is located more than 7 km from the head at high water, there is the occurrence of a separation between the FSI and the TMZ within the range of 1-3 km (Uncles & Stephens, 1993a). Dominant freshwater input into the Tamar estuary is provided by the river Tamar with typical monthly average flow rates decreasing from a maximum of 38 m^3 s^{-1} in January to a minimum of 5 m^3 s^{-1} in June (Uncles *et al.*, 1983). The freshwater input into the estuary is however also complimented by the Tavy and Lynher (Uncles & Stephens, 2001).

The transport of fine-sediments and the formation of a TMZ are also affected by intra-tidal variations in salinity stratification (Hamblin, 1989; Geyer, 1993). In the Tamar estuary, the formation of the TMZ occurs around a salinity of 5 (Grabemann *et al.*, 1997).

Generally, during spring and neap tides, observed salinity stratification and current layering in the upper reaches of the estuary are significantly greater during the ebb phase of the tide as compared to the flood phase (Uncles & Lewis, 2001). During the ebb flow there is reduced vertical mixing and enhanced rates of deposition as well as reduced SPM loads in the upper water column. This occurs following the erosion of bed sediment due to the stability in the water column located down-estuary of the FSI (Uncles *et al.*, 1994). In the upper reaches of the estuary, SPM levels are highest behind the FSI, and as the FSI moves down-estuary, high SPM readings are observed in the freshwater reaches (Grabemann *et al.*, 1997). Variation in salinity affects the composition and physiology of microbial population in estuarine systems. Changing salinity may therefore provide some influence on any differences in the type and rate of bacterial activity along the Tamar estuary.

1.2.2.3. Surface water temperature

Temperature plays a key ecological role within an estuary by determining the range of fauna and flora over an annual time period (Beukema, 1990). Variations in temperature may also influence interactions between physical and biological processes in estuaries (Uncles & Stephens, 2001). Spatial variation in water temperature within estuarine systems are predominantly controlled by mixing of coastal and river waters (Miller, 1999). In temperate estuaries, temperature serves as an important factor that determines seasonal variations in microbial activity (Tison *et al.*, 1980; Schultz Jr. *et al.*, 2003; McManus *et al.*,

2004). Processes that are microbially-driven (e.g. CH_3I production/removal) could be expected to be similarly dependent on temperature.

In the Tamar estuary, maximum surface water temperatures are recorded in August (16.8-18.4 °C), whilst minimum surface water temperatures are recorded in December (5.9-9.4 °C). Strong variations in temperature reflect the seasonality in the estuary (Miller, 1999). Between April and July, the temperature of freshwater is at least 3 °C above that of coastal waters and the trend is reversed by August (Miller, 1999).

1.2.2.4. Dissolved oxygen

In the Tamar, dissolved oxygen concentration decreases with increasing heterotrophic bacterial activity. Therefore an oxygen minimum is located at the seaward reaches of the TMZ due to river transport down the estuary. The presence of oxygen minima are usually observed during the months of May, June and August (Jackson *et al.*, 1987). Oxygen profiles in the middle estuary (from spring to late summer) indicate the presence of an oxygen minimum in this location (Morris *et al.*, 1982). Dissolved oxygen (DO) depletion during this period is largely due to an oxygen demand exerted in the very low (0-2) salinity region (Morris *et al.*, 1982) by heterotrophic microbial processes (Goosen *et al.*, 1997). With respect to atmospheric exchange, oxygen concentrations along the Tamar estuary are always near equilibrium with the exception of small mid-estuarine oxygen sag especially during the winter (Morris *et al.*, 1982).

1.2.2.5. Light Intensity

Naturally, there is the exposure of bacteria to solar radiation in the surface-most layers of aquatic systems (Morán *et al.*, 2001). Photochemical degradation of complex DOM of higher molecular weight into smaller, biologically-labile organic molecules could be caused by UV radiation (Lindell *et al.*, 1995; Tranvik & Bertilsson., 2001). The inhibition

of bacterial production (Jeffrey *et al.*, 1996; Pakulski *et al.*, 1998), bacterial biomass (Sieracki & Sieburth, 1986) and metabolically important enzymes (Müller-Niklas *et al.*, 1995) by overexposure to sunlight has also been reported.

Over a period of time, UV radiation is capable of stimulating natural bacterial assemblages while short-term exposure to enhanced UV radiation could reduce bacterial activity in bacterial communities (Gustavson *et al.*, 2000). For example, according to Dumestre *et al.* (1999), light intensity could seriously inhibit the growth of methanotrophs that are found in the water column especially around oxyclines.

However, due to the presence of high suspended particles in some estuarine systems especially in the TMZ, light penetration is greatly reduced (Joint & Pomroy, 1981; Jackson *et al.*, 1987). Therefore, regardless of the high nutrient availability associated with estuarine systems, primary production in some estuaries (especially in the upper reaches where the concentration of suspended particles are mostly at its maximum) is low (Joint & Pomroy, 1981; Van Spaendonk *et al.*, 1993; Krompkamp *et al.*, 1995) compared to coastal waters and open oceans. Estuaries with high turbidity such as the Tamar have reduced light penetration and consequently a low primary productivity (Jackson *et al.*, 1987). One of the established sinks of CH_3I in the aquatic environment is photolysis. Therefore reduced light penetration may imply a reduction in the influence of light intensity on the consumption of CH_3I in the Tamar estuary.

1.2.3. Bacterial activity and the effect of some physico-chemical properties in estuarine systems

Bacterial activity within estuarine systems is influenced by both physical and chemical factors (Bent & Goulder, 1981). These influences include the effect of physico-chemical properties on the structure, composition and activity of microbial community associated

with the water column (e.g. Relexans *et al.*, 1988; Crump *et al.*, 1999; Goosen *et al.*, 1999; Herman & Heip, 1999; Böckelmann *et al.* 2000).

The growth rates and physiological states of bacterial cells are related to changes in physico-chemical factors (including freshwater discharge, nutrient availability, temperature, salinity, SPM and pH) in estuaries (Palumbo *et al.*, 1984).

Due to river discharges into estuarine systems, there are relatively high organic carbon and nutrient concentrations to support microbial activity. As a result of this, the standing stock and production of bacteria in estuaries relative to other aquatic systems is high (Ducklow & Carson, 1992; Ducklow & Shiah, 1993). The distribution of nutrients however decreases down the estuary and this is reflected in lower bacterial activity in the lower estuary.

The species composition of bacterial communities in estuaries changes from fresh- to marine waters (Valdés and Albright, 1981; Mantoura, 1987; Prieur *et al.*, 1987). These changes in bacterial community structure however require adequate growth rates and a relatively long residence time (Crump *et al.*, 2004) to sustain them. In general, cell sizes of bacterial species in the upper reaches are larger than those present in the lower reaches (Goosen *et al.*, 1995). The increased size of bacterial cells in the upper reaches of estuaries may be due to a faster growth as a result of high organic nutrient availability in this region of the estuary (Palumbo *et al.*, 1984).

The function and composition of heterotrophic microbial communities associated with the TMZ are also affected by the quality and supply of organic substrates (Eiler *et al.*, 2003) transported down the estuary into the TMZ. However, most of the bacterial taxa present are affected differently by DOM composition (van Hannen *et al.*, 1999; Crump *et al.*, 2003) and the concentration of dissolved organic carbon (DOC) (Eiler *et al.*, 2003).

Temperature is known to provide the strongest correlation with bacterial abundance and productivity (McManus *et al.*, 2004). For example, Hoch & Kirchman, (1993), Shiah & Ducklow, (1995) have shown that temperature is an important factor limiting bacterial growth, especially during winter (McManus *et al.*, 2004).

At the optimal growth temperature, a maximum amount of substrate is degraded through microbial activity (Tison *et al.*, 1980). However, at extreme temperatures above the optimal growth temperature, bacteria are stressed, while decreasing the temperature to just below the minimum required for growth will only make them temporarily inactive (Ranneklev & Bååth, 2001). The optimal growth temperature for bacterial growth depends on the type of bacterial community. For a group of temperate methylotrophic bacterial community amongst the α-proteobacteria (e.g. *L. methylohalidivorans, R. gallaeciensis*), the optimal growth temperature is 27 °C (Schaefer *et al.*, 2002).

According to Nedwell & Rutter (1994), the affinity of bacteria for organic substrates can be highly temperature sensitive. The parabolic relationship that exists between temperature and the specific growth rate has been found to apply amongst bacterial communities (Wijtzes *et al.*, 1995). This may be because there is a decreased uptake of nutrients by bacteria across their cell membranes at temperatures below the optimal growth temperature (Quinn, 1988; Reay *et al.*, 1999) as a result of alterations in physical characteristics of the cell membrane (Reay *et al.*, 1999). This effect is temporary, therefore in the event of an increase in temperature, bacteria may immediately become active again (Ranneklev & Bååth, 2001). At very high temperatures (above the optimum), however, the growth of bacteria is limited as a result of protein denaturing (Eppley, 1972; Russell, 1990).

During both cold and warm seasons, there are bacteria communities that are prevalent in estuarine systems, but these communities differ both in abundance and diversity. Therefore

seasonal changes (warming and subsequent cooling periods) can be considered as two phases that exert different pressures on a bacterial community (Horwath & Elliott, 1996). The first phase selects a more thermophilic community when there is an increase in temperature whilst the second phase selects a mesophilic community when there is a decrease in temperature (Horwath & Elliott, 1996). Although heterotrophic bacterial activity in estuaries decreases with decreasing seasonal temperature, the optimal temperature could be several degrees above the *in situ* temperature (Tison *et al.*, 1980).

In addition to temperature, spatial variability in bacterial properties in estuaries is most strongly related to salinity (Schultz Jr. *et al.*, 2003; McManus *et al.*, 2004). In relation to this, some authors (e.g. Valdés and Albright, 1981; Mantoura, 1987; Cunhua *et al.*, 2000) have provided both field and experimental evidence which suggest that bacterial activity in estuarine systems is affected by salinity. For instance, based on the work of Cunhua *et al.* (2000) in the Ria de Aveiro estuary in Portugal, more than 80% of the variation in bacterial abundance was found to be associated with the combined effects of temperature and salinity.

The low salinity (<10) regions of estuaries are identified as regions of severe physiological stress for both freshwater and marine organisms (Kinne, 1971). For example, the bacterial communities that are transported down the river begin to experience salt stress within the TMZ (Flameling & Kromkamp, 1994). Also as a result of lytic processes due to osmotic stress (Troussellier *et al.*, 2002), estuaries register a net loss of freshwater bacterial cells when they encounter marine water (Painchaud *et al.*, 1995a). Within estuaries, the concentrations of freshwater bacterial cells have been found to peak at low salinity (1-3) immediately downstream of the freshwater zone and then decrease down the estuary (Painchaud *et al.*, 1995b). With decreasing salinity, marine bacteria are affected by

freshwater influence. The structure of bacterial communities in estuarine systems therefore varies with changing salinity (Troussellier *et al.*, 2002).

As a function of changing salinity, bacterial production also changes substantially during tidal cycles in an estuary (Goosen *et al.* 1995). Changes in hydrodynamic conditions associated with tidal cycles therefore serve as an important factor that also controls microbial community structure in estuarine systems (Eldridge & Seiracki, 1993). According to Hyun *et al.* (1999), it is common to have two distinctive bacterial communities present in tidal estuaries. These are (i) allochthonous freshwater bacterial communities which are adversely affected by an increase in salinity at low tides and (ii) autochthonous estuarine bacterial communities that are well adapted to changes in salinity at high tides which therefore benefit from the high concentration of nutrients in the upper reaches of an estuary.

In the estuarine environment (especially within the TMZ), microbial activity is dominated by heterotrophic processes (Goosen *et al.*, 1999) and this could be enhanced by the availability of suspended and resuspended particulate substrate with a long residence time in the TMZ (Herman & Heip, 1999). This is because besides temperature and salinity, a positive relationship between the concentration of SPM and the heterotrophic bacterial production rate in estuaries exist (Goosen *et al.*, 1999). According to some authors (e.g. Bent & Goulder, 1981; Joint & Pomroy, 1982; Plummer *et al.*, 1987; Painchaud & Therriault, 1989), a good correlation has also been found between particle concentration and the number of attached bacteria in a number of estuarine systems. For example, the density of attached bacteria recorded in the Humber estuary (North-East England) was found to be closely dependent on the concentration of the suspended particles to which they were attached (Bent & Goulder, 1981).

In the aquatic environment, bacterial communities are known to respond to changes in pH (Bååth, 1996; Pennanen *et al.*, 1998). According to Wijtzes *et al.* (1995), reduced pH below or increased pH above the optimal pH for growth, could lead to inhibited growth or even the death of bacteria in a given medium. Observations made in estuaries (Morris *et al.*, 1982) indicate that, recorded freshwater pH values are normally lower (within the range 6.7-7.5) whilst showing a general pattern of increasing pH with increasing salinity. Optimal pH for bacterial growth also depends on the group or even the species of bacteria. In coastal waters and estuarine systems, the optimal pH for growth of some group of methylotrophic bacteria has been given as 7.7 (Schaefer *et al.*, 2002) or in a range of 7.0-7.3 (Goodwin *et al*, 1998). Within the short term, the pH of natural waters is very responsive to changes in the inorganic carbon system (Morris *et al.*, 1982). Changes in estuarine inorganic carbon occur as a result of variation in enrichment from land (Butler & Tibbitts, 1972).

Investigating estuarine physical and chemical variables will therefore aid in the interpretation of variations in bacterial activity to be investigated in the Tamar estuary. Presented in Table 1.1 is a comparison of bacterial activity and abundance in some temperature estuaries.

From Table 1.1, bacterial activity evidently varies between several temperate estuaries. Bacterial abundance in one estuary may be high but will have low specific bacterial activity and vice versa. For example, the Humber estuary, UK which has the highest recorded bacterial abundance of 45.4×10^9 cells L^{-1} in Table 1.1 also has a mean specific bacterial activity of only 2.1 fg C d^{-1} $cell^{-1}$ as compared to a mean specific activity of 41.5 fg C d^{-1} $cell^{-1}$ from a maximum recorded bacterial abundance of 6.5×10^9 cells L^{-1} in the Westerchelde, Netherlands. These differences in bacterial activity may be due to diverse

estuarine dynamics (e.g. differences in SPM concentrations, varying POM and DOM concentrations) that may exist between the estuaries.

Table 1.1: A summary of bacterial abundance and productivity in temperate estuaries. Units of bacterial productivity have all been converted into mg C m^{-3} d^{-1} for ease of comparison.

Estuary	Abundance (x 10^9cells L^{-1})	Rate of bacterial productivity (mg C m^{-3} d^{-1})	Mean Bacterial Sp. Activity (fg C d^{-1} $cell^{-1}$)	Source
Humber, UK	1.8 – 45.4	1.7 – 146.4	2.1	Bent & Goulder (1981)
Chesapeake Bay, USA	0.5 – 25.0	0.5 – 480.0	10.1	Ducklow & Shiah (1993)
Delaware Bay, USA	0.7 – 13.0	1.5 – 184.0	8.1	Hoch & Kirchman (1993)
Elbe, Germany	2.0 – 7.5	19.2 – 108.0	12.0	Goosen *et al.* (1999)
Westerchelde, Netherlands	2.0 – 6.5	48.0 – 384.0	41.5	Goosen *et al.* (1999)
Gironde, France	1.5 – 7.0	2.4 – 67.2	5.6	Goosen *et al.* (1999)
York River estuary, USA	1.4 – 9.6	7.2 – 108.4	8.2	Schultz Jr. *et al.* (2003)
Narragansett Bay, USA	0.4 – 7.5	1.4 – 148.0	11.6	Staroscik & Smith (2004)
Mobile Bay, USA	1.3 – 7.3	14.0 – 179.0	17.7	McManus *et al.* (2004)

1.3. Particle Aggregates (estuarine and marine aggregates)

Particle aggregates are a concentration of mostly POM in the form of marine/estuarine snow (floccules of dead algae) and faecal pellets that are obtained mainly from planktonic particles and activities (Trent *et al.*, 1978; Alldredge & Silver, 1988; Alldredge & Gotschalk, 1990) and are enriched in nutrients, especially inorganic phosphorus and nitrogen (Shanks & Trent, 1979).

Particle aggregates are ubiquitous in the marine environment (Herndl, 1988; Turley, 1992), lakes (Grossart & Simon, 1993), rivers (Berger *et al.*, 1996; Böckelmann *et al.*, 2000) and estuarine environment (Eisma *et al.*, 1980; Eisma, 1986).

Marine and estuarine particle aggregates are very similar in that they all have both organic and inorganic constituents and provide substrate for microbial activity in the aquatic environment. The differences between marine and estuarine aggregates lie in the nature of their constituents, source of their constituents and their mode of formation. Marine aggregates are composed of mostly organic detritus (dead phytoplankton), zooplankton faecal pellets, lithogenic materials and some natural exopolymers. Their formation begins from the photic zone where most of the constituents originate and is facilitated by mostly wind-driven circulation of surface water. Sticky faecal pellets and the available exopolymers serve as matrices for the formation of aggregates whilst they continue to sink to the bottom of the ocean. On the other hand the composition of estuarine aggregates are mostly mineral grains (quartz, clay mineral, and calcite), resuspended bed materials and detrital material originating mostly from the banks of the estuary and water mass brought in by river discharge. Sticky materials that serve as matrices for aggregation in the estuarine environment are mostly the polymeric materials provided by benthic diatoms. The formation of aggregates occurs mainly through flocculation facilitated by the mixing process of the estuarine water mass which include tidal forcing, gravitational circulation as well as turbulent shear.

Depending on the characteristics of the environment within which they occur, sizes of particle aggregates may range between a few micrometers and several centimeters (Alldredge & Silver, 1988; Grossart & Simon, 1993; Zimmerman-Timm *et al.*, 1998). Within the marine environment, particle aggregates are much larger (Wörner *et al.*, 2000). On the other hand, due to the effect of tidal physical forcing, sizes of particle aggregates in

the estuarine environment have been reported to reach only 1 mm in diameter (Eisma, 1986; Wörner *et al.*, 2000).

1.3.1. Formation of Particle Aggregates

The formation of these macroaggregates in the aquatic environment is facilitated by the coupling of both physical processes, such as surface mixing enhanced by wind activity and thermohaline circulation (Kumar *et al.*, 2001) in the marine environment, water circulation through tidal forcing and river discharges in the estuarine environment, biological processes that involve the self-aggregation of dead algae (Ittekkot, 1996; Boyd *et al.*, 1999), the excretion of faecal materials by meso-grazers (Ittekkot, 1996), bacterial and algal production of mucus that facilitate adhesion (Biddanda & Pomroy, 1988) and chemically through ionic attraction between particles. Macroaggregation however, begins with the formation of adhesive mucus matrices from pico- and microaggregates that act as nuclei for adsorption onto other components, such as faecal pellets (Turley, 1992), as well as other microaggregates.

In the estuarine environment, flocculation (a process by which particles are caused to clump together) is the main process through which aggregation occurs. Flocculation normally occurs at the fresh water/salt water interface where physical changes facilitate bonding of particles and eventually settling to the bottom (Mead & Moores, 2005). Concentration of suspended particles, turbulent shear of the water body, differential settling of the particles, and the concentration of adhesive organic compounds in the water are some of the important factors in estuarine systems that influence particle flocculation (Eisma *et al.*, 1991b; Mead & Moores, 2005).

The characteristics of flocs in estuarine systems may vary over short and long time scales. This includes the rapid adjustment of the aggregates sizes to changes in turbulent shear

(Gibbs *et al.* 1989; Chen *et al.*, 1994, Fennessy, *et al.*, 1994) and seasonal variations in the source and type of primary particles (Fettweis *et al.*, 1998; van Leussen, 1994). Through encounter frequency, turbulent shear facilitate an increase to aggregate sizes but as turbulent shear increases, it becomes limiting to the aggregate size by tearing them apart (van Leussen, 1994; Berhane *et al.*, 1997).

During periods of reduced turbulent energy, differential settling of particles may dominate the aggregation dynamics. Larger aggregates are formed from primary particles in the middle of the water column when an increase in encounter frequency occurs without the destructive turbulent shear (e.g. van Leussen, 1994; Dyer, 1989).

Extracellular polymeric substances (EPS) produced by benthic diatoms (Underwood *et al.*, 1995) through the excretion of glucan (an intracellular carbohydrate storage product) (Hoagland *et al.*, 1993) play a crucial role in the estuarine environment. Among several functions, the purpose of benthic diatom EPS production is to provide adhesive film by which the diatoms attach themselves to the substratum surface (Busscher *et al.*, 1995), to facilitate cell aggregation and biofilm formation (Cescutti *et al.*, 1999; Burdman *et al.*, 2000; Decho, 2000) and to aid their movement to and from the surface of the sediment (Happey-Wood & Jones, 1988; Escher & Characklis, 1990; Underwood & Smith, 1998). Due to the adhesiveness in nature (ten Brinke, 1994; van Leussen, 1999), EPS aid the binding of sediment grains together (Meadows *et al*, 1990; Paterson *et al.*, 1990; Madsen *et al*, 1993; Underwood *et al.*, 1995) thereby increasing sediment cohesion (Paterson, 1989) and facilitating aggregate formation in the estuary.

The constituents of EPS are mainly polysaccharides, proteins, humic substances, nucleic acids and lipids (Decho, 1990; Cooksey, 1992; Nielsen *et al.*, 1997; Law & Bale, 1998; Azeredo & Oliveira, 2000; Flemming & Wingender, 2001). Therefore due to their richness

in organic carbon EPS serves as an important source of carbon (Bhaskar & Narayan, 2005) for the microbial community in the mid-estuarine region.

Beside the physical changes, the rate of estuarine flocculation is also enhanced by the total surface charge on the particles that result in bonding of the particles (Dyer, 1994). This occurs through chemical interactions between negatively charged particles and positively charged dissolved ions (e.g. Al, Fe, Ca and Mg) in the water causing the particles to stick together. Biological activity (especially during warm temperatures) also promotes the growth of aggregates in estuarine systems (Fugate & Friedrichs, 2003; Mikes *et al.*, 2004; Chang *et al.*, 2006). However, estuarine aggregates are very fragile and therefore can be easily broken up in sufficiently vigorous current flow (Mead & Moores, 2005; Chang *et al.*, 2006) as well as being affected by salinity changes (Eisma *et al.*, 1991a).

Through the influence of turbulence caused by wave action and current flow of the flood and ebb tides in estuaries, resuspension of bed sediments occur (Allen, 1973). This is because as the velocity of the current flow is increased there comes a point where the force is strong enough to lift sediment from the bed into the flow of water (Allen, 1973). During slack water (low current speeds) estuarine aggregates can be large enough to settle onto the sediment bed (Mead & Moores, 2005). The process of aggregate formation and disintegration during the course of a tidal cycle therefore plays an important role in the resuspension, transport and deposition pattern of sediments in tidal estuaries (Mikkelsen & Pejrup, 1998; Manning, 2004; Chang *et al.*, 2006).

Dense populations of microorganisms, of which bacteria form a major part, have been found to inhabit these nutrient-rich macroaggregates (Karl, 1982). This bacterial community has several metabolic states ranging from actively growing states to completely moribund states (Turley, 1993).

According to Caron (1991) the role of aggregates as niche for microorganisms becomes more importance when nutrients are scarce. In the marine environment, nutrient enrichment of the aggregates declines as the aggregates become smaller in sizes (Zimmermann-Timm *et al.*, 1998) and as they get older through loss of nutrients to the surrounding water (Hendl, 1988). However, in the estuarine environment, aggregates do not get old and lose their nutrients since there is a continuous formation of new aggregates as a result of high organic material abundance coupled with tidal sedimentation and resuspension (Zimmermann-Timm *et al.*, 1998).

1.3.2. Bacterial Association with Particle Aggregates

In the aquatic environment, bacterial communities become associated with aggregates (Turley & Mackie, 1994) during the formation of aggregates in surface water and also as the aggregates descend towards the bottom (Jacobsen & Azam, 1984; Lochte & Turley, 1988; Turley & Mackie, 1995). These associated communities of bacteria may be found both in the internal part (e.g. Gowing & Silver, 1983) as well as on the surface (Hansen & Bech, 1996) of the aggregates. The external population originates from passive collection during the sedimentation of faecal pellets from the surrounding water (Jacobsen & Azam, 1984; Peduzzi & Herndl, 1986; Nagasawa & Nemoto, 1988). The internal population may originate from faecal pellets as either enteric or as ingested species (Gowing & Silver, 1983) or from the external population (Lawrence *et al.*, 1993) that penetrates the peritrophic membrane of the faecal pellets (Turner, 1979). However, colonization generally occurs from outside the aggregates (Jacobsen & Azam, 1984; Hansen & Bech, 1996).

The assemblages of bacteria found associated with particle aggregates may be less diverse (Bidle & Fletcher, 1995) but in most cases, more active (Alldredge *et al.*, 1986; Iriberri *et al.*, 1987) compared to their free-living counterparts depending on conditions. Although

the total number and biomass of free-living bacteria predominate in the aquatic environment (Simon *et al.*, 1990; Unanue *et al.*, 1992) and account for a much more heterotrophic activity (e.g. Azam & Hodson, 1977; Ducklow & Kirchman, 1983), the proportion of attached bacteria is minor (e.g. 6.8%) in the overall bacterial abundance (Kirchman & Mitchell, 1982; Unanue *et al.*, 1992; Turley & Mackie, 1994; Turley & Stutt, 2000) but contribute significantly (e.g. 10-38%; Iriberri *et al.*, 1987) to the bacterial decomposition and regeneration of nutrients on a microscale (Goldman, 1984).

The group of bacteria associated with aggregates has a structure that differs from free-living forms (Weiss *et al.*, 1996). Most often, the attached bacteria have larger cells with comparatively higher abundance in the same volume of water (Caron *et al.*, 1982; DeLong *et al.*, 1993). Between 85% and over 95% of them belong to the Phylogenetic group, Proteobacteria (α, β and γ-class) thereby representing the largest single group that harbours the majority of the gram-negative bacteria found in aquatic systems (Weiss *et al.*, 1996). Their contribution to heterotrophic activities in the aquatic environment therefore depends on their abundance, metabolic rate as well as the availability of their particulate microhabitat (Alldredge *et al.*, 1986).

A number of factors are known to affect microbial activities such as growth and decomposition of particulate organic carbon (POC) in the aquatic environment. These factors include changes in pressure (Turley, 1993) and temperature (Pomeroy & Deibel, 1986) associated with depth. A comparison of the two groups of bacteria has however revealed that free-living bacteria are less tolerant to changes in pressure than attached bacteria (Turley, 1993) although this phenomenon will not prevail in the estuarine environment due to shallow water depths in estuaries.

1.3.3. Bacteria-aggregate relationship and biogas production

Aggregates provide a suitable microenvironment for the microbially mediated transformation of POM (Karl, 1982; Tranvik & Sieburth, 1989) into DOM with the aid of extracellular enzymes (Cho & Azam, 1988). This occurs in the form of remineralization through decomposition (Silver & Alldredge, 1981; Caron *et al.,* 1982) in the aquatic environment.

After becoming attached to aggregates, bacteria remain active and can increase their population density (Kaltenböck & Herndl, 1992) and begin the enzymatic breakdown of the POM (Noji *et al.,* 1991; Delille & Razouls, 1994). The rate of degradation of faecal pellets depends upon the type of diet from which it was obtained (Hansen *et al.,* 1996). During the process of degradation, most of the aggregates could be consumed by the associated bacterial assemblage within 2-3 days (Pomeroy *et al.,* 1984). Therefore attached bacteria play an important role in the conversion of POM into DOM in the aquatic environment through extracellular enzyme activity (Alldredge & Gotschalk, 1990; Karner & Herndl, 1992; Smith *et al.,* 1992).

Although there are extensive discussions in the literature concerning the role of microbial assemblages in the conversion of POM into DOM in the aquatic environment (e.g. Jacobsen & Azam, 1984; Karner & Herndl, 1992; Smith *et al.,* 1992; Hansen & Bech, 1996), very little has been reported that directly link bacterially mediated degradation of aggregates to the production of biogas in the aquatic environment. For example, Bianchi *et al.* (1992) reported anaerobic production of methane (CH_4) by methanogens that were associated with aggregates with suitable oxygen-depleted microzones (Alldredge & Cohen, 1987) whilst Conrad & Seiler (1988) reported an observed increased in CH_4 concentration associated with the accumulation of decaying organic matter. In addition, there have been reports (e.g. Oremland, 1979; Burke *et al.,* 1983) that connect methanogens to actively

reducing microenvironments such as particle aggregates, since methanogens cannot survive in oxygenated environments. On the other hand, several studies (discussed in section 1.7) have reported with regards to bacterial mediation in the aquatic environment, the degradation (oxidation) of several halocarbons (e.g. methyl halides).

It is therefore essential to carry out investigations into the specific roles of the microbial community associated with particle aggregates, in the production and emission of CH_3I and other halocarbons into the environment.

1.3.4. The role of particulate organic matter in the cycling of biogases and nutrients in the aquatic environment

In the aquatic environment, vertical and horizontal transfer of biogenic materials that facilitate the cycling of nutrients and gases takes place. Within the marine environment, particulate matter is removed from the surface ocean to the ocean floor through descent and this may result in the sequestration of biogenic materials. Estuarine environments on the other hand serve as major pathways through which the transfer of dissolved and particulate biogenic materials (of continental origin) from rivers to the marine environment occur (Frankignoulle & Middelburg, 2002).

The biological pump (see Figure 1.1) simply describes the formation, modification and sedimentation of POM which facilitate the conveyance of biogenic materials and other accompanying materials from the surface water to the aquatic floor where they become embedded in the sediment. This process involves a series of complex interactions within the biological community, especially the microbial assemblage both found in the free form and those associated with sinking particulate materials.

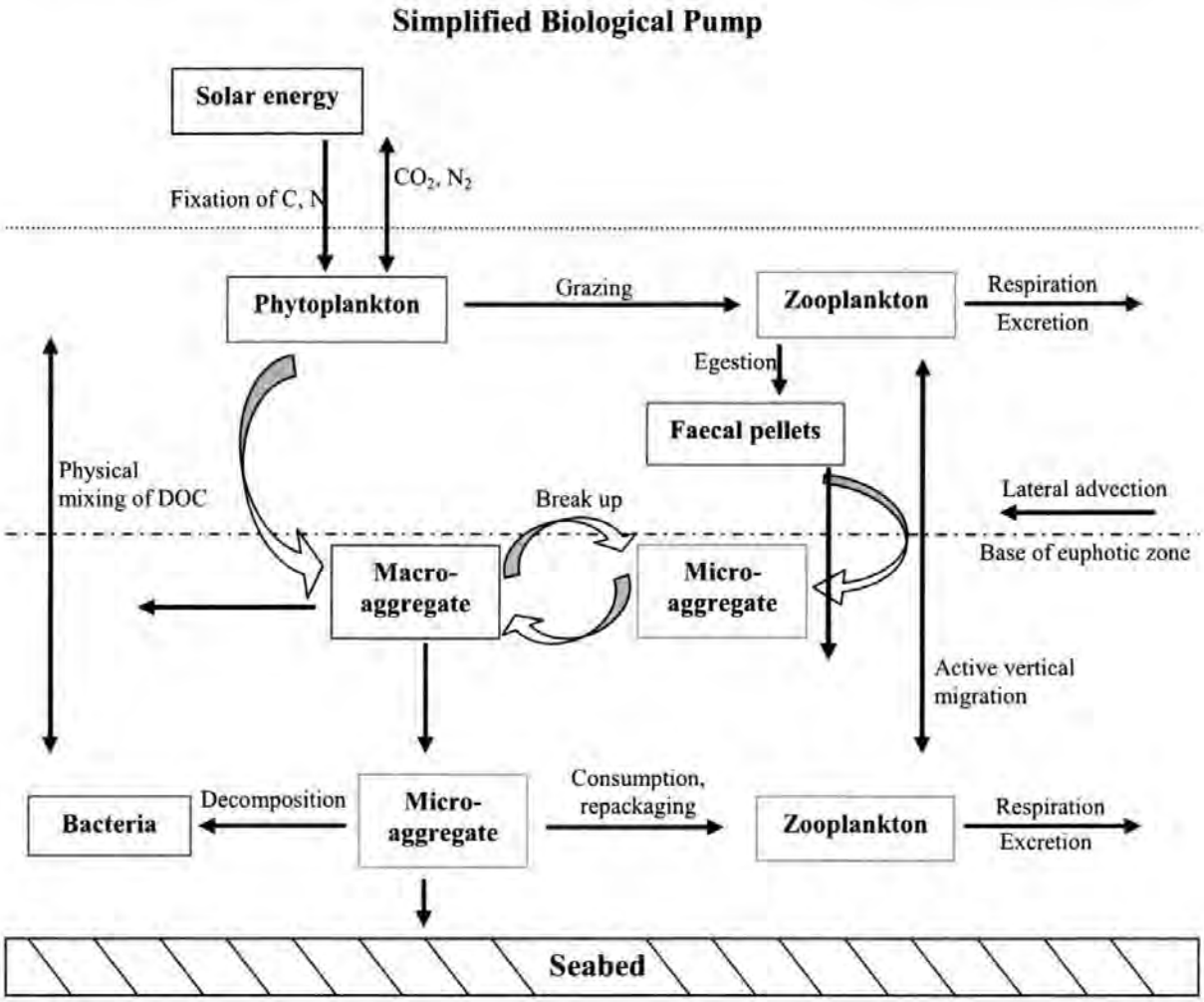

Figure 1.1: A schematic diagram of a simplified biological pump. Atmospheric CO_2 (or N_2 gas) fixed by autotrophs in the upper ocean is transported to deep waters (below the mixed layer) by various processes. Phytoplankton become senescent and sink out as aggregates, or are consumed by herbivores that produce sinking faecal pellets. Aggregates may then be decomposed by bacteria or consumed by animals (redrawn from OCTET).

In the marine environment, the net flux of biogenic materials and the processes that reintroduce the inorganic constituents into the system is in one direction therefore coupling processes are required to return some of the inorganic constituents to the surface. These include the upwelling of nutrient-rich deep water, diffusion across the thermocline and seasonal wind-driven deep mixing. These processes result in the release of a sizeable quantity of CO_2 to the surface water along with nutrients in the form of DOM and POM.

The biological pump therefore forms an important part of the global CO_2 and subsequently carbon cycle. In the estuarine environment, the process of biological pumping of biogenic material may be absent due to the periodic resuspension of sedimentary materials and high microbial activity that results in the decomposition of POM in the water column. However, with respect to the role of estuaries in global CO_2 cycling, estuarine environment have been reported (Kempe 1982; Frankignoulle *et al.*, 1996; Frankignoulle *et al.*, 1998) to show significant supersaturation of CO_2 in comparison to the atmosphere as a result of intricate biological and physico-chemical processes. Therefore the estuarine environment is potentially an important site for the emission of biogases (Frankignoulle & Middelburg, 2002).

Since aquatic systems interact with the atmosphere both physically (e.g. Gallego & Cessi, 2000) and chemically (e.g. Seitzinger *et al.*, 1984; Boyle, 1988; Law & Owens, 1990), there results a gaseous exchange between the water and the atmosphere. The most important mechanism by which gaseous exchange occurs is through diffusion across the water-atmospheric interface (Millero, 1995; Nightingale, 2003) during physical mixing. In the marine environment, gaseous exchange (especially CO_2) occur when warm water mass is carried from low latitudes to higher latitudes by oceanic surface currents (Munk & Wunsch, 1998; Garrett, 2003) and this water mass cools during the transition resulting in the absorption of CO_2 whilst sinking into deep water (Garrett, 2003). When such cold water mass returns from deep water to the surface, it warms up and loses CO_2 to the atmosphere (Stewart, 1992). On the other hand, gaseous exchange in the estuarine environment is facilitated by tidally-induced mixing.

Another mechanism that enhances the transfer of atmospheric CO_2 into the aquatic systems is assimilation by phytoplankton (Stewart, 1992). On the other hand, autotrophic and heterotrophic respiration (Siegenthaler & Sarmiento, 1993) combined with microbial

remineralization of organic matter (e.g. Karner & Herndl, 1992; Smith *et al.*, 1992; Simon *et al.*, 2002) in surface water through rapid oxidation (Suess, 1980; Martin *et al.*, 1987) release CO_2 back into the atmosphere.

1.4. Cycling of iodine in the environment

CH_3I (the halocarbon to be investigated in this study) is considered one of the main iodine carrier from the aquatic environment to the atmosphere (Rasmussen *et al.*, 1982; Hemann *et al.*, 1987; Moore & Tokarczyk, 1993). The production and consumption of CH_3I in the environment therefore serve an important and integral part of the iodine cycle. Cycling if iodine in the environment depends on both the identified and unidentified sources and sinks of iodine compounds through biotic and abiotic means. Iodine in the aquatic environment is known to exist predominantly in three forms (iodate, iodide and nonvolatile dissolved organic iodine). Iodate predominates in the deep ocean (McTaggart *et al.*, 1994; Campos *et al.*, 1996b) whilst iodide is chiefly present in coastal waters as a result of iodate reduction through biological activity (Elderfield & Truesdale, 1980; Upstill-Goddard & Elderfield, 1988; Farrenkopf *et al.*, 1997; Cook *et al.*, 2000). A simplified schematic diagram of iodine cycle in the environment has been provided in Figure 1.2.

According to Carpenter (2003), marine algae may be the main source of organic iodine in seawater involving the application of enzymes such as transferases (Wuosmaa & Hager, 1990). From several published work (e.g. Manley *et al.*, 1992; Nightingale *et al.*, 1995; Carpenter *et al.*, 2000), there is the indication that macroalgae are capable of producing all the detected atmospheric volatile organic iodine. On the other hand microalgae have been described to be responsible for the reduction of iodate into iodide in the aquatic environment (e.g. Udomkit & Dunstan, 1991; Moisan *et al.*, 1994). As stated by Carpenter *et al.* (2000) macroalgae production of iodine compounds such as CH_3I alone is not enough to account for the measured levels in surface coastal waters.

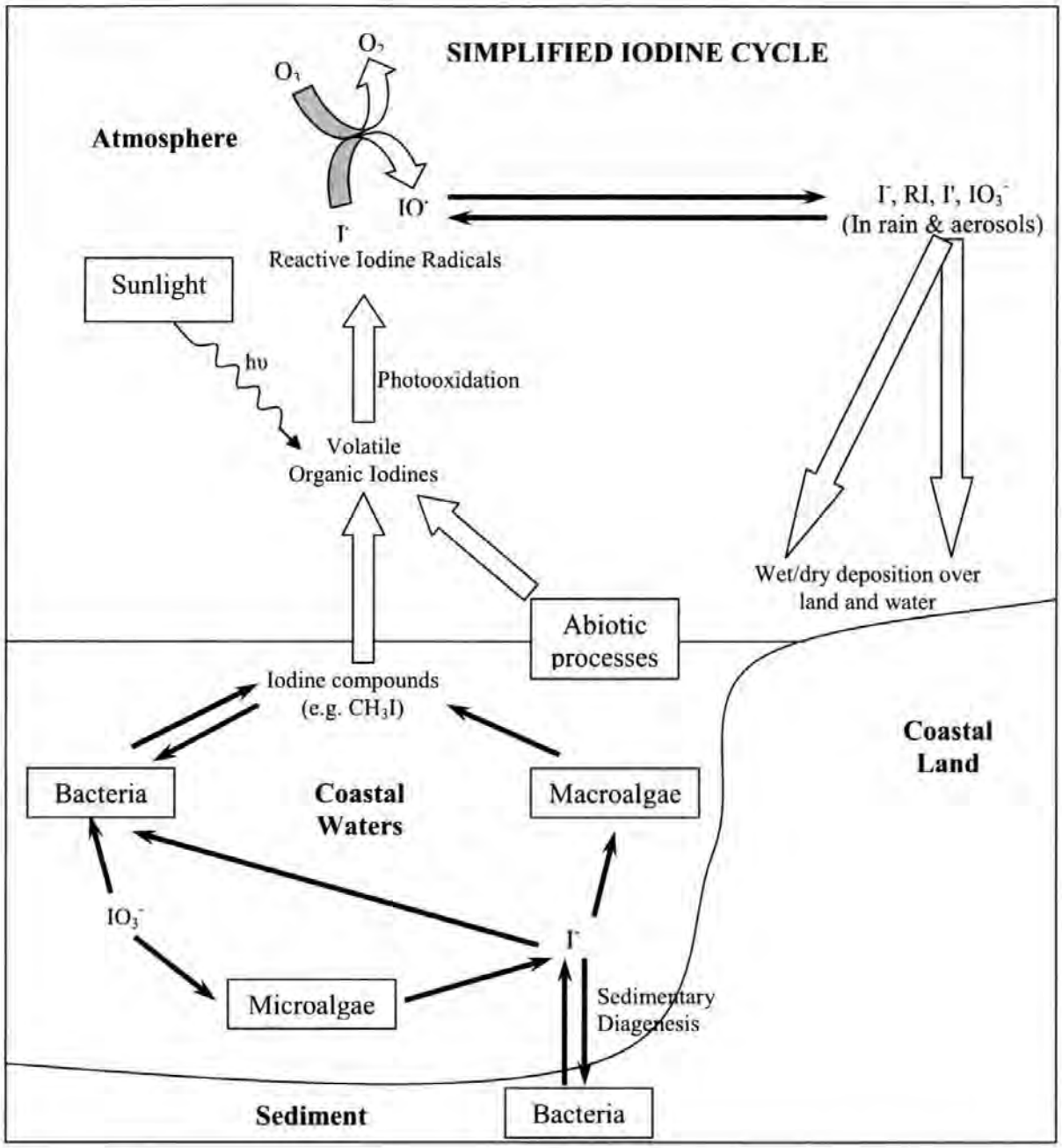

Figure 1.2: A simplified diagram of the iodine cycle in the environment involving reactive iodine radicals (I·), iodine monoxide radicals (IO·), inorganic iodide (I^-), inorganic iodate (IO_3^-), and soluble organic iodine compounds (RI).

Therefore other sources of iodine in the aquatic environments including microbial processes (e.g. Manley & Dastoor, 1988; Amachi *et al.*, 2001) and abiotic processes such as photochemical production in surface water (Moore & Zafiriou, 1994) have been reported. In an estuarine environment, sedimentary diagenesis of organic matter by microbial community may also result in the production and scavenging of inorganic iodine thereby influencing iodine concentration in porewaters and subsequently the overlying water (Upstill-Goddard & Elderfield, 1988).

Through water to air flux, volatile iodine compounds that end up in the atmosphere undergo photooxidative changes to form reactive iodine radicals ($I^{\cdot}$) which may act as catalyst in the tropospheric ozone destruction (Chameides & Davis, 1980) forming iodine monoxide radicals ($IO^{\cdot}$) in the process. A portion of the monoxide radicals is partitioned to the aerosol phase (e.g. I^-, IO_3^-) which are eventually dry/wet deposited on coastal land and water (Carpenter, 2003).

1.5. Halocarbons and their importance in the global environment

Halocarbons are organic compounds whose chemical compositions are primarily made up of carbon and halogens (chiefly chlorine (Cl), bromine (Br) and iodine (I)). Several of these compounds contain a multiple of the same or different halogen atoms thereby forming multi-halogenated compounds.

Halogenated compounds include multi-halogenated compounds such as bromo, chloro methane (CH_2BrCl) di-iodo methane (CH_2I_2) and monohalomethanes (CH_3X) that have been found to affect atmospheric, tropospheric and stratospheric chemistry thereby contributing significantly to global climatic change (e.g. Dickinson & Cicerone, 1986; Forster & Joshi, 2005). Halogenated compounds are of particular importance as a result of their major role in stratospheric ozone depletion (Prather & Watson, 1990) when their resulting halogen radicals react with the ozone to form molecules of halogen oxide and oxygen (i.e. $X^{\cdot} + O_3 \rightarrow XO + O_2$). Their sources are both natural (Goodwin *et al.*, 1997a) and anthropogenic (Lovelock, 1975; Mellouki *et al.*, 1992). Even though disturbances in the atmospheric balance of halocarbons arise from human activities, their baseline concentrations in the atmosphere are determined primarily by natural activities (Harper, 1993).

Among others, including wood-rotting fungi (Harper, 1985), volcanic emissions (Rasmussen *et al.*, 1980), coastal salt marshes (Rhew *et al.*, 2000), terrestrial plants (Attieh *et al.*, 1995), soils and sediments (Keppler *et al.*, 2000), the oceans have been identified as one of the main sites for the natural emission of halocarbons (Moore *et al.*, 1996). The main anthropogenic source is through biomass burning (Crutzen & Andreae, 1990), whilst industrial inputs into rivers through evaporation may also act as an important source (Dyrssen *et al.*, 1990).

The natural production of halocarbons may be biotic through either bacterially-mediated production (Manley & Dastoor, 1988; Tait & Moore, 1995), directly from algae (Ekdahl *et al.*, 1998) or by abiotic means through chemical (Vogel *et al.*, 1987) and photochemical (Moore & Zafiriou, 1994) reactions. The actual mechanism of biotic production of halocarbon from algae is not well understood. However, it has been hypothesized that they are secondary waste compounds formed during oxidative stress caused by chemical compounds such as hydrogen peroxide, superoxide and hydroxyl radicals (Pedersen *et al.*, 1996) or they act as a defence mechanism against epiphytism and grazing (Hay, 1992).

1.6. Monohalomethanes

Monohalomethanes, which are represented chemically as CH_3X are low-molecular-weight halocarbons (simplest forms of methyl halides) containing single halide atoms of Cl, Br and I. They are important among the halocarbons because they serve as the primary carriers of halides in the atmosphere (Singh *et al.*, 1983). They are mainly obtained naturally from oceans, volcanic activity and forest fires (Palmer, 1976; Rasmussen *et al.*, 1980). The production of CH_3X in natural water involves several species of macroalgae and microorganisms (Wuosmaa & Hager, 1990; Scarratt & Moore, 1996; Scarratt & Moore, 1998), with microorganisms appearing to be the dominant contributors (Singh *et*

al., 1983). A number of pathways for the synthesis of CH_3X have been identified. These include the following;

(1) Enzymatic synthesis through direct methylation of an iodide ion which then undergoes a spontaneous but slow transformation to yield more stable forms of methyl chloride (CH_3Cl) and methyl bromide (CH_3Br) after the formation of methyl iodide (CH_3I) through nucleophilic substitution reactions (Lovelock *et al.*, 1973; Zafiriou, 1975). These reactions are based on the relative reactivity of the halide ions in seawater shown in equations (1.1), (1.2) and (1.3).

$$CH_3I + Cl^- \rightarrow CH_3Cl + I^-, \quad \textbf{(1.1)}$$

$$CH_3I + Br^- \rightarrow CH_3Br + I^- \text{ or} \quad \textbf{(1.2)}$$

$$CH_3Br + Cl^- \rightarrow CH_3Cl + Br^- \quad \textbf{(1.3)}$$

(2) Synthesis through a haloperoxidase-mediated reaction in the presence of hydrogen peroxide (shown in equation (1.4)) although there has been no published evidence to support this (Neidelman & Geigert, 1983).

$$CH_4 + X^- + H^+ + H_2O_2 \rightarrow CH_3X + 2H_2O. \quad \textbf{(1.4)}$$

(3) Synthesis in marine algae, fungi and halophytic plants through a transmethylation reaction with the aid of S-adenosyl-L-methionine dependent chloride transferase (Harper & Hamilton, 1988; Wuosmaa & Hager, 1990).

(4) Abiotic alkylation of halide ions during the oxidation of organic matter in the presence of electron acceptors such as Fe (III) in soils and sediments (Keppler *et al.*, 2000).

(5) Synthesis through photochemical reactions (Moore & Zafiriou, 1994).

1.6.1. Methyl Iodide

CH_3I has a global annual ocean to atmosphere flux rate of between 0.9-2.5 x 10^9 mol y^{-1} (i.e.128-355 Gg y^{-1}) (Moore & Groszko, 1999) and this makes it one of the major and most dominant of all iodide compounds responsible for the delivery of iodine into the atmosphere from aquatic environments (Rasmussen *et al.*, 1982; Hemann *et al.*, 1987; Moore & Tokarczyk, 1993) as well as the main source to land (Nightingale, 2003). In the aquatic environment, CH_3I is substantially oversaturated in all surface waters (Moore & Groszko, 1999) which results in water-atmospheric flux. In the atmosphere, CH_3I is quickly destroyed through the process of photolysis and therefore has a short lifetime of just typically five days (Zafiriou, 1974). The resulting free iodine is subsequently removed from the atmosphere and deposited through precipitation (Campos *et al.*, 1996a). However, a model by Solomon *et al.* (1994) indicates that, irrespective of its short lifetime; it still has the potential to control the lower stratospheric ozone.

From published work, biological production by algae is considered as the main natural source of CH_3I (Gschwend *et al.*, 1985; Manley & Dastoor, 1987; Manley & Dastoor, 1988) in coastal waters. In the open ocean, phytoplankton are mainly responsible for its production, rather than macroalgae (Klick & Abrahamsson, 1992; Manley & de la Cuesta, 1997; Scarratt & Moore, 1999). However, photochemical production through the interaction of seawater and sunlight has been recognized as the main source in regions of low biological activity, such as the tropical Atlantic Ocean (Moore & Zafiriou, 1994; Nightingale, 2003). Chemical formation in seawater that involves methylcobalamin (Manley, 1994) also contributes to the global budget of CH_3I. In the estuarine environment, high microbial degradation of organic matter may be responsible for its production

The production rate of CH_3I has been found to be about three fold higher in coastal waters than offshore waters (Moore & Zafiriou, 1994) even though it is substantially

supersaturated in all surface ocean waters (Moore & Groszko, 1999). The destruction of CH_3I through nucleophilic substitution reaction involving chloride ions probably serves as its main sink in the ocean (Manley & de la Cuesta, 1997).

1.7. Bacterial Degradation of Methyl Iodide

1.7.1. Process of Bacterial Degradation of Methyl Halides

Bacteria responsible for the removal and degradation of methyl halides in the environment constitute a collective group of bacteria known as methylotrophs (methylotrophic bacteria). They form part of the group of aerobic bacteria that depend on one-carbon compounds which are found in a more reduced state than formic acid as sources of carbon and energy and assimilate formaldehyde as a major source of cellular carbon (Hanson & Hanson, 1996).

The process of methyl halide degradation by the methylotrophs occurs through the employment of a number of possible pathways. The possible mechanism involved in such pathways has been illustrated by Vannelli *et al.* (1998) for CH_3Cl as the carbon source (see Figure 1.3) with each pathway following the general stoichiometry as shown in equation (1.5).

$$CH_3Cl + 3/2O_2 \longrightarrow CO_2 + H_2O + HCl \qquad \textbf{(1.5)}$$

Methylotrophic bacteria are known to be capable of utilizing a variety of one-carbon compounds, which include methane, methanol, methylated amines, methyl halides, and sulphur containing methylated compounds involving the use of certain enzyme systems. A typical enzymatic degradation process is illustrated in a suggested pathway (Figure 1.4) for CH_3Cl utilization (McDonald *et al.*, 2002).

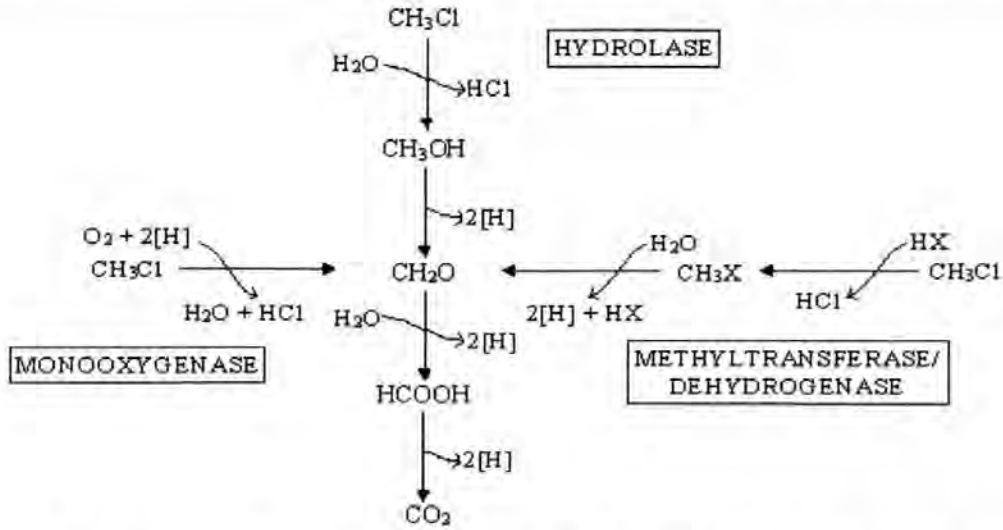

Figure 1.3: A diagram illustrating the possible pathways of methyl halide degradation (redrawn from Vannelli *et al.*, 1998).

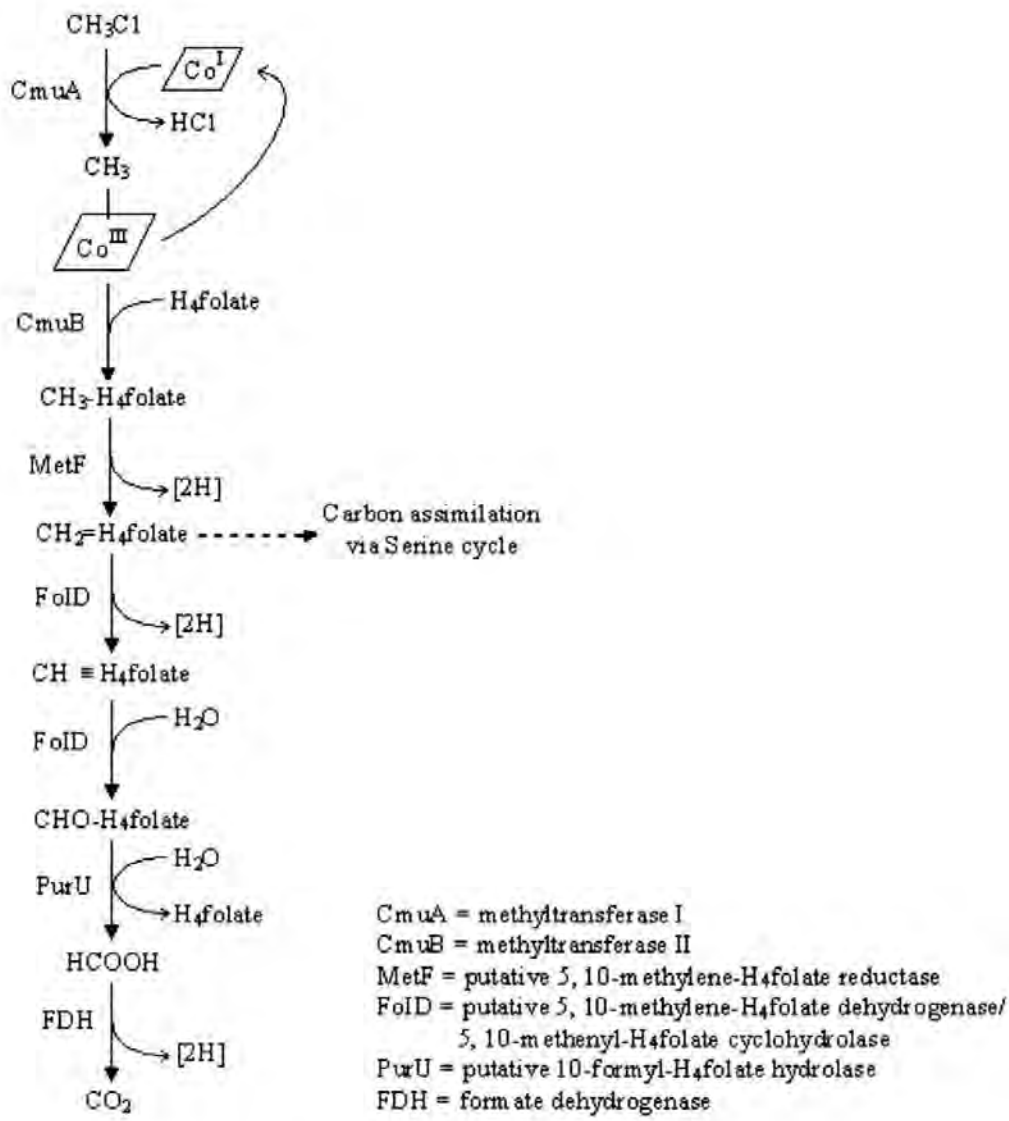

Figure 1.4: A suggested pathway for the enzymatic degradation by methylotrophs (redrawn from McDonald *et al.*, 2002).

1.7.2. Strains of Methyl Halide Degraders

Several strains of these methyl halide-oxidizing bacteria have been found in or isolated from different environments, including the soil (Connell Hancock *et al.*, 1998; Miller *et al.*, 1997), aquatic environments (Goodwin *et al.*, 1997b; Goodwin *et al.*, 1998; King & Saltzman, 1997), and recently from leaf litter in the forest (Coulter *et al.*, 1999).

Besides the mentioned aerobic bacteria, some anaerobic groups of bacteria including acetogens (Magli *et al.*, 1995) and methanogens (Oremland *et al.*, 1994a) have been found to be capable of using methyl halides. According to some authors there is an indication that methyl halides are co-metabolized by methanotrophs (Schaefer & Oremland, 1999), nitrifying bacteria (Stirling & Dalton, 1979; Rasche *et al.*, 1990), and ammonia-oxidizing bacteria (Duddleston *et al.*, 2000; Rasche *et al.*, 1990). This is probably because methanotrophs have significant genetic similarities to methylotrophs. These similarities include the common expression of cofactors that shuttle C_1 units of various oxidation states between enzymes (Christoserdova *et al.*, 1998; Krum & Ensign, 2000; Vannelli *et al.*, 1999; Vorholt *et al.*, 1999). Irrespective of the contribution by these anaerobic bacteria, the majority of methyl halide degraders are aerobic bacteria (McDonald *et al.*, 2002).

In the aquatic environment, bacteria that are capable of growing on methyl halides have not been described in detail even though several terrestrial facultative methylotrophs belonging to the genera *Hyphomicrobium* and *Methylobacterium* (Doronina & Trotsenko, 1997) have been described.

Some strains of aquatic methylotrophs that have been found to degrade methyl halides in natural water include *Methylobacter marinus A45* (Goodwin *et al.*, 1997b), *Methylobacterium sp. CM4* (Vannelli *et al.*, 1999), *Methylomicrobium album BG8* (Han & Semrau, 2000), as well as some ammonia-oxidizing bacteria (Rasche *et al.*, 1990). These

C_1-utilizing methylotrophs therefore represent an important functional group of bacteria in the aquatic environment (Sieburth *et al.*, 1993; Visscher *et al.*, 1992) because they significantly contribute to the regulation of methyl halide flux to the atmosphere.

The process of methyl halide degradation amongst methylotrophs appears to be constitutive at low-level concentrations (Schaefer & Oremland, 1999) but non-constitutive at high concentrations (Schaefer *et al.*, 2002). At low-level concentrations, this observation is supported by the findings obtained by Hines *et al.*, (1998) which indicate that methylotrophs are responsible for all removal of CH_3Br at levels below 1 ppmv.

Although the enzymatic system responsible for the metabolism of methyl halides by aquatic methylotrophs could be highly specific (Schaefer & Oremland, 1999), some bacterial strains have been found to grow on all three methyl halides (Connell Hancock *et al.*, 1998). This suggests that their metabolism is likely to be facilitated by a common enzymatic system (Schaefer & Oremland, 1999). This is manifested in some strains that have the ability to degrade more than one of the methyl halides at the same time. For example, in an experiment conducted by Schaefer *et al.*, (2002), the methylotrophic strain *L. methylohalidivorans MB2*^{T} was able to demonstrate an exponential growth on all three methyl halides. Also, during the degradation of CH_3Cl by the strain *Methylobacterium sp. CM4*, CH_3I was readily dehalogenated in the culture medium (Vannelli *et al.*, 1998). However, in a situation where all three methyl halides are present as the carbon and energy source, the enzymatic degradation of CH_3Br is inhibited by CH_3I and CH_3Cl through competition for a potential enzyme active site (McDonald *et al.*, 2002), with CH_3I as the most potent inhibitor. This inhibition activity also seems to follow the general order of chemical reactivity of the three methyl halides with CH_3I being the most reactive whilst CH_3Cl is the least reactive (Schaefer & Oremland, 1999).

The destruction of methyl halides in the environment is also achieved through abiotic means including chemical and photochemical reactions. For example, nucleophilic substitution with sulphide has been shown to consume CH_3Br in anaerobic sediments producing sulphur gas in the process (Oremland *et al.*, 1994b). These processes such as hydrolysis (Keuning *et al.*, 1985; Elliott & Rowland, 1995) have been found (Miller *et al.*, 2001) to compete with biotic processes of degradation in the environment.

Except in a few cases where CH_3I has been applied alongside other methyl halides to investigate its co-metabolic effect (especially on methylotrophic CH_3Br oxidation), there has been no report that directly addresses the role of methylotrophs in the degradation of CH_3I. In one such experiment (Connell Hancock *et al.*, 1998), there occurred an accumulation of iodide in the experimental medium when CH_3I was used as an alternative electron donor and carbon source. This consequently resulted in a well-pronounced growth of the methylotrophs. The graph indicating this growth is shown in Figure 1.5.

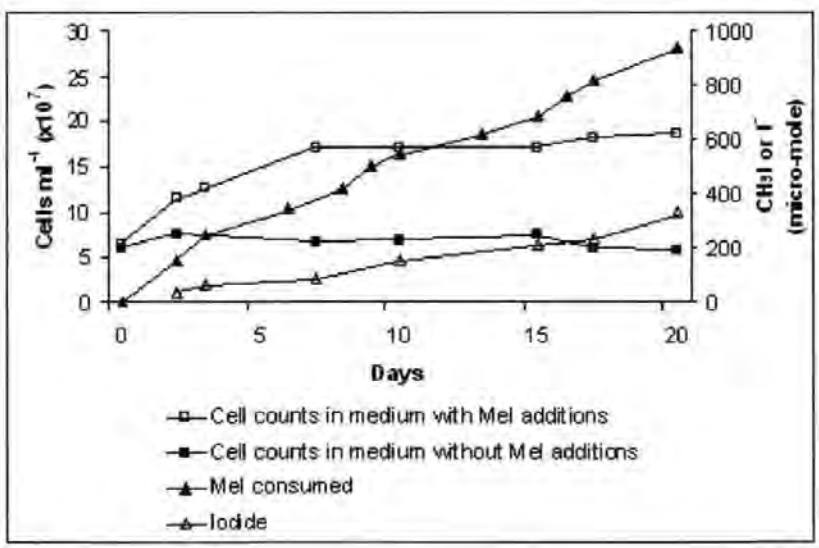

Figure 1.5: A graph showing a typical growth pattern of methylotrophs (e.g. strain IMB-1) on CH_3I (redrawn from Connell Hancock *et al.*, 1998).

Schaefer *et al.* (2002) also reported a consumption of CH_3I during their experiment involving the methylotroph *MB2*^{T}. The use of CH_3I alongside CH_3Br and CH_3Cl as a source of carbon and energy was described by Schaefer & Oremland (1999). Therefore evidence exists of CH_3I degradation by bacteria in the aquatic environment. Bacterial CH_3I removal mentioned in this thesis henceforth refers to the total amount of CH_3I utilized by bacteria that end up in their cells and as CO_2 through their oxidation of CH_3I.

CH_3I is an important compound in the cycling of iodine in the environment. Information regarding the role of CH_3I in the description of the global iodine cycle has been limited to the marine environment. This is because no published data supporting and explaining estuarine cycling of CH_3I was found. Therefore there is a crucial gap in our knowledge of the role estuarine processes play in the global iodine cycle especially with regards to microbially-mediated production and removal of CH_3I.

From the literature reviewed in this chapter, a number of uncertainties have resulted in the following questions;

1. Is an estuary a source or sink of atmospheric CH_3I?

2. Does bacterial-aggregate relationship in the estuarine environment facilitate the production of CH_3I?

3. Can methylotrophic bacterial activity result in the removal of CH_3I in the estuarine environment?

4. How do estuarine physico-chemical properties influence the bacterial processes that facilitate the production or consumption of CH_3I in the estuarine environment?

1.8. Aims and Objectives

Based on the questions that arose from the literature review, the following aims and objectives have been formulated for the present study;

1. To investigate and estimate the methyl iodide (CH_3I) production from estuarine particle aggregates through controlled laboratory experiments and the contribution of the produced CH_3I to water-air CH_3I flux.

2. To assess the possible role of the microbial community associated with particle aggregates in CH_3I production and evaluate the effect of the aggregation process on the rate of change of CH_3I concentration in estuarine water.

3. To develop a method that could be effectively used to investigate and assay the process of CH_3I removal by methylotrophic bacteria.

4. To investigate CH_3I removal by methylotrophic bacteria in an experimental medium.

5. To investigate the spatial and seasonal variations in CH_3I concentration and the rate of bacterial CH_3I removal with reference to key physical, chemical and microbial variables along the Tamar estuary.

To investigate the above aims and objectives, an estuarine system was considered because as discussed in section 1.2 of this chapter, estuarine systems with their high biodiversity and with the prevalence of a wide range of physico-chemical conditions play a very important role in the biogeochemical processes of the environment. In addition, high concentration of POM and DOM in estuaries compared to other aquatic systems (e.g. marine systems) reflects in a relatively higher microbial activity. This makes estuaries important systems for the study of microbial processes that facilitate the cycling of ecologically important gases and compounds. The Tamar estuary has been chosen for this study because it is one of the most extensively studied estuarine systems in the United Kingdom. It exhibits a strong TMZ with high suspended matter concentration (Uncles &

Stephens, 1989; Uncles & Stephens, 1993b) that provides potentially a suitable substrate for microbial activity. Also the Tamar estuary has a close proximity to research establishments (University of Plymouth, Plymouth Marine Laboratory, and Marine Biological Association). A description of the Tamar estuary has been provided in section 5.3 of Chapter Five.

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1. Introduction

In order to address the different aims and objectives set out in Chapter One, the application of various methods and procedures are required. In this respect, published techniques that have already been applied in similar fields by other researchers were adapted and used for the present study. This chapter details all the methods and techniques employed.

2.2. Preparation of Chemicals

All analytical procedures employed in this investigation involved the use of a number of chemicals. A complete list of all chemicals and their preparation has been provided (refer to Appendix 1 and 2). In addition to the list, a table highlighting the purpose of each chemical in the analytical procedures has also been given (see Appendix 1).

2.3. Applied Analytical Methods

2.3.1. Gas Chromatography (GC) – Methyl Iodide Detection

2.3.1.1. Principles of Gas Chromatography

Dissolved gaseous compounds in natural water samples maybe analysed by gas chromatographic systems (GC) equipped with appropriate detectors. A GC fitted with an electron capture detector (ECD) was used in the present study to determine methyl iodide (CH_3I) concentrations in estuarine or seawater samples using the methods described by Nightingale (1991) and Krysell & Nightingale (1994). The procedure required the following steps; stripping and trapping, injection, separation, detection and computation (See Figure 2.1).

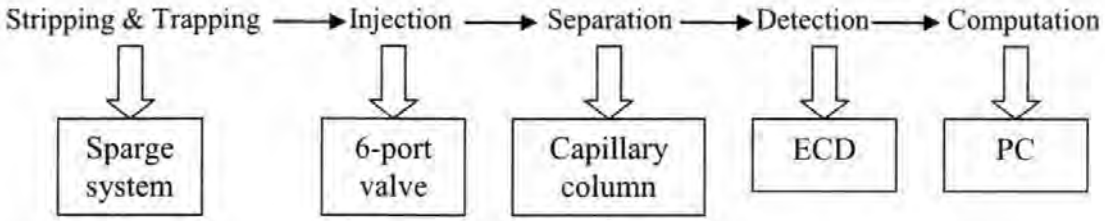

Figure 2.1: A flow diagram illustrating the sequence of analytical stages involved using a GC.

These steps were accomplished with the use of carrier and make-up gases, helium (He) and nitrogen (N_2) respectively. The system (shown in Figure 2.2) used for the analysis of CH_3I was a GC system (Shimadzu GC-14A) fitted with a ^{63}Ni ECD and a zebron GC capillary column (Phenomex ZB-624) of length: 75m, ID: 0.53mm and df: 3.00μm designed for the separation of volatile organic compounds (VOCs). The column has a liquid stationary phase (cyanopropyl-phenyl-dimethyl-polysiloxane) which is adsorbed onto the surface of an inert solid. The ECD consists of a sealed stainless steel cylinder containing radioactive Nickel-63. The Nickel-63 emits beta particles (electrons) which collide with the make-up gas molecules (N_2), ionizing them in the process. This forms a stable cloud of free electrons in the ECD cell. When electro-negative compounds enter the cell, they immediately combine with some of the free electrons, temporarily reducing the number remaining in the electron cloud. The resulting change in current is recorded as an electronic signal by a computer.

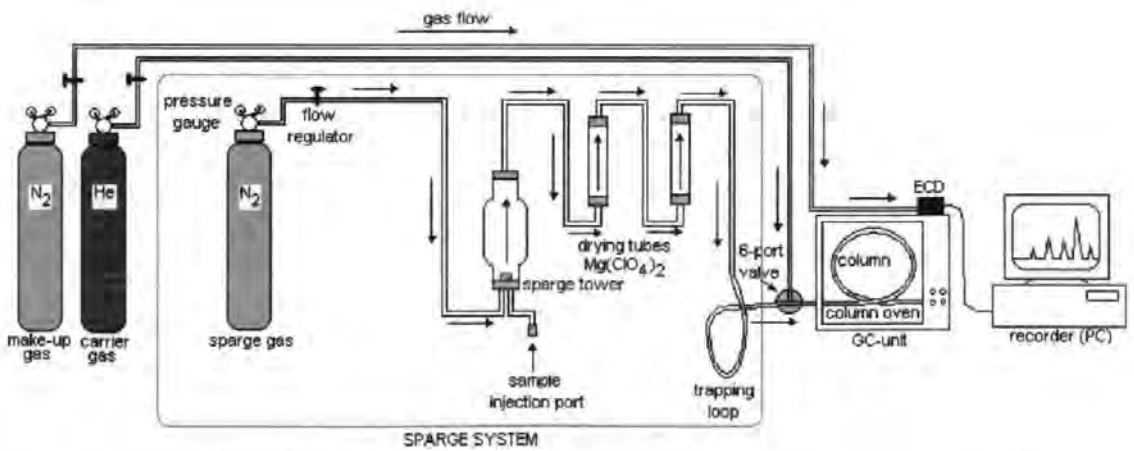

Figure 2.2: A schematic diagram of the gas chromatograph setup used in this study.

Analysis involved the following: (i) Sample vapourisation; (ii) injection into the separation column; (iii) transport through the column by He gas; (iv) separation of the mixture by the capillary column; (v) detection of CH_3I by the ECD.

2.3.1.2. Cryogenic focusing

The process of cryogenic focusing is necessary for the determination of VOCs in order to achieve distinctive separation of the compounds as well as optimizing the recovery of the introduced compounds. It is a process whereby a mixture of volatile compounds is trapped in a loop at a very low temperature (-130°C) before being introduced into the separation column. Since CH_3I in water samples is in the dissolved state, it needs to be 'stripped off' from solution into the gaseous state before cryogenic focusing may be applied. This was achieved with the aid of a sparging system (see Figure 2.2) which comprises of a compressed gas, sparging tower, drying tubes and a trapping loop.

For the present study, a known volume (typically 10 mL) of the water sample to be analysed was injected into the sparging tower having a sparge gas (N_2) delivery rate of 60 mL min^{-1}. For 20 minutes, the dissolved gases were forced to volatilize. The stripped gases were then passed through a series of two drying tubes filled with magnesium perchlorate ($Mg(ClO_4)_2$). The gas mixture finally entered the trapping loop where trapping occurred through freezing at a temperature of between -130°C and -150°C with the aid of liquid nitrogen.

2.3.1.3. Injection and analysis

After freeze-trapping of gaseous compounds, the samples were then injected into the GC column from the sparging system. This was facilitated by a 6-port valve that allows for

flow direction and redirection of sparge gas and carrier gas through the trapping loop and into the separation column.

The trapped samples were injected into the capillary column by the carrier gas (delivery rate of approximately 5 mL min^{-1}) which acts as the mobile phase. To achieve this, the trapped samples were rapidly heated in the trapping loop using a hot water bath (95-100 °C). The volatile mixture of compounds then traveled along the separation column inside the oven which had been pre-programmed to operate between initial and final temperatures of 70 and 90 °C, respectively.

Separation occurred as a result of the differences in solubilities and affinities of the various compounds for the stationary phase of the separation column. Owing to different retention times of the compounds, separation occurred and subsequently they entered into the ECD, which was set at a temperature of 320 °C. The detector responses to the compounds were recorded by the computer software (ChromPerfect) as analogue signals in the form of peaks. Peak areas were computed by the software for each compound presented, which were then used to quantify their amounts. Figure 2.3 presents a typical chromatogram with CH_3I as the compound of interest.

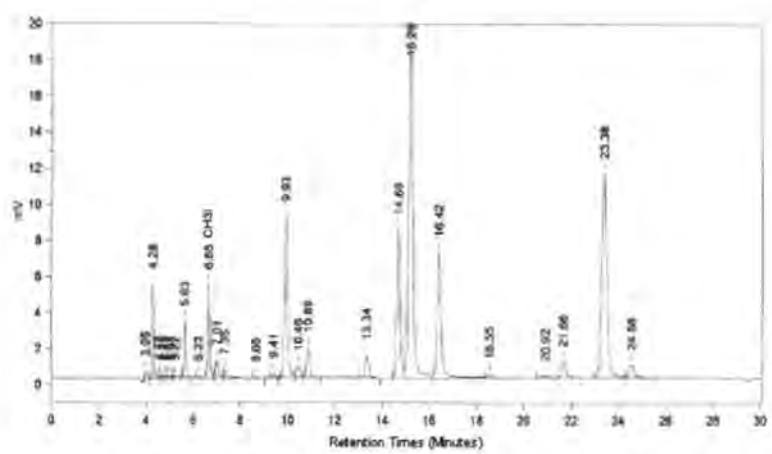

Figure 2.3: A typical chromatogram showing different peaks representing different compounds in a Tamar estuary water sample (Station 4, salinity of 34.9; sample collected on 12th July 2005) with varying retention times (minutes). CH_3I peak appears on the chromatogram at 6.65 minutes.

2.3.1.4. Data Processing

The amount of CH_3I in each injected water sample was estimated from the generated peak area value by the ChromPerfect software using the linear equation of a calibration curve for CH_3I.

The amount of CH_3I present in each replicate water sample was estimated by inserting the peak areas into the linear equation of the form $y = ax + b$ for the CH_3I calibration curve.

Where y = peak area and x = amount.

The mass concentration (ng L^{-1}) of CH_3I was subsequently calculated based on the volume of water sample analysed. This was then converted into a concentration (pmol L^{-1}).

2.3.2. Bacterial Production

2.3.2.1. Principles of the method

Bacterial production has been quantified by the rate of protein synthesis. This may be measured by the rate of incorporation of radiolabelled leucine or thymidine into bacterial protein (e.g. Fuhrman & Azam, 1982; Chin-Leo & Kirchman, 1988; Smits & Reimann, 1988). However the simplest, most economical and efficient method is the one described by Smith & Azam (1992). In place of filtration, as applied in other methods, this method employs the use of centrifugation in the separation of ^{3}H-leucine incorporated protein from the unincorporated label. The procedure required the following stages: (i) incubation; (ii) extraction and counting; (iii) data processing.

2.3.2.2. Incubation

Incubated samples comprised of three live replicates plus two tri-chloroacetic acid (TCA)-killed replicate controls taken from each water sample. Incubation was carried out in pre-labelled 2 mL capacity microcentrifuge tubes. An adjustable-volume pipette (Eppendorf) was used to pipette 89 µl of TCA (100%) into all control sample tubes in order to terminate bacterial activity. This was followed by the addition of 1.7 mL of the water sample. To each sample tube, 5 µl of L-[4, 5-^{3}H] leucine (specific activity of around 5 TBq mmol^{-1} stored at 4 °C), was added to achieve a final sample concentration of 20 nmol L^{-1}. The choice of concentration was based on the determination of the minimum concentration that could productively be used for bacterial incubation without generating excessive waste whilst reducing cost (see Appendix 3 and 4). The caps of the microcentrifuge tubes were then screwed on and the samples thoroughly mixed using a Vortex Genie 2 (Scientific Industries).

The tubes containing the samples were then placed in a Thermo-Tote Incubator (Scientific Device Lab Inc.) and incubated for approximately 1 hour (a statistically optimized duration, refer to Section 2.5.2 for details) at the *in situ* temperature of the samples. After 1 hour, 89 µl of 100% TCA solution was added to all samples to terminate bacterial uptake of the substrate. All samples were then mixed and left on the bench for 30 minutes at room temperature before proceeding with extraction.

2.3.2.3. Extraction and counting

The extraction procedure involved the separation of ^{3}H-leucine incorporated bacterial protein from unincorporated leucine labels. The samples were placed in a centrifuge (Sorvall RMC 14) and then centrifuged at 12000 rpm for 10 minutes. The centrifuged

samples were then aspirated in a fume cupboard (all waste was kept in a capped bottle for proper disposal).

This was followed by washing any remaining unincorporated leucine labels with the addition of 1.5 mL TCA (5%) and vortex mixing. The samples were then centrifuged and aspirated again as described above. Finally 1 mL scintillation cocktail was added (Wallac OptiPhase 'HiSafe'3) directly to the microcentrifuge tubes and then stored in the dark to await counting. Tritium counting (^{3}H-leucine) was conducted by placing the microcentrifuge tubes into scintillation vials and loading them into an automated Wallac liquid scintillation counter (LSC) for radioassaying. Counts per minute (CPM) were measured over a 3 minute period. CPM were converted to disintegrations per minute (DPM) using the internal standards method.

Conversion of CPM to DPM takes into account chemical quench, colour quench and the type of cocktail during the calculation of the final activity values. This conversion may also include corrections for chemiluminescence and half-life where necessary.

$$\text{DPM} = \frac{x}{e} = \frac{(y/t)}{e} \qquad \textbf{(2.1)}$$

Where x = count rate (CPM)
e = counting efficiency
y = number of counts (collected during time t)
t = counting time (minutes)

The counting efficiency is a function of two quench indices (total quench level and colour quench level) provided by the instrument.

2.3.2.4. Data Processing

DPM of tritium were automatically generated by the counter with the software 1414 WinSpectral (Wallac). The raw data were further processed by loading them onto a spreadsheet (Microsoft Excel). Leucine incorporation rate (pmol L^{-1} h^{-1}) was estimated by taking into account the volume of sample, specific activity (Ci $pmoL^{-1}$), decay factor of leucine, length of incubation, and dilution factor, in addition to DPMs from the control replicates.

DPM of each replicate was calculated by subtracting the mean DPM of the control sub-replicates from the DPM of each of the live sub-replicates. Uptake (pmol) by bacteria was estimated by converting DPMs into Curies (1 Curie = 2.22×10^{12} DPM) and dividing by the ratio of the specific activity and decay factor of tritium. Incorporation rate (pmol L^{-1} h^{-1}) was then estimated by the relationship between the uptake, incubation period, sample volume and the dilution factor:

$$\text{Incorporation Rate (pmol } L^{-1} h^{-1}) = \frac{\text{Uptake Rate (pmol } h^{-1})}{\text{Sample Vol. (1 L)}} \quad \textbf{(2.2)}$$

$$\text{Uptake Rate (pmol } h^{-1}) = \frac{\text{Uptake (pmol)}}{\text{Incubation duration (h)}} \quad \textbf{(2.3)}$$

$$\text{Uptake (pmol)} = \frac{(\text{DPM} - \text{Control})/2.22 \times 10^{12}}{\text{Sp. Activity (Ci pmol}^{-1})} \quad \textbf{(2.4)}$$

Where 2.22×10^{12} is a conversion factor

2.3.3. Bacterial Enumeration

2.3.3.1. Description of method

In this study, the technique of analytical flow cytometry (AFC) was applied in the enumeration of bacteria in water samples. This is a modern generic technique for optically analyzing and quantifying particles suspended in a medium (Shapiro, 1995). The analysis is multi-parametric providing data relative to five parameters; forward and side angles of detection, and three channels (blue, green and red) measuring fluorescence properties as a result of light scattering by particles present. This therefore requires the particles to fluoresce and this is achieved by staining the samples with a fluorescent chemical (e.g. Zubkov *et al.*, 2000b; Tarran *et al.*, 2001; Zubkov *et al.*, 2001; Duhamel & Jacquet, 2005).

Typically for bacterial enumeration analysis, the fluorescent chemical used is SYBR Green I stain. SYBR Green I stain is a highly sensitive fluorescent double stranded stain dissolved in dimethylsulphoxide (DMSO) that binds to the nucleic acid of the bacteria in the water sample. Its fluorescent signal is greatly enhanced when it becomes bound to nucleic acids, thereby exhibiting an excellent signal-to-noise ratio with no background fluorescence. The method of SYBR Green I application is explained in more detail by Marie *et al.* (1997).

2.3.3.2. Sonication

During the study, the process of sonication was applied. This was to dislodge any particle-attached bacteria whilst avoiding the destruction of the cells (Turley & Hughes, 1992). Prior to sonication, replicate water samples for bacterial enumeration were fixed in 20% formaldehyde solution and stored at 4 ^{o}C in the dark. Samples for the estimation of total bacterial numbers were ultra-sonicated on ice with a vibra cell sonicator (Sonics) in

disposable centrifuge tubes (Corning) programmed at a temperature of 15 °C, amplitude of 19% and a pulse rate of 1 second on, 1 second off prior to analysis. In some sets of samples, both sonicated and un-sonicated samples were analysed for bacterial cell numbers to give total (attached + free-living) and free-living bacteria respectively. Particle-attached bacterial numbers were estimated by the difference. During the aggregation experiment (See Chapter Three), sonication was applied only in samples used in Study Two.

2.3.3.3. Staining and counting

Staining of the replicate samples were conducted using a 1% SYBR Green I stain while counting was achieved using a FACSort flow cytometer (Becton Dickinson).

500 µL of the water sample was pipetted into a pre-labelled 5 mL polystyrene round-bottom tube (Becton-Dickinson). Then 45 µL of 10% tri-potassium citrate solution was added to each of three replicates in order to improve both the staining quality and the stability of SYBR fluorescence. This was immediately followed by the addition of 5 µL of 1% SYBR green I stain.

The samples were then vortex mixed and kept in the dark at room temperature for 1 hour for the bacterial cells to absorb the stain. Each replicate was subsequently analysed for bacterial cell numbers by running it through the flow cytometer. Due to the presence of a high concentration of particles in general and particularly bacteria in water samples from the Tamar estuary, minimum flow rate and analysis duration was used. The flow rate used was typically 15 µL min^{-1}, which was operated at a sample voltage of 6.25V and analysed for 1 minute in order to improve the resolution and accuracy of the count whilst preventing the creation of very large files for each count (see Figure 2.4a-b).

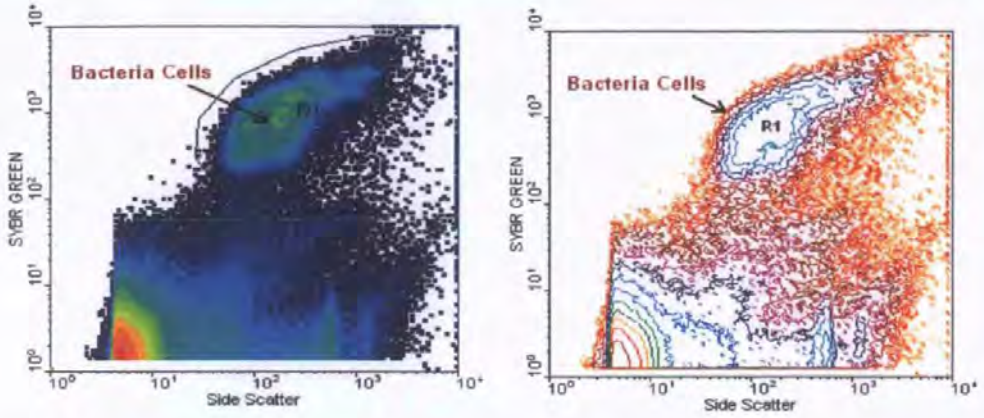

Figure 2.4a-b: A typical plot showing bacterial enumeration of a water sample from the Tamar estuary (Station 4, salinity of 34.9 on 12th July 2005), using WinMIDI. 2.4a is a density plot and 2.4b is a contour plot of the same sample analysed.

2.3.3.4. Bacterial enumeration by epifluorescent microscopy (EFM)

In order to ascertain the reliability of the AFC method in the enumeration of bacteria in water samples, a second application method by epifluorescent microscopy (EFM) was employed for comparison. The method of EFM is a relatively simple technique used to estimate bacterial abundance in water samples after retention of bacteria on black membrane filters (Hobbie *et al.*, 1977; McFeters *et al.*, 1999). The method involves staining bacteria cells with the fluorescent dye 4',6-Diamidino-2-phenylindole (DAPI) and directly counting using a microscope equipped with a fluorescent lamp capable of illuminating the fluorescing cells against the black membrane filter background.

Due to the high concentration of bacterial cells in estuarine systems, water samples were generally diluted to a desirable ratio (1:1000). Replicate water samples of 10 mL each (using diluted sample) were pipetted into sample vials and stained with 150 µL of 15 µg mL^{-1} DAPI and incubated in the dark for 15 minutes. The samples were then vacuum-filtered onto a nuclepore polycarbonate filter (Whatman; 0.2 µm pore size, 25 mm Ø) placed on top of a 1 µm filter (to prevent break in the 0.2 µm filter) at a pressure of 5 mmHg. After the filtration, the top 0.2 µm filter (with sample) was removed and placed on

a glass slide. 30 µL of antifade fluid was pipetted onto the filter and a cover slide was placed on top. The samples were then counted using a Nikon epifluorescent microscope equipped with 100W mercury light, x10 oculars and Nikon filter cube (with total magnification of x400). Bacterial abundance was estimated with the following equation.

$$\text{Bacterial Abundance (cells mL}^{-1}) = Xb \times Cf \times \frac{1}{V} \quad \textbf{(2.5)}$$

Where Xb = Mean number of bacteria per field of view

V = Initial vol. of sample (mL) used before dilution

$$Cf = \text{Conversion factor} = \frac{\text{Area of filtration}}{\text{Area of field of view}} \quad \textbf{(2.6)}$$

2.3.3.5. Data Processing

Data for bacterial numbers (flow cytometry) were generated by the software CellQuest. The actual bacterial numbers (cells L^{-1}) were estimated taking into consideration the flow rate of the system, duration of analysis and the dilution factor. As a result of fluctuations in flow rate, the actual flow rate of the system for each set of samples to be analysed was estimated by an initial run of a flow-set fluorospheres (Beckman Coulter) of known concentration used as a standard.

150 µL of flow-set fluorospheres (beads) was removed using a pipette into a polystyrene tube. 1.3 mL of Milli-Q water was immediately added to the tube containing the beads and vortex mixed to achieve a dilution factor of ten.

Three replicates of the diluted beads were passed through the flow cytometer as already described. The flow rate of the system was then estimated using bead numbers obtained

from CellQuest and the dilution factor. Cell numbers for all samples were estimated using the resulting flow rate.

2.3.4. Bacterial methyl iodide breakdown

2.3.4.1. Description of method

Bacteria are known to be capable of using a wide range of organic compounds as a carbon source either for protein synthesis or for the generation of energy. The analytical procedure used involved the investigation of CH_3I as a substrate for bacterial activity. It therefore provided the methodology for assaying the rate of CH_3I breakdown. Similar work based on bacterial degradation of other methyl halides has already been carried out (e.g. Goodwin *et al.*, 1997b; Goodwin *et al.*, 1998; Schaefer & Oremland, 1999; Schaefer *et al.*, 2002).

The radioassaying using labelled $^{14}CH_3I$ was based on an adaptation of analytical methods developed by Connell *et al.*, (1997) and Smith & Azam (1992). The method requires the ability to estimate the quantity of CH_3I in a sample that is either incorporated or oxidized by bacteria. A full account on the development and the application of this analytical procedure has been provided in Chapters Four and Five.

2.3.4.2. Incubation

The incubation procedure employed here generally follows the same principles explained in Section 2.3.2.2. Three replicates of estuarine samples and two replicate controls were used. Termination of bacterial activity in the control samples was, however, achieved through autoclaving. Typically, a final $^{14}CH_3I$ concentration of 300 nmol L^{-1} per replicate was used and incubated at *in situ* temperature for 45 minutes in pre-labelled 2 mL capacity microcentrifuge tubes placed in a Thermo-Tote Incubator (Scientific Device Lab Inc.).

2.3.4.3. Extraction and counting

The process of extraction here differed from that outlined in Section 2.3.2.3. It comprised of two separate stages which involve the separation of $^{14}CH_3I$ incorporated into bacterial protein from unincorporated $^{14}CH_3I$ label. The unincorporated fraction of the substrate was subsequently separated into oxidized and completely unused fractions.

To separate $^{14}CH_3I$ incorporated into bacterial protein from unincorporated label, the samples were centrifuged at 12000 rpm for 10 minutes and the supernatant aspirated into new labelled microcentrifuge tubes for the next stage of separation. Oxidized $^{14}CH_3I$ in the form of $^{14}CO_2$ gas dissolved in the supernatant was separated from the unused substrate through the process of precipitation by the addition of sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) and strontium chloride hexahydrate ($SrCl_2.6H_2O$) to the supernatant. This batch of sub-samples was also centrifuged and the remaining supernatant (containing the unused substrate) aspirated. All samples were washed with Milli-Q to remove excess substrate present. 1 mL of scintillation cocktail was then added to all samples and then counted for carbon 14 disintegration in a scintillation counter. For more details refer to Chapter Four.

2.4. Field Data Collection

Measurements of physico-chemical parameters from the field can be helpful in the interpretation of trends and variations in biological activities that are observed in the natural environment. Most of these parameters were measured directly in the field with the aid of electronic probes. These field measurements were taken together with water sampling from the research boat MBA Sepia at four locations (Morwellham, Tuckermarsh, Cargreen and The Narrows) along the Tamar Estuary.

2.4.1. Collection of water samples

Water samples for laboratory work during this study were collected from four locations along the Tamar estuary during each sampling date using stainless steel bucket. Information regarding the description of the Tamar estuary, the sampling locations and the protocol of sampling has been provided in Chapter Five.

2.4.2. Temperature

Surface water temperature readings (<1m deep) were made with a Multiparameter Water Quality Monitor 6600-M (YSI Inc.) from all selected sampling locations. In addition, some readings were made from a continuous flow of water pumped onboard along the Tamar. Guidelines for the calibration of all probes of the instrument were obtained from YSI Inc.

2.4.3. Salinity

Salinity measurement at the selected sampling locations along the Tamar was also achieved using the Multiparameter Water Quality Monitor 6600-M (YSI Inc.). Surface water samples at each location were collected with a bucket and the probe immersed in the samples to allow salinity readings to be recorded.

The YSI probe was calibrated using 10 mg L^{-1} and 1000 mg L^{-1} chloride standard solutions. A 3-point calibration with two portions of 10 mg L^{-1} and one portion of 1000 mg L^{-1} solutions was performed. The first point calibration began with the sensors of the probe carefully immersed in 300 mL of the 1000 mg L^{-1} chloride standard solution (at ambient temperature). About 1 minute was allowed to elapse for temperature equilibration and 'Chloride' selected from the Calibration Menu to continue. '3-3-Point' was subsequently selected and the concentration value of the solution (1000 mg L^{-1}) entered. The sensors were allowed to stabilize and then continued to the second calibration with 300 mL of 10 mg L^{-1} (at ambient temperature). The second point calibration proceeded in the same

manner as the first. After the second point calibration was completed by entering the concentration value of 10 mg L^{-1}, the probe was immersed in 300 mL of chilled (<10°C) 10 mg L^{-1} chloride standard solution for the third point calibration. 5 minutes was allowed to elapse in this case for temperature equilibration before proceeding. The concentration value of 10 mg L^{-1} was also entered and the sensors allowed to stabilize in the solution. After there was no change in the chloride reading, the 'Enter' button was depressed to complete the third and final part of the calibration.

2.4.4. pH

The measurement of pH at the sampling location along the Tamar was carried out using the Multiparameter Water Quality Monitor 6600-M (YSI Inc.) in the same manner described in Section 2.4.1 and 2.4.2.

To enable the YSI probe to accurately measure pH, a 2-point calibration was conducted. pH 7 and pH 10 buffer standards were used. 300 mL of pH 7 buffer standard was placed in a clean calibration cup and the sensors of the probe carefully immersed into the solution. Temperature was allowed to equilibrate for 1 minute before selecting 'ISE1 pH' from the Calibration Menu to access the pH calibration choices. This was followed by the selection of '2-2-Point' and the value of the pH buffer (7) entered at the prompt. All sensors were allowed to stabilize in the solution. When the pH reading indicated no significant change for about 30 seconds the 'Enter' button was depressed. After pH 7 calibration was completed, the process was continued with 300 mL of a pH 10 buffer standard solution in exactly the same way as the first part of the calibration. With all calibrations on the instrument completed, the probe was thoroughly rinsed with Milli Q water.

2.4.5. Dissolved Oxygen

In situ dissolved oxygen (DO) measurement of water samples were taken using a DO meter H19142 (Hanna Instruments). The probe of the meter (due to the small size of the DO meter) was immersed in a bucket of surface water sample from each sampling station and gently swirled to allow a continuous flow of water across the membrane of the probe. DO readings were taken (while still swirling) after the readout had stabilized. Guidelines for the calibration of the meter were obtained from Hanna Instruments. Through rapid saturation from the atmosphere, this approach could result in over-estimation of DO concentration. To minimize the interference of oxygen saturation from the atmosphere, DO measurements were carried out as quickly as possible after collection of the water samples. Readings are also taken quickly (10-15 seconds) after the probe is immersed in the water. In addition DO concentration readings obtained from this approach were limited only to the use as a guide in understanding DO distribution in the estuary.

Before the DO meter was calibrated, the membrane of the probe was filled with electrolyte and the probe was connected to the meter. For accurate calibration, the meter was initially switched on for 15 minutes to condition the probe. The protective cap of the probe was then removed to commence the calibration procedure. There were 2 parts to the calibration of the probe; Zero Calibration and Slope Calibration.

For zero calibration, the probe was dipped into HI 7040 zero oxygen solution (Hanna Instruments) and swirled gently for approximately 3 minutes. The LCD readout was then allowed 2 minutes to stabilize and the zero D.O. calibration trimmer adjusted until the display read zero.

The slope calibration was performed in air. The probe was rinsed using copious amounts of clean water to remove any residual zero oxygen solution. The tip of the probe was then

dried and a few minutes were allowed for the readout to stabilize. The CAL button was pressed and the slope trimmer adjusted to read 100% while still holding the CAL button. This completed the calibration procedure of the instrument.

2.4.6. Suspended Particulate Matter (SPM) analysis

Water samples from the various sampling locations were analysed in the laboratory to obtain the dry weight of the SPM. The analysis was conducted as follows;

Pre-ashed (450 °C, 6 hours) glass microfibre filters (Whatman GF/C 55 mm Ø) kept in a desiccator were weighed with an electronic balance prior to use. The filters were then mounted on a filtration apparatus operated using a vacuum pump. 250 mL of homogenized sample water from each sampling station was measured with a measuring cylinder and then filtered. Salt residue in each sample was removed by rinsing the sample with Milli Q.

The filtered SPM together with the filter papers were dried in the oven overnight at 40 °C. The dried filter papers were then repeatedly weighed and returned to the oven to continue drying while recording the values each time they were weighed until there was no change in weight. The dry weight of SPM from each location was then estimated per unit volume of sample water from the final weight of the filters.

2.5. Analytical Performance

In order to ensure that the analytical techniques being used were reproducible and accurate, several studies were conducted to evaluate the analytical performance of the applied techniques.

2.5.1. Gas Chromatography (GC)

The analytical technique involving the use of a GC system is prone to several analytical errors (over a period of time). The reliability of the analytical results of a GC system depends primarily on the extent of the system response to the compound being analysed and changes in its sensitivity over time. To define the analytical performance of the GC, a number of studies were conducted. These include defining the linearity of the instrumental response, precision, stability of the detection system and standard solution, intercalibration of analytical systems (i.e. assessment of accuracy), blanks and detection limit, and comparison with an internal standard.

2.5.1.1. Linearity of instrumental response

The calibration of the GC system during the various stages of the analytical work was achieved using separately prepared CH_3I standards.

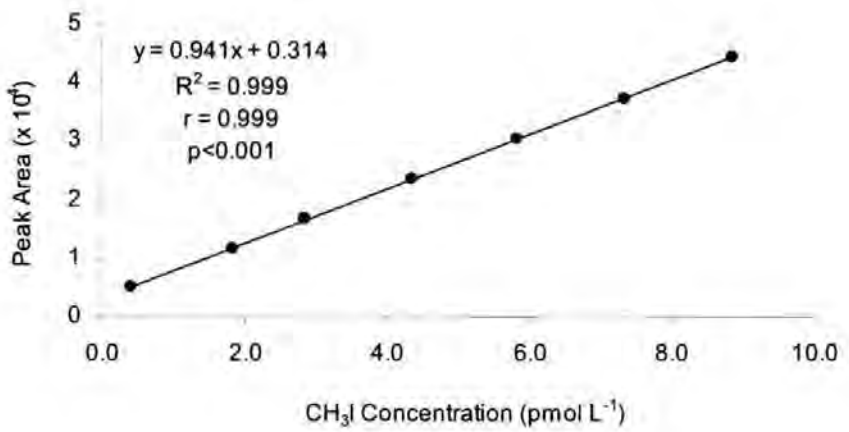

Figure 2.5: A typical calibration curve (2003) obtained from analysed standard used for the estimation of CH_3I concentration in water samples from L4 and sampling stations along the Tamar estuary.

Typical CH_3I standards ranging up to 8.7 pmol L^{-1} were used for all calibrations during the course of the study. These were prepared from 99.9% w/v pure CH_3I standard (Sigma-Aldrich). Figure 2.5 shows an example of a calibration line generated from the analysis of

a series of CH_3I standard solutions. The line represents the system calibration obtained and used in the quantification of CH_3I present in samples during an aggregation experiment (See Chapter Three). With a correlation coefficient of 0.999 and p-value<0.001 there is a strong linearity extending from the instrumental response of the minimum standard concentration to the highest standard concentration used in this calibration. With respect to other calibration analyses made during the course of the study, good linearity similar to that shown in Figure 2.5 was obtained and was adequate for all sample analyses (with r > 0.9).

2.5.1.2. Precision

Table 2.1: Typical precision of replica analysis of seawater (n = 4) at station L4 of Plymouth. (30/07/03).

Water replicates	Volume (mL)	Peak Area	CH_3I amount (pg)	Concentration. (pmol L^{-1})
1	10	40330.5	11.21	7.90
2	10	40767.4	11.34	7.99
3	10	40971.9	11.41	8.04
4	10	40690.3	11.32	7.98
Mean				**7.98**
sd				**0.06**
Precision				**0.71%**

The purpose of GC calibration is not only to aid in the quantification of compounds of interest but also to acknowledge its sensitivity, precision and hence reliability during periods of operation. The analysis of 3 replicate water samples for CH_3I concentration was therefore carried out prior to/during the aggregation experiment in 2003 (analytical precisions ranging between 0.1-11%). Typical results obtained (See Table 2.1) with 4 replicate water samples from L4 (30^{th} July, 2003) indicated excellent analytical precision (< 1%). The mean concentration of CH_3I of the L4 samples was also estimated as being

7.98 ± 0.06 pmol L^{-1} which is consistent with values presented in the literature (refer to Table 2.3).

2.5.1.3. Stability of detection system and standard solution

A study was carried out in order to assess (i) how stable the instrumental sensitivity was over a period of time and (ii) how long the CH_3I standards could be stored after preparation without affecting the overall analytical accuracy. A volume of prepared CH_3I standard solution containing an estimated amount (7.2 pg) was analysed over a 4-day period.

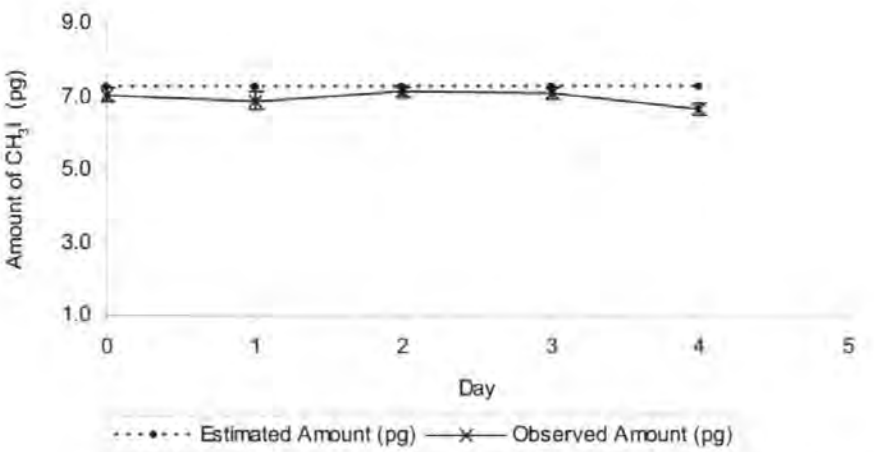

Figure 2.6: A graph showing the stability of CH_3I standard solution over a 4-day period (18^{th} – 22^{nd} August 2003).

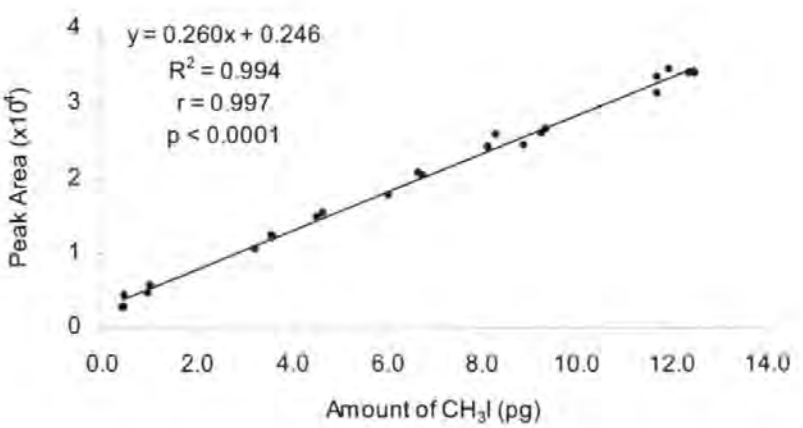

Figure 2.7: A graph showing a linearity of CH_3I standard solution prepared and analysed on each day over a 5-day period (18^{th} – 22^{nd} August 2003) to illustrate stability of the instrumental detector's sensitivity.

Based on a student's *t* test, the observed amount of CH_3I on the fourth day was statistically different ($p < 0.001$, $df = 4$) from the estimated amount. Therefore from the results (Figure 2.6), a CH_3I standard was stable for a period of at most 3 days. Beyond this period, the observed amount declined making the standard unreliable.

The analyses of CH_3I standards were conducted over a 5-day period (fresh standard prepared each day) to monitor the stability of the sensitivity of the instrumental detector. The overall result of these analyses (a line between amount of standard and the observed peak) has been presented in Figure 2.7. The gradients of the curves for these plots (18^{th} - 22^{nd} August 2003) ranged between 0.25 - 0.27 x 10^4 with an average of 0.26 x 10^4. The result provided evidence that instrumental stability over the study period was acceptable.

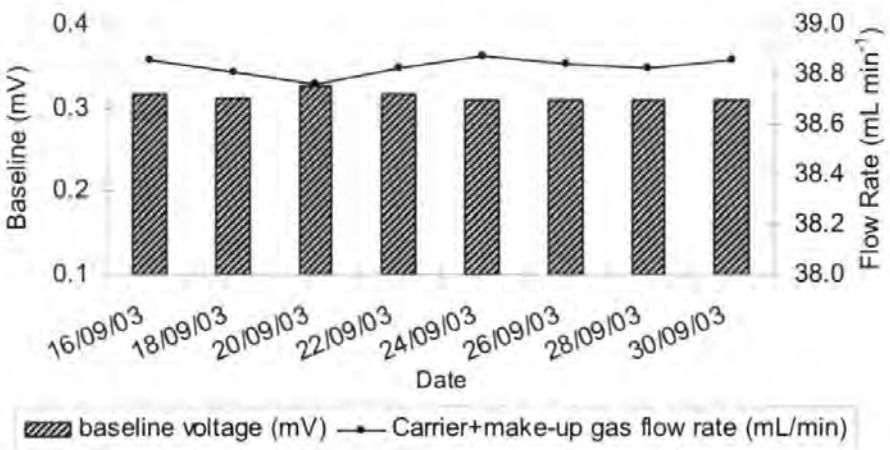

Figure 2.8: A plot of drift in the flow rate of carrier and make-up gases that affect the stability of the GC detector's baseline during the course of study in 2003.

Since baseline drift of the instrumental detector is more often affected by changes in gas flow, the baseline voltage of the detector and the total flow rates of the gases (carrier and make-up) were monitored and recorded. As shown in Figure 2.8, there occurred a minor variation in the flow rate over a 14-day period (i.e. 38.83 ± 0.04 mL min^{-1}). However this

resulted in a negligible drift in the baseline voltage of the instrument (i.e. 0.313 ± 0.006 mV).

2.5.1.4. Intercalibration of analytical systems

Intercalibration of analytical systems is recommended for the application of analytical techniques in assaying (e.g. Rasmussen & Khalil, 1981; Sharp *et al.*, 2004). Normally, Certified Reference Materials (CRMs) are used to assess a procedure's analytical accuracy. However, no such CRM is available for CH_3I determinations. It was therefore important to compare the performance of the GC system used in these analyses with another GC system used by a different operator. The performance of the system used in the present study was assessed by (i) comparing CH_3I measurements with a permeation tube standard (P.tube) and (ii) comparing CH_3I with another GC system at Plymouth Marine Laboratory, England (performed by L. Goldson). Replicate water samples from the local station L4, Plymouth were analysed for CH_3I concentration by both GC systems at the same time. Results of the comparison are presented in Table 2.2.

Table 2.2: A comparison of CH_3I concentration observed in a water sample from L4, Plymouth. (12th December 2005) using different approaches. Std-1, Std-2 and Std-3 are 1st, 2nd and 3rd prepared CH_3I standards; P-Tube is CH_3I standard in a permeation tube.

GC Analyses					
Calibration Source	**Standard prepared on**	**Standard analysed on**	**Date of water sample analysis**	**CH_3I Conc. (pmol L^{-1})**	**sd**
Std-1	12-Dec-05	12-Dec-05	14-Dec-05	1.42	0.08
Std-2	13-Dec-05	13-Dec-05	14-Dec-05	1.45	0.07
P.Tube	14-Dec-05	14-Dec-05	14-Dec-05	1.53	0.08
Std-3	10-Jan-06	10-Jan-06	14-Dec-05	1.42	0.09
L. Goldson			14-Dec-05	1.48	0.07

The comparison based on the estimation of CH_3I concentration using the calibration curves is also graphically presented on Figure 2.9. Even though the resulting CH_3I concentrations from the different calibrations (Std-1, Std-2, Std-3, P.tube and L. Goldson) slightly differed

from each other, there were no statistical differences (based on ANOVA at 5% significant level) between them ($p = 0.418$). This therefore indicates an excellent relative performance of the analytical protocol used in the current studies.

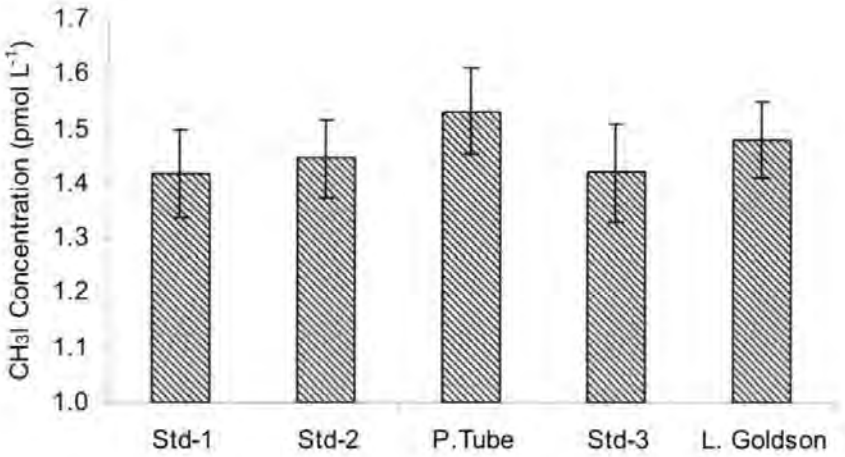

Figure 2.9: Statistical comparison of the various calibration curves in the monitoring of system reliability using CH_3I standard (2005). Water sample of unknown CH_3I concentration was obtained from the local sampling station L4 (Plymouth) on 12th December 2005 and analysed on 14th December 2005. Std-1, Std-2 and Std-3 are results obtained using calibration curves from analysed 1st, 2nd and 3rd prepared CH_3I standards; P-Tube is the result obtained using calibration curve from analysed CH_3I standard in a permeation tube. L. Goldson is the result from Laura Goldson of PML who analysed the same samples on the same date as above for comparison.

2.5.1.5. Blanks and Detection Limit

The value of the blank was determined by running sparge gas (N_2) for the same duration and trapped at the same temperature as the analytical water sample (20 minutes and -130°C respectively). The detection limit of CH_3I was calculated as described below.

Mean Conc. of Blank + (3*SD)

$= 0.105 + (3 * 0.069)$

$= 0.311$pg

$= 0.031$ ng L^{-1} (0.22 pmol L^{-1})

This value remained 0.311 pg or less during the course of the research period. The estimated detection limit was also compared with detection limits reported in recent publications. A summary of this comparison is presented in Table 2.3.

From Table 2.3, it is apparent that the recorded detection limit (0.22 pmol L^{-1}) of the GC system during the analysis of water samples from the local station L4 are comparable to those quoted in the literature, although the precision of < 1% indicates a better reliability of the instrumental performance for the present study compared to those reported in the literature. Based on all the various steps taken, it is clear that the analytical procedure involving the use of the GC system is reliable in the assaying of CH_3I in water samples.

Table 2.3: A table of CH_3I analytical results presented in the literature for the analyses of natural water samples.

Zone	CH_3I detection limits (pmol L^{-1})	Surface Conc. (pmol L^{-1})	Analytical Precision (%)	source
Labrador sea		2.5		Moore & Groszko, 1999
Pacific Ocean		2 – 6		Moore & Groszko, 1999
Open Ocean		7.1		Singh *et al.*, 1983; Reifenhauser & Heumann, 1992
Inshore waters		155		Lovelock, 1975; Singh *et al.*, 1983
Southern North Sea	0.14	37.4	5	Campos *et al.*, 1996a
Lab. Culture (red algae)	0.99	130		Scarratt & Moore, 1999
	1.41		5-14	Nightingale *et al.*, 1995
Weddell Sea (surface water)	0.21	24.7	17	Tanhua, 1995
Weddell Sea (ice pore water)	0.21	38.1	17	Tanhua, 1995
Bosphorus Strait	0.14	4.9		Fogelqvist *et al.*, 1996
L4 (Plymouth)	0.22	8.0	0.7	Present Study
Incubation Expt. (Day2)		5.4	5.7	Present Study

2.5.1.6. Comparison with an internal standard

To augment confidence in the sensitivity of the GC detector, a common chlorofluorocarbon (trichloro-fluoro methane also known as Freon 11) found in aquatic environment at relatively constant concentration was used as an internal standard.

Chrolofluorocarbons (CFCs), also known as Freons are stable colourless liquids or gases that are anthropogenically introduced into the environment usually from products such as aerosol propellants and refrigeration. They are chemically stable and have long lifetimes in the environment.

Fluorotrichloromethane (Freon 11) is used either as fumigant, insecticide or propellant. In the analyses of water samples in this study, its presence was manifested in the appearance of a peak approximately one minute before the peak appearance of CH_3I on a chromatogram. Since the level of Freon 11 in the aquatic environment is quite constant and is easily determined by GC, its presence over a period of time may be used as an internal standard in monitoring changes in the sensitivity of the GC system.

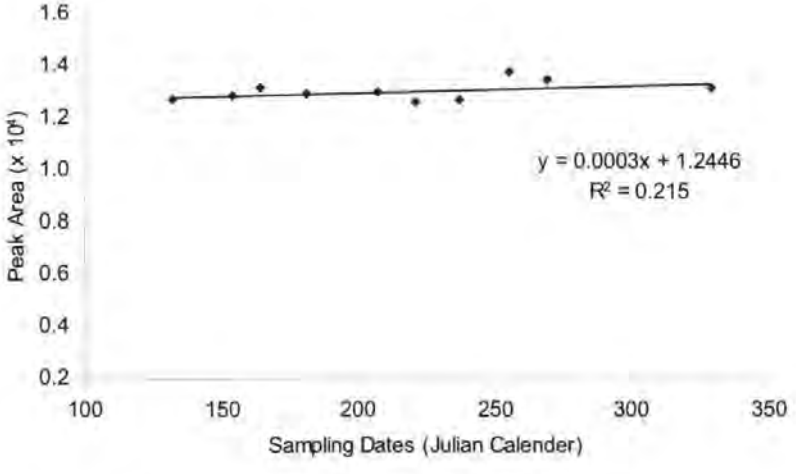

Figure 2.10: A plot of fluorotrichloromethane (Freon 11) from sampling station 4 as an internal standard for the GC system (2005).

Figure 2.10 which represents Freon 11 in the analysed water samples shows a stable value over the study period (also see Figure 2.11). With Freon 11 peak areas varying between 1.26-1.36 x 10^4 there is an indication that the GC system over the study period was very stable.

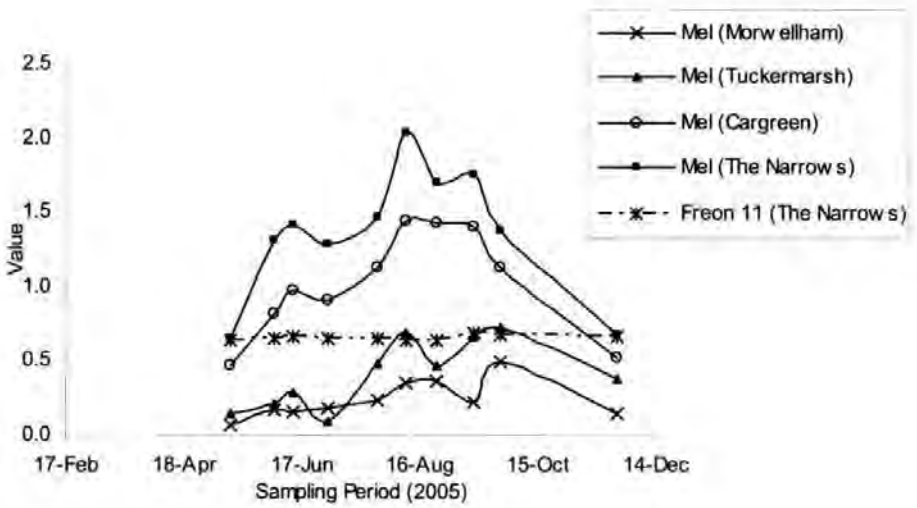

Figure 2.11: Relative changes in Freon 11 value (from station 4) to the observed CH_3I concentrations of the 4 sampling stations in the monitoring of system reliability (2005).

2.5.2. Bacterial Production

In order to establish an adequate substrate concentration and incubation time with which to incubate bacteria, preliminary experiments on water samples from L4, Plymouth were performed. Different substrate (^{3}H-leucine) concentrations of 10, 20, 40 and 100 nmol L^{-1} were each incubated for a period of 20, 40, 60, 120, 180 and 360 minutes respectively. Each sample comprised of four live and two dead control replicates. The results are presented in Figure 2.12.

It is apparent that bacterial leucine incorporation rate is optimum (linear growth phase) at the concentrations of 20, 40 and 100 nmol L^{-1}. There is statistically no difference between bacterial incorporation rate of the introduced 20, 40 and 100 nmol L^{-1} leucine respectively for the first 60 minutes of the study. Based on these results, a substrate concentration of 20

nmol L^{-1} and an incubation time of 60 minutes were used providing reliable results, whilst cutting down time, excess use of the substrate as well as producing less radioactive waste.

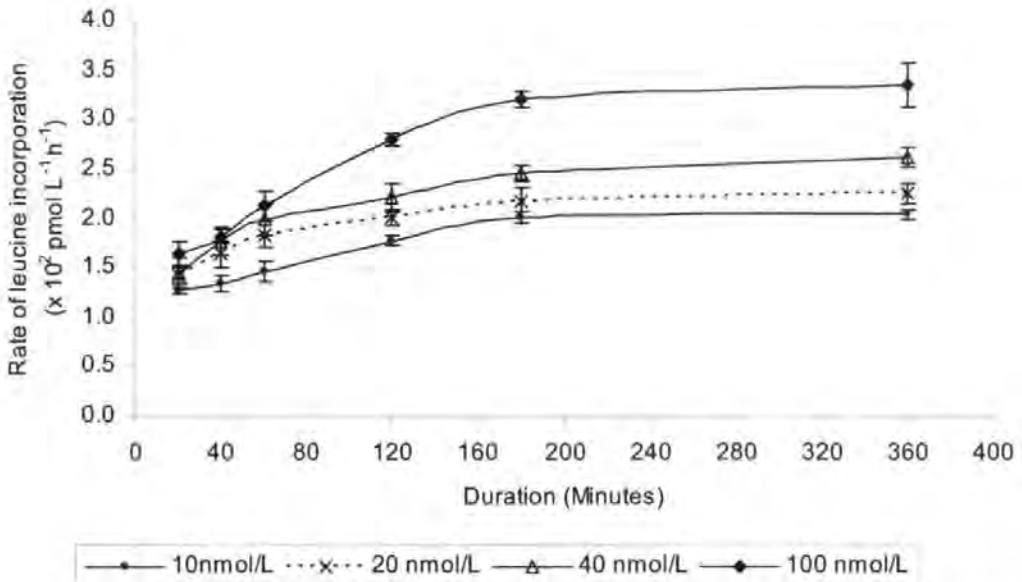

Figure 2.12: Rate of bacterial production at different substrate concentration and incubation duration. All samples were incubated at 15 °C for ease of comparison.

2.5.3. Bacterial Enumeration

The reliability of the enumeration process using flow cytometry depends upon the stability of the system. The system could experience changes in flow rate and if unchecked could make cell enumeration unreliable. This anomaly is eliminated by measuring the flow rate of the system each time it is used. The procedure involved in this is described in section 2.3.3.5 of this chapter.

In order to ascertain the relative performance of the analytical technique employed (AFC with SYBR® green I) for the measurement of sample bacterial abundance, a second method of enumeration (EFM with DAPI) was used in addition to flow cytometry. One set of water samples (25th November 2005) was analysed using both techniques. The results are presented in Figure 2.13 and 2.14.

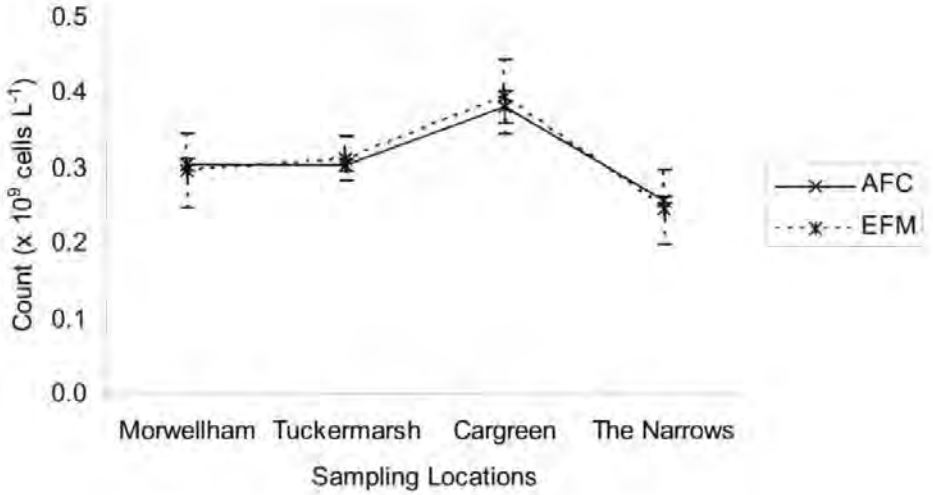

Figure 2.13: A plot of bacterial enumeration results (with error bars) comparing two techniques; AFC and EFM. Water sample used (three replicates for each technique) was obtained from the Tamar estuary and analysed on 25th November 2005.

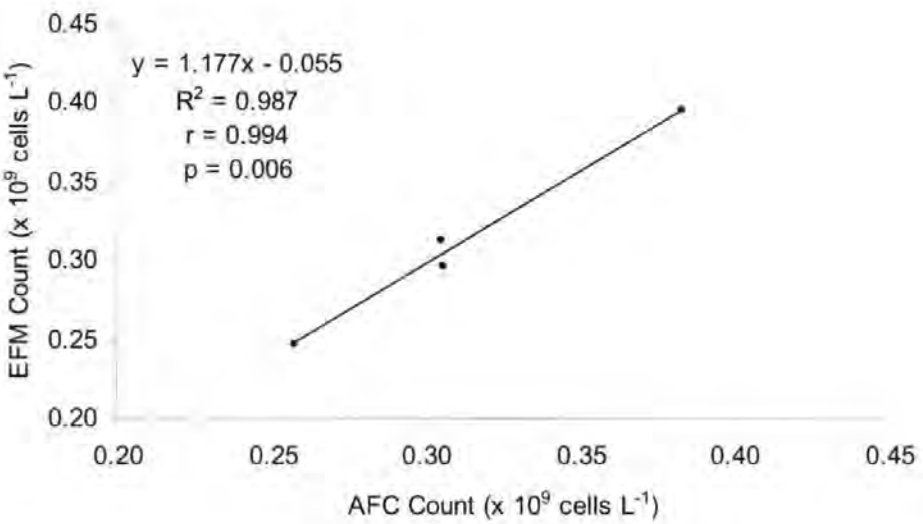

Figure 2.14: A correlation plot between the two bacterial enumeration techniques (AFC vs. EFM).

From the comparison of the two enumeration methods, there is a statistically significant correlation ($p = 0.006$) between estimates of bacterial abundance by the two methods with a linear ratio very close to 1:1 (with a slope of 1.18×10^9). However, based on the observed standard deviations of replicate counts from both methods (ranging between 1.5 – 5.4% and 9.1 – 20% for AFC and EFM respectively), the error associated with AFC is far lower than that of the EFM counts (see error bars in Figure 2.13). It is therefore reasonable to conclude that the method of flow cytometry used in the enumeration of bacterial

abundance in this study is reliable and more precise than that of EFM. This approach has been used by others (e.g. Jochem, 2001) for the same reasons.

2.6. Summary

This chapter has considered the various analytical techniques employed in the investigations in the present study and their analytical reliability and reproducibility. The main area of investigation of the study involved the assaying of CH_3I concentration in natural water samples and bacterial activity with respect to their abundance as well as substrate breakdown. Analytical approaches pertaining to these areas of investigation were addressed. These include GC (for CH_3I assaying), radioassaying (which include ^{3}H-leucine incorporation for bacterial production and $^{14}CH_3I$ breakdown), as well as bacterial enumeration methods (i.e. AFC and EFM). A step-by-step approach to each of these analytical techniques was provided. In addition, several experiments were carried out to define the analytical performances of the various techniques employed in this study. With regards to GC, these included linearity of the instrumental response, precision, stability and performance of the detection system, blanks and detection limit, comparison with an internal standard and another GC system of a different operator. The reliability of the bacterial production analytical technique was established through assaying with several substrate concentrations for different durations, whilst the relative performance of the bacterial enumeration technique was established by comparison of two techniques.

CHAPTER THREE

Effect of aggregation and associated bacteria on methyl iodide production

3. Effect of aggregation and associated bacteria on methyl iodide production

3.1. Introduction

As established in Chapter One, according to the literature (refer to section 1.3.3), through microbial activity, the association of bacteria with aggregates may result in the decomposition of POM into DOM with biogases as by-products. This chapter is to investigate the specific role of the microbial community that is found associated with aggregates in the production and emission of CH_3I into the environment.

3.2. Aims and Objectives

i. To investigate CH_3I production from estuarine macroaggregates through controlled laboratory experiments.

ii. To estimate the rate of CH_3I production by the microbial community through their decomposition of organic matter in estuaries.

iii. To evaluate the effect of aggregation processes on the rate of microbially-mediated production of CH_3I in estuarine waters.

3.3. Methodology

3.3.1. Field and laboratory work

To achieve the objectives, an experimental design that closely simulates the *in situ* conditions of the aquatic environment is required. The design should also link bacterial activity to biogenic gas production. The strategy employed in this study involved establishing a series of laboratory controlled experiments. Water samples, from a site

where the TMZ of the Tamar estuary is localized at the time of sampling (located with the aid of a Multiparameter Water Quality Monitor; see Figure 3.1) were subjected to laboratory simulated aggregation experiments. This was achieved by rotating them with the aid of electric rollers under controlled physical and biological conditions (*in situ* temperature and no illumination to eliminate possible primary production). A batch of samples was aggregated in this way (Rolled) whilst another batch was left standing on a bench to minimize aggregation (Unrolled). Each of the 2 batches comprised of 2 sets (see Figures 3.2 and 3.3). One set was treated with antibiotics to render the bacteria present inactive (Dead) whilst the other received no treatment (Live). Each treatment had 3 replicates (sub-samples). Sub-samples from each set were used for the analyses of CH_3I concentration, bacterial activity and abundance.

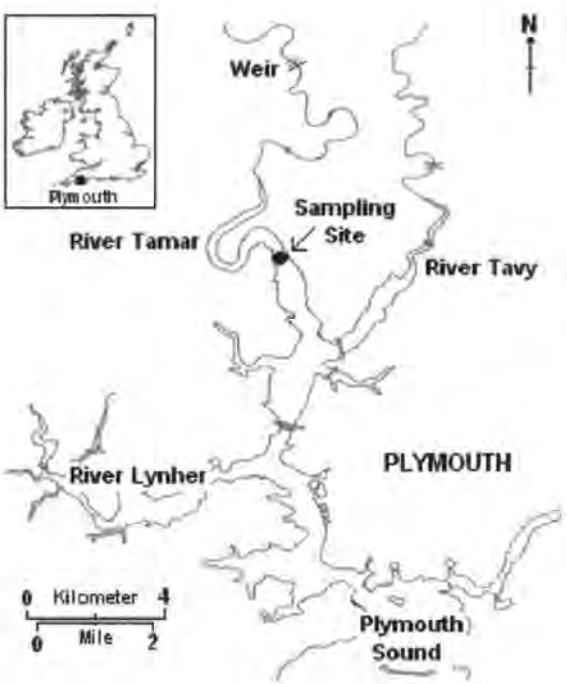

Figure 3.1: The Tamar estuary showing the site from which water was collected and used in the laboratory-controlled aggregation experiment.

Water sampling for two laboratory-controlled experiments was conducted on the 16th September 2003 (temperature of 17 °C, salinity of 34) and 21st November 2003 (temperature of 11 °C, salinity of 17) respectively on board the research boat MBA Sepia

at high water. Bulk surface water samples (< 1m) were collected using a stainless steel bucket and stored in a 10 litre glass reagent bottle (Pyrex) with a glass stopper (avoiding any headspace) before transport to the laboratory. However, water samples used for Study One were not stored at the *in situ* temperature during transportation from the field to the laboratory resulting in a fluctuation in the temperature of the samples and prompted the storage of all subsequence water samples (Study Two) at the *in situ* temperature during transportation from field to the laboratory.

From this experiment, an estimation of the rate of microbially-mediated production of CH_3I could be made from the difference in CH_3I concentration between the contrasting experimental treatments. This information together with bacterial abundance in the experimental medium could also be used to estimate the total global production of CH_3I by bacteria in the aquatic environment. This estimation (as presented in section 3.4.9) is based on the assumption that all bacteria present in the aquatic environment contribute to the production of CH_3I. This assumption is however limited as it is more likely that a subset of the total bacteria present are actually involved in the removal of CH_3I in the aquatic environment.

3.3.2. Aggregation Experiment

3.3.2.1. Background

In order to achieve the simulated aggregation process in the study, a similar approach to that of Shanks & Edmondson (1989) was adapted. This involved a laboratory roller-system that results in the production of aggregates during rotation (30 rpm) of natural water samples (see Figure 3.2a).

Two separate studies were conducted with respect to this (see Figure 3.3). The first study was carried out to investigate (1), the possible role of bacteria in CH_3I production during the decomposition of POM and (2), the influence of the aggregation process on bacterial CH_3I production. The second study was conducted to address some additional questions arising from the first study (see Result and Discussion sections).

Glass sample bottles (30 mL reagent bottles with glass stoppers) were filled with the water sample and sealed with the glass stoppers, whilst avoiding any headspace in the process. Three replicates (each bottle representing one replicate and a unique experiment on its own) were used for each sampling time point. Each replicate bottle supplied 10 mL for CH_3I concentration analysis, 7.5 mL for bacteria production, and 5 mL for bacterial enumeration. Analytical methods adopted are fully discussed in Chapter Two.

Figure 3.2a-b: Images of (**a**) the roller system used in the laboratory-facilitated aggregation experiment (Experiments 1 and 2) and (**b**) benched sample bottles (Unrolled samples; Experiment 1 only) used in comparison with (a) to determine the impact of aggregation on bacterial CH_3I production.

3.3.3. Experimental Designs

Details of the experimental approach used in Study One are highlighted in Figure 3.3 and 3.4.

Study One

The aggregation experiment in this study was conducted in the dark to eliminate possible autotrophic CH_3I production. Sample aliquots were taken over 14 days at 2 days intervals.

Set 1: Rolled samples at x 30 rpm (see Figure 3.2a) which had no treatment. These were used to evaluate the role of bacterial association with aggregates in the production of CH_3I.

Set 2: Rolled samples at x 30 rpm which had been treated with 1 mL of an antibiotic solution (antimycotic cocktail), comprising of penicillin G (10000 units mL^{-1}), streptomycin sulphate (10 mg mL^{-1}), and amphotericin B (25 µg mL^{-1}) in order to prevent any activity of bacteria. These served as controls for investigating the role of bacteria in CH_3I production through the decomposition of POM.

Set 3: Unrolled samples (see Figure 3.2b) which had no antibiotic treatment. These acted as controls for observing the absence of particle aggregation on the rate of POM decomposition by bacteria which may result in CH_3I production.

Set 4: Unrolled samples that had been treated with an antibiotic cocktail (as in Set 2) to served as controls for both bacteria decomposition and the process of aggregation on CH_3I production.

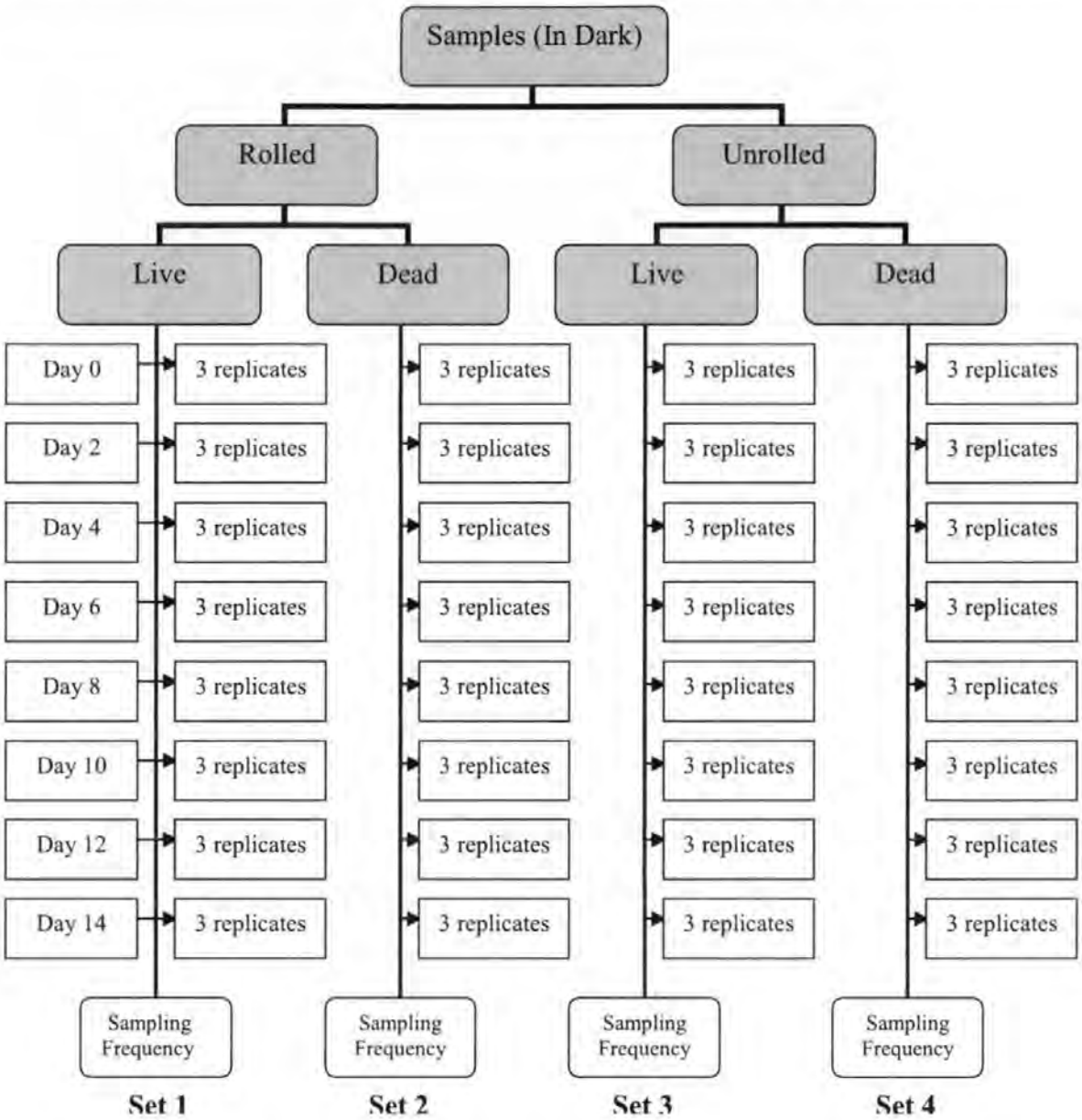

Figure 3.3: Experimental design for the laboratory facilitated aggregation process (Study One) showing periods (days) of sampling and analyses and the number of replicate samples used at each sampling time point.

Study Two

In this study, several changes to the initial experimental design were introduced (see Figure 3.4). Sample analyses were carried out for a period of 8 days (since most of the contribution made by aggregate associated bacteria during the first study occurred within 8 days).

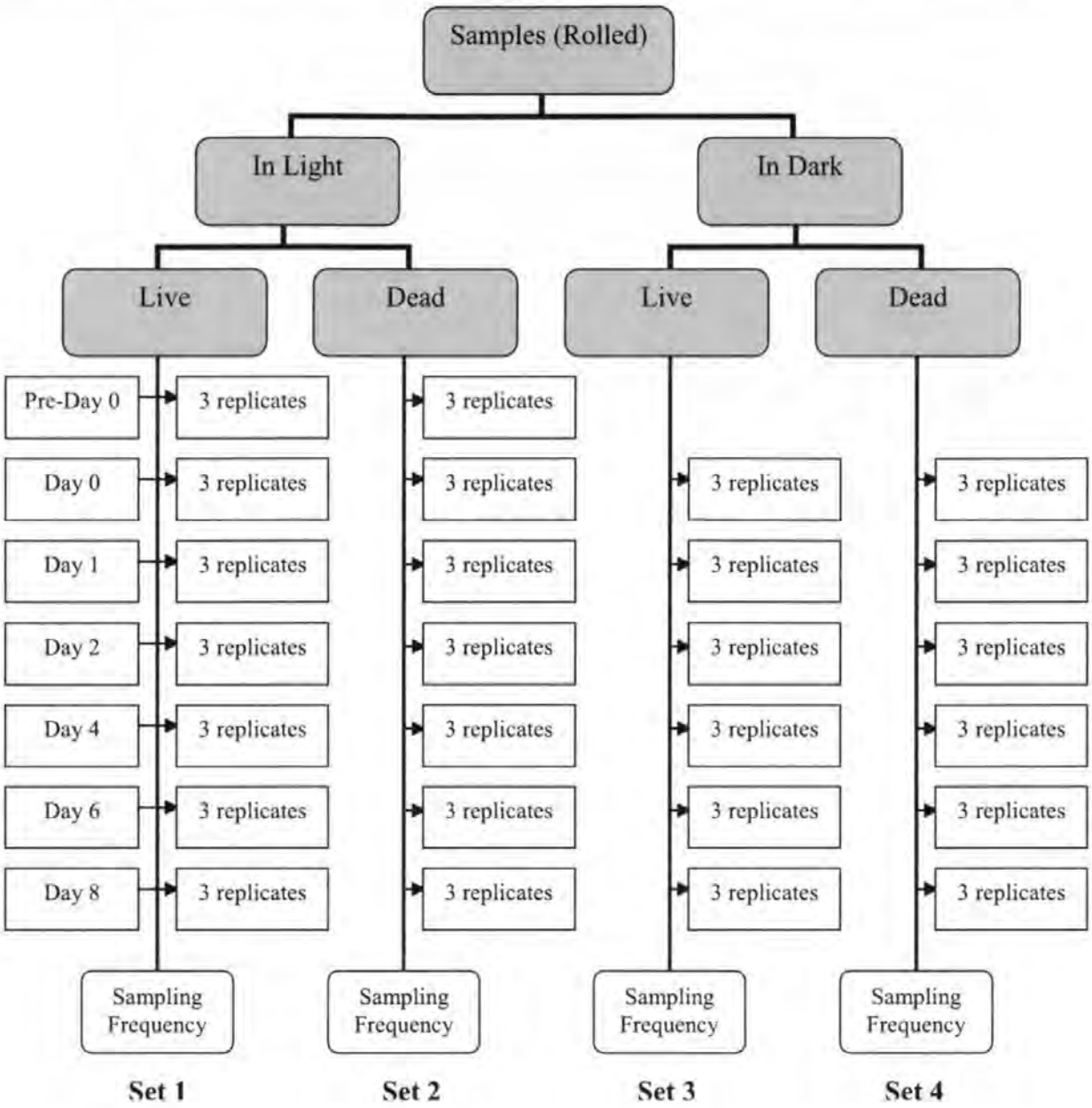

Figure 3.4: Experimental design for the laboratory facilitated aggregation process used in Study Two. It shows periods (days) of sampling and analyses and the number of replicate samples used each period.

In addition, the following changes were made to the original experimental design during Study Two.

1. The experiment comprised of only rolled samples (i.e. Live-Rolled and Dead-Rolled).

2. It had two sets; one set was run in the dark whilst the other set was carried out in the light, (both were at the *in situ* temperature of the sample water).

3. The sampling period was limited to Day 00, Day 01, Day 02, Day 04, Day 06 and Day 08. However Pre-Day 00 samples were included. These were used to assess any changes in the CH_3I concentration that may have occurred during transport of samples from the field to the laboratory. Three replicates for each treatment (i.e. Live and Dead) sample bottles were filled at source.

4. Estimations of two separate bacterial abundances (for methods see Chapter Two) were made, one for free living and the other for total bacterial numbers, whilst numbers for aggregate-attached bacteria were estimated from the difference between the two.

3.3.4. General laboratory procedures

In the laboratory, replicate samples were prepared by the initial additions of 1 mL antibiotic antimycotic solution (Sigma-Aldrich A5955) into labelled replicate bottles for the treated samples using a calibrated adjustable-volume digital pipette (Eppendorf). This was followed by the serial addition of water samples from the 10 litre bottle (after gently mixing through swirling) into all replicate bottles beginning with the treated samples (with the exception of the pre-day 00 replicate samples which were prepared and treated on the field).

Samples to be aggregated (Rolled) were all placed on low profile rollers (Stovall) operated at a speed of 30 rpm while static replicate sample bottles were placed on a bench (see Figure 3.2a-b). All samples were kept in a culture cabinet set at the *in situ* temperature (approximately 17 °C for Study One and 11.2 °C for Study Two). All samples for Study One were however incubated in constant darkness whilst half the samples of Study Two (Live-in-Light and Dead-in-Light) were cultured in constant fluorescent light condition to evaluate the effect of light.

Table 3.1: The experimental conditions used for the laboratory-controlled aggregation experiments (Study One and 2).

CONDITIONS	STUDY ONE	STUDY TWO
Filtration	No filtration	No filtration
Addition of nutrients	No addition	No addition
Light	Continuous darkness	Continuous light (for In Light samples), continuous darkness (for In Dark samples)
***In situ* Temperature (°C)**	Not measured (approx. 17)	11.2
Simulated Temperature (°C)	17	11
Salinity	34.3	17.1

3.4. Results and discussion

3.4.1. Temporal change in CH_3I concentration (Study One)

In this section, the results obtained from CH_3I concentration analyses of the samples over the study period are presented.

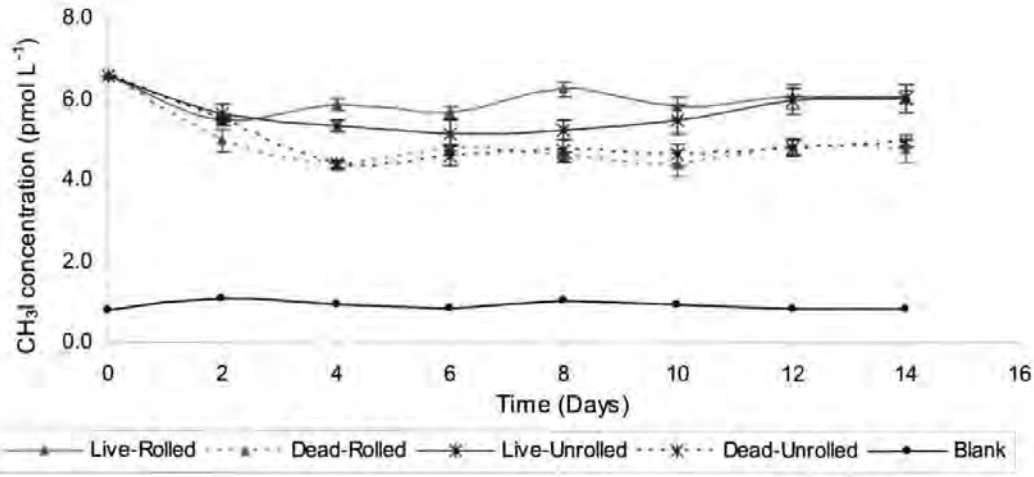

Figure 3.5: A plot of CH_3I concentration (pmol L^{-1}) from Study One with error bars representing the standard deviation of three replicate samples. It shows changes in recorded CH_3I concentration between the various treatments and over the 14-day study period. A blank reading was taken each period prior to the analyses of the replicate samples and is also presented in the graph representing the stability of the analytical measurement.

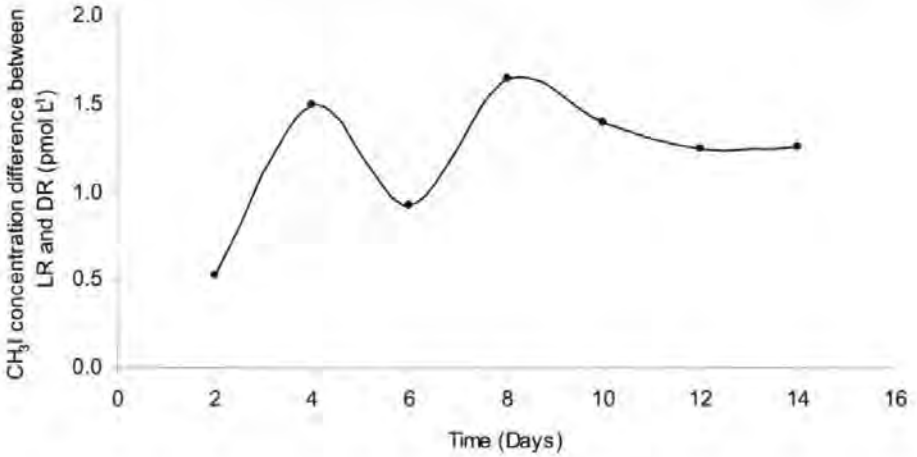

Figure 3.6: A plot of CH_3I concentration difference (pmol L^{-1}) between Live-Rolled (LR) and Dead-Rolled (DR) samples at each sampling day over a 14-day period. This is to highlight the net increase or decrease in bacterially-mediated CH_3I production during Study One.

From the results, a difference is confirmed between the two sets of treatments (Live-Rolled and Dead-Rolled samples) by a maximum concentration difference of 1.64 pmol L^{-1} CH_3I recorded on day 8 of the experiment representing bacterially-mediated CH_3I production in the Rolled (aggregated) samples. A maximum concentration difference of 1.16 pmol L^{-1} CH_3I was also recorded between Live-Unrolled and Dead-Unrolled samples during day 12 which represents bacterially-mediated CH_3I production in the Unrolled (non-aggregated) samples. During the study, a net increase in the rate of bacterially-mediated CH_3I production was observed between day 2 and 4 (estimated as 0.49 pmol L^{-1} d^{-1}; i.e. (conc of day 4 – conc of day 2)/days), and between day 6 and 8 (estimated as 0.36 pmol L^{-1} d^{-1}; i.e. (conc of day 8 – conc of day 6)/days) (see Figure 3.6). From these values, the mean daily rate of bacterially-mediated CH_3I production is estimated as 0.42 ± 0.09 pmol L^{-1} d^{-1}. These observations indicate that bacterial activity contributed to the production of CH_3I while making it possible to distinguish between bacterially-mediated CH_3I production from the background CH_3I concentration in the sample treatments.

With both sets of treatments (Rolled and Unrolled) beginning with theoretically the same amount of POM and bacterial cells, the influence of aggregation on bacterial CH_3I production was experienced in the Rolled samples although the pattern of CH_3I concentration change over time in both the Rolled and Unrolled Live samples were identical on several occasions (see Figure 3.5). A statistical test (student's *t*-test) between Rolled and Unrolled Live samples at the 95% confidence level of significance (refer to Appendix 5) shows that concentrations of CH_3I recorded during day 4, 6 and 8 (5.88 ± 0.13, 5.69 ± 0.14, and 6.26 ± 0.17 pmol L^{-1} respectively) for the Rolled samples were significantly higher than the comparative values (5.34 ± 0.13, 5.15 ± 0.32, and 5.22 ± 0.24 pmol L^{-1}) for their Unrolled counterparts. These results show that the rate of bacterial CH_3I production was facilitated by the process of aggregation in the medium. On average, bacterial activity contributed about 22% and 15% of the total CH_3I recorded during the study in Rolled and Unrolled samples respectively and the difference of 7% between the two representing the effect of the presence of aggregates. These were estimated from the difference between the recorded CH_3I concentration of the Live and Dead samples and may represent the percentage of bacterially-mediated CH_3I production in the medium.

Considering Figure 3.5 further, there was a decrease in the CH_3I concentration during the first two days of the experiment. This initial decrease may have arisen as a result of (a) disturbed biological conditions during the first couple of days, (b) physical changes during sampling and sample preparation during transport from the field to the laboratory which may have resulted in the loss via volatilization or (c) through adsorption onto the walls of the experimental bottles.

3.4.2. Rate of leucine incorporation by bacteria (Study One)

The results of bacterial leucine incorporation during Study One (shown in Figure 3.7) follow the general pattern of bacterial growth (lag, growth, stationary and death phases) in succession.

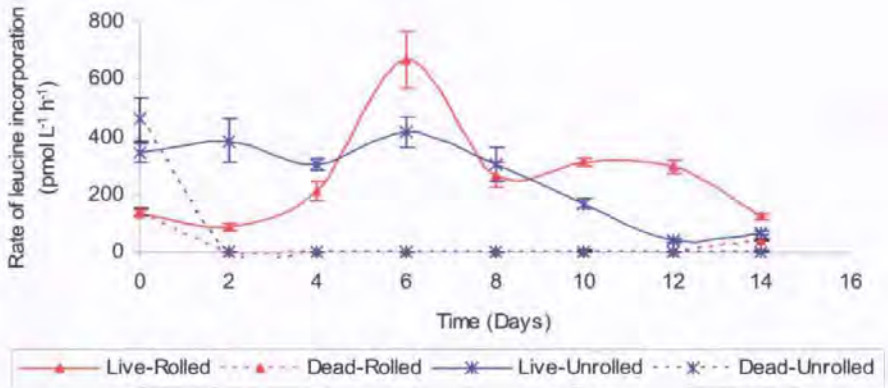

Figure 3.7: The rate of leucine incorporation (pmol L^{-1} h^{-1}) by bacteria as observed in Study One of the aggregation experiment, highlighting changes in bacterial leucine uptake between the different treatments and over the study period.

The maximal incorporation rate (peak production) which stood at 670 ± 90 pmol L^{-1} h^{-1} and 420 ± 50 pmol L^{-1} h^{-1} for Live-Rolled and Live-Unrolled samples, respectively, were both recorded on the sixth day of the experiment. An estimated difference of 250 pmol L^{-1} h^{-1} (student's t-test; $P<0.01$), representing about 37.3% of the total, is observed between the two sample sets at this time. This percentage may represent the rate at which attached bacteria contributed to the overall bacterial activity in the experimental medium. This observation therefore suggests that differences in conditions that facilitate aggregate formation (Rolled and Unrolled in this case) influenced bacterial activity that lead to differences in leucine incorporation rates between the two experimental media. Results from the two sets of treated samples (Dead-Rolled and Dead-Unrolled) also indicate the effectiveness of the antibiotics used in the treatment in inhibiting bacterial growth and activities although the effectiveness seems to decrease after Day 12.

3.4.3. Bacterial abundance (Study One)

High rate of bacterial activity (leucine incorporation) may not necessarily be the result of increase in cell abundance. In order to establish a possible link between the increase in bacterial activity and CH_3I production in the experimental media, there was the need to also estimate the specific bacterial activity (a ratio of leucine incorporation to cell numbers) for each treatment and time point. Bacterial abundance of each sample set was therefore estimated. The changes in bacterial abundance (cells L^{-1}) with time are presented in Figure 3.8.

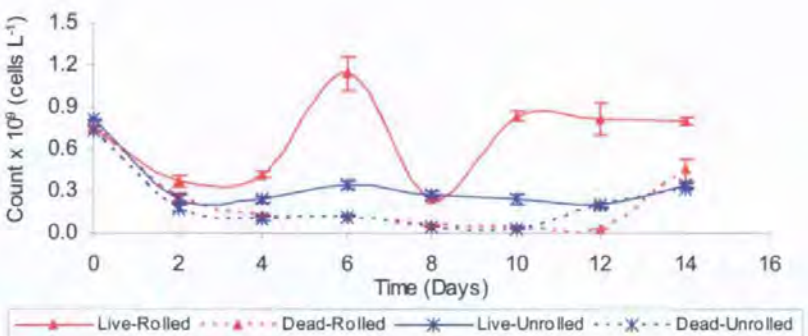

Figure 3.8: A graph showing variations in the cell abundance of free-living bacteria (cells L^{-1}) for the four different experimental treatments recorded during the course of Study One.

A rapid increase followed by a sharp decline in bacterial abundance observed between Day 4 - 10 of Study One (especially in the Live-Rolled samples) conforms to the growth pattern of bacteria in natural systems. During the availability of nutrients, an increase in bacterial abundance (growth phase) is observed through rapid bacterial growth (between day 4 – 6 and day 8 -10; Figure 3.8). Such rapid growth results in the depletion of available nutrients and is translated into a sharp decline (death phase) in bacterial abundance (between day 6 - 8; Figure 3.8). This pattern of bacterial activity (also observed in Figure 3.7) is cyclical through nutrient availability and depletion.

Between the two Live samples (Live-Rolled and Live-Unrolled), there was a distinct difference in total cell abundance per unit volume of water sample over the entire 14-day period ($5.34 \times 10^9 \pm 0.41 \times 10^9$ cells L^{-1} and $2.69 \times 10^9 \pm 0.19 \times 10^9$ cells L^{-1} respectively). By ignoring readings for day 0, the highest counts of 1.14×10^9 cells L^{-1} and 0.34×10^9 cells L^{-1} for the two Live samples (Rolled and Unrolled respectively) were recorded on the sixth day of the experiment with the maximum difference of 0.79×10^9 cells L^{-1}. These coincided with the period of highest bacterial activity indicating that the increase in the overall bacterial activities at that period was a result of an increase in bacterial abundance. Over the 14-day period, the mean bacterial abundance for the Live-Rolled samples (a representative of the estuarine environment) was $0.66 \pm 0.32 \times 10^9$ cells L^{-1}.

Low cell counts for the Dead samples reveals the stability of the antibiotic cocktail in inhibiting increase in bacterial abundance over the study period. However, it also shows that the effect of the antibiotics on bacteria in the experimental media began to wear off after the tenth day of the experiment. This lead to a rise in cell numbers as could be seen during day 12 and 14 of the experiment (see Figure 3.8).

3.4.4. Integration of Study One

In this section, statistical analyses of the relationships between the measured variables during Study One have been performed. The analyses were performed using the Pearson's correlation. The results have been presented as a matrix in Table 3.2.

Based on the correlation matrix presented in Table 3.2, a number of plots that exhibit statistically significant relationships between the measured analytical variables were performed. These included comparisons between (i) recorded CH_3I concentration and specific bacterial activity (Figure 3.9a-d), (ii) bacterial leucine incorporation rate and specific bacterial activity (Figure 3.10a-d), (iii) bacterial leucine incorporation rate and

bacterial abundance (Figure 3.11a-d), and (iv) bacterial leucine incorporation rate and recorded CH_3I concentration (Figure 3.12a-b).

Table 3.2: A Pearson correlation matrix of the measured variables for the various experimental treatments during Study One. Values in bold are p-values of the analyses with those significant at 5% underlined. [CH_3I] = CH_3I concentration (pmol L^{-1}), B.Nos = bacterial abundance (cells L^{-1}), ^3H = bacterial leucine incorporation (pmol L^{-1} h^{-1}), RC[CH_3I] = rate of change of CH_3I (pmol L^{-1}), P/N = bacterial specific activity (pmol cell^{-1} h^{-1}).

Live samples on rollers (Live-Rolled)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	-0.160			
	0.487			
^3H	-0.018	0.608		
	0.940	**0.003**		
RC[CH_3I]	0.857	-0.143	0.118	
	0.000	**0.537**	**0.610**	
P/N	0.474	-0.254	0.526	0.401
	0.030	**0.266**	**0.014**	**0.072**

Live samples without rollers (Live-Unrolled)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	-0.126			
	0.588			
^3H	-0.717	0.066		
	0.000	**0.777**		
RC[CH_3I]	0.432	-0.086	-0.348	
	0.051	**0.711**	**0.122**	
P/N	-0.693	-0.186	0.950	-0.367
	0.001	**0.419**	**0.000**	**0.102**

Dead samples on rollers (Dead-Rolled)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	0.250			
	0.275			
^3H	-0.167	0.663		
	0.468	**0.001**		
RC[CH_3I]	0.100	-0.304	-0.087	
	0.666	**0.181**	**0.708**	
P/N	-0.205	0.548	0.962	-0.000
	0.372	**0.010**	**0.000**	**0.999**

Dead samples without rollers (Dead-Unrolled)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	0.378			
	0.091			
^3H	0.074	0.275		
	0.749	**0.228**		
RC[CH_3I]	-0.001	0.087	0.129	
	0.995	**0.706**	**0.578**	
P/N	-0.062	-0.359	0.563	0.191
	0.788	**0.110**	**0.008**	**0.407**

The rate of CH_3I change was calculated as the difference in CH_3I concentration between successive time-points (days) whilst the specific activity (P/N) was estimated as the ratio between the rate of bacterial production (P) and bacterial abundance (N) at each time point (day).

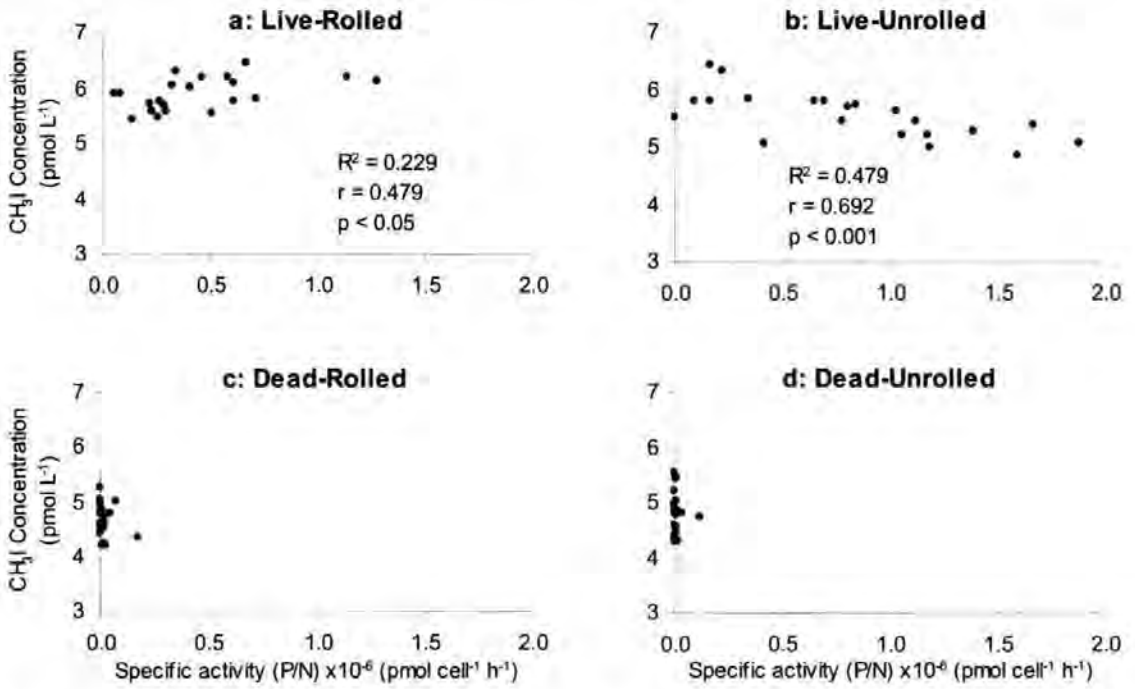

Figure 3.9a-d: Graphs showing the relationships between CH_3I concentrations (pmol L^{-1}) and bacterial specific activity (pmol $cell^{-1}$ h^{-1}) over the study period (Study One). (**a**) Live-Rolled, (**b**) Live-Unrolled, (**c**) Dead-Rolled, (**d**) Dead-Unrolled.

According to Figure 3.9a-d there is a linear relationship between bacterial specific activity and methyl iodide concentration. Figure 3.9a with a positive gradient indicates that an increase in specific bacterial activity resulted in an increase in CH_3I concentration in the Live-Rolled samples whilst Figure 3.9b with a negative gradient indicates that an increase in specific bacterial activity resulted in a decrease in CH_3I concentration in the Live-Unrolled samples. Lack of aggregation in the Unrolled samples therefore reveals how the microbially-mediated CH_3I production rate is affected by the absence of aggregates. This is depicted by the gradual decline in net CH_3I concentration of the Live-Unrolled sample over time (more consumption than production) from the beginning of the experiment until the sixth day when the balance between consumption and production shifted in favour of higher production (refer to Figure 3.5).

The linear relationships (Figure 3.9a-b) although statistically significant in both treatments (Live-Rolled and Live-Unrolled), was weaker in the Live-Rolled samples ($r^2 = 0.229$) in

comparison with the Live-Unrolled samples ($r^2 = 0.479$). With regard to the Dead-Rolled and Dead-Unrolled samples, the relationships were statistically not significant.

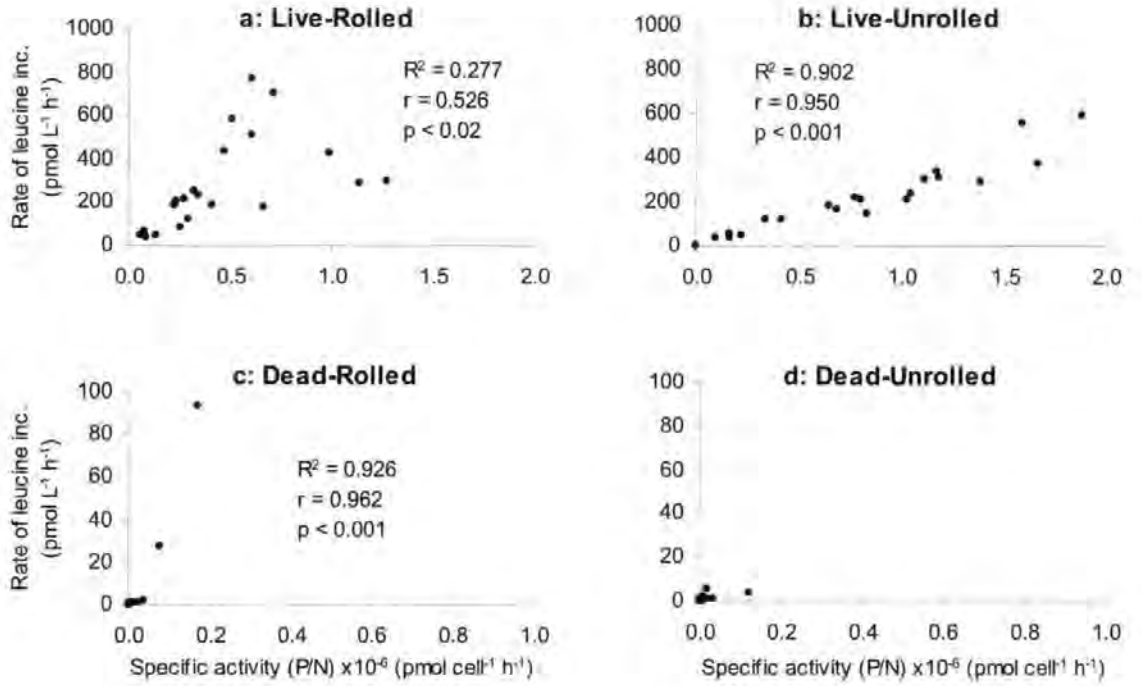

Figure 3.10a-d: Graphs showing the relationships between the rates of bacterial leucine incorporation (pmol L^{-1} h^{-1}) and specific activity (pmol $cell^{-1}$ h^{-1}) over the study period (Study One). (**a**) Live-Rolled, (**b**) Live-Unrolled, (**c**) Dead-Rolled, (**d**) Dead-Unrolled. Note the differences of the scales of the axes.

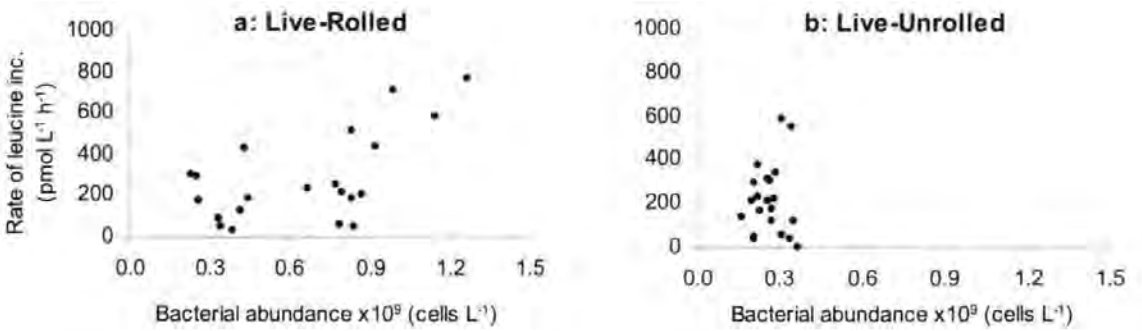

Figure 3.11a-b: Graphs showing the relationships between the rate of bacterial leucine incorporation (pmol L^{-1} h^{-1}) and bacterial abundance (cells l^{-1}) over the study period (Study One). (**a**) Live-Rolled, (**b**) Live-Unrolled.

As shown in 3.11a, the rate of bacterial leucine incorporation in Live-Rolled samples increased with increasing bacterial abundance which is subsequently reflected in specific

bacterial activity (see Figure 3.10a-d). Bacterial leucine incorporation rate in relation to specific bacterial activity, was statistically significant in all treatments (Figure 3.10a-d) whilst in comparison with bacterial abundance, the relationship was found to be statistically significant only in the Live-Rolled treatment (Figure 3.11a).

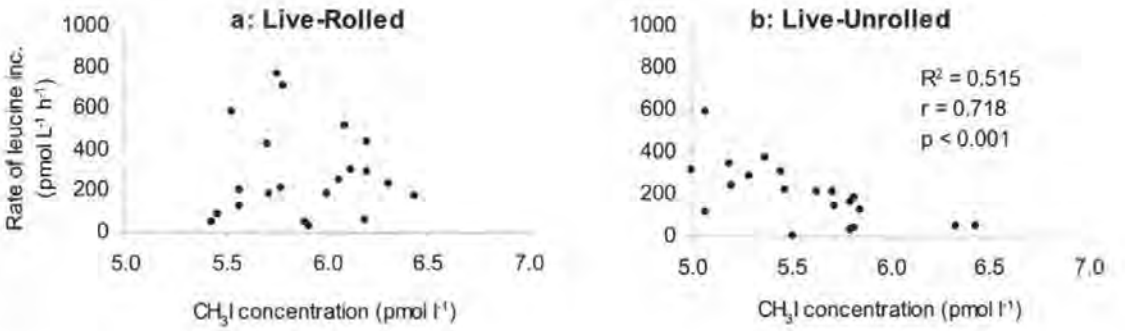

Figure 3.12a-b: Graphs showing the relationships between the rate of bacterial leucine incorporation (pmol L^{-1} h^{-1}) and CH_3I concentrations (pmol L^{-1}) over the study period (Study One). (**a**) Live-Rolled, (**b**) Live-Unrolled.

Among the parameters used in the Pearson's correlation analyses (see Table 3.2), CH_3I concentration was found to have a statistically significant relationship (i.e. at 5% level of significance) with bacterial leucine incorporation rate in only the Live-Unrolled treatment (Figure 3.12b). CH_3I concentration was also found to be significantly correlated to bacterial specific activity in the two Live samples (Live-Rolled and Live-Unrolled). Since the relationship is directly proportional (as shown in Figure 3.9a), it may be an indication that there is enhanced bacterially-mediated CH_3I production facilitated by the process of aggregation (thus exhibiting a net CH_3I production with increasing bacterial activity). On the other hand, in the absence of significant aggregation, there occurred a net removal of CH_3I with increasing bacterial activity (Figure 3.9b).

The observations made in Figure 3.9a and 3.9b may be interpreted as the presence of both production and consumption of CH_3I by the microbial community in the Live sample treatments (and in the Tamar estuary). The presence of both production and consumption

of CH_3I could be further reinforced by the linear correlation existing between CH_3I concentration and bacterial leucine incorporation rate in the two Live treatments. Although the linear relationship in the Live-Rolled treatment was statistically not significant ($p > 0.10$), CH_3I concentration did not decrease with increasing bacterial activity as exhibited in the Live-Unrolled treatment (see Figure 3.12a-b). These variations in bacterial activity may however depend on physical, chemical and biological conditions of each medium that determine bacterial growth pattern and the community structure.

3.4.5. Temporal change in CH_3I concentration (Study Two)

A second experiment (Study Two) was conducted with certain modifications (highlighted in section 3.3.3) to the experimental design of Study One.

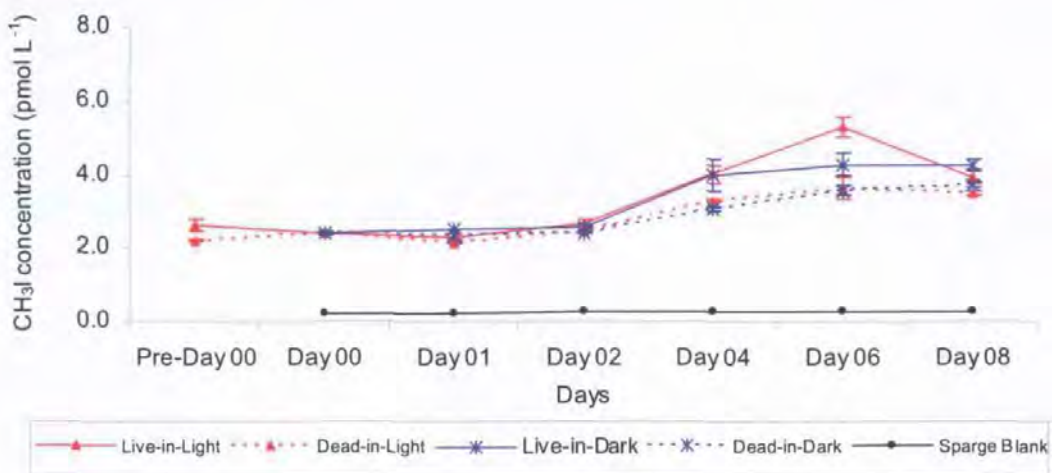

Figure 3.13: A plot of CH_3I concentration (pmol L^{-1}) from Study Two with error bars representing the standard deviation of three replicate samples. It shows changes in recorded CH_3I concentration between the various treatments and over the 8-day study period. A blank reading was taken each period prior to the analyses of the replicate samples and is also presented in this graph representing the stability of the analytical instrument.

Unlike the first study, results from Study Two (Figure 3.13) which included a comparison between rolling samples in the light and in the dark, presented no statistical difference between the Live and the Dead samples for the first 2 days (with the exception of samples

in light) but showed statistically different observations for the next 4 days (Between Day 2 and 6) (refer to Appendix 6). Peak CH_3I production for both treatments (light and dark) occurred at the same period (Day 6) as observed in Study One.

The rates of bacterially-mediated production of CH_3I were estimated as 0.35 ± 0.15 pmol L^{-1} d^{-1} and 0.38 pmol L^{-1} d^{-1} for aggregated samples in the dark (Live-in-Dark) and in the light (Live-in-Light) respectively. These were estimated from periods of exponential bacterial growth (day 2 to day 6 for Live-in-Light samples and day 2 to day 4 for Live-in-Dark samples). From these estimates, bacterially-mediated production of CH_3I in the presence of light is statistically indistinguishable ($p > 0.05$) from production in the dark.

The initial decrease in CH_3I concentrations for all the treatments observed during the first two days of Study One were however absent in Study Two. Comparison between results of Live samples (Live-in-Light) for Pre-Day 0 and Day 0 indicate that some CH_3I is lost (as observed in Study One) if samples are exposed to fluctuations in physical conditions (probably temperature). A decrease in environmental temperature during sampling for Study Two coupled with a close monitoring and prevention of such fluctuation experienced during Study One was therefore greatly reduced in the second study.

Results for the antibiotically treated samples, which were used as controls, indicate that either the effect of the antibiotics started wearing off much earlier than in the first Study or there was non-bacterial contribution to CH_3I production from day 4 of the experiment.

3.4.6. Rate of leucine incorporation by bacteria (Study Two)

The leucine incorporation rate by bacteria in Study Two (Figure 3.14) also followed the general pattern of bacterial growth. Periods of peak leucine incorporation occurred at different occasions for the two different treatments (Live-in-Light and Live-in-Dark).

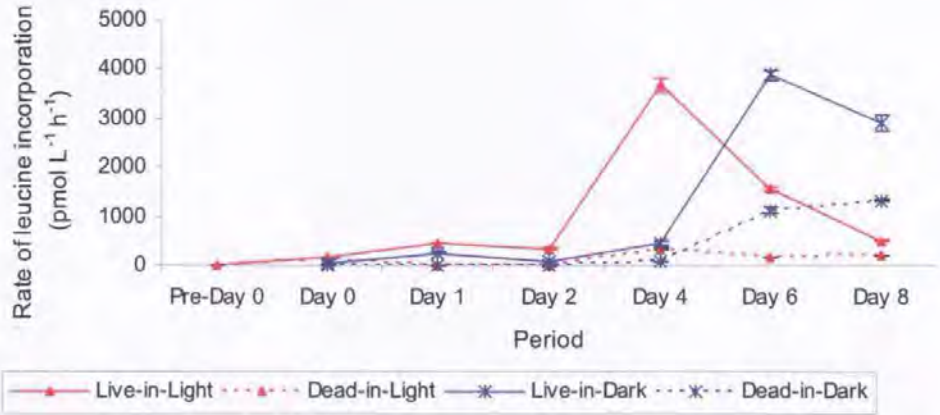

Figure 3.14: A graph showing the rate of leucine incorporation (pmol L^{-1} h^{-1}) by bacteria as observed in Study Two of the aggregation experiment showing changes in bacterial leucine uptake between the different treatments and over the study period.

That of the samples kept in the dark occurred on the sixth day (same as in Study One) whilst that of the samples kept in the light occurred on the forth day of the experiment. The maximum production rate of $36.7 \times 10^2 \pm 1.4 \times 10^2$ pmol L^{-1} h^{-1} and $38.9 \times 10^2 \pm 1.0 \times 10^2$ pmol L^{-1} h^{-1} recorded for Live samples in the light and in the dark, respectively, are statistically the same (P>0.10).

Although there is statistically no difference between the maximum production of the two treatments (Live-in-Light and Live-in-Dark), since they are both obtained from rolling Live samples, the much earlier occurrence of the peak leucine incorporation of Live-in-Light samples indicates that bacteria growth in light may be faster than that in the dark, which may be as a result of added DOC from primary production (Azam *et al.*, 1992; Ducklow & Carlson, 1992).

3.4.7. Bacterial abundance (Study Two)

Bacterial abundance obtained from Study Two was categorized into free-living and aggregate-attached bacteria. This was to enable the assessment of the proportion of

attached bacteria which from the first study, appeared to be the major contributors to bacterially-mediated CH_3I production.

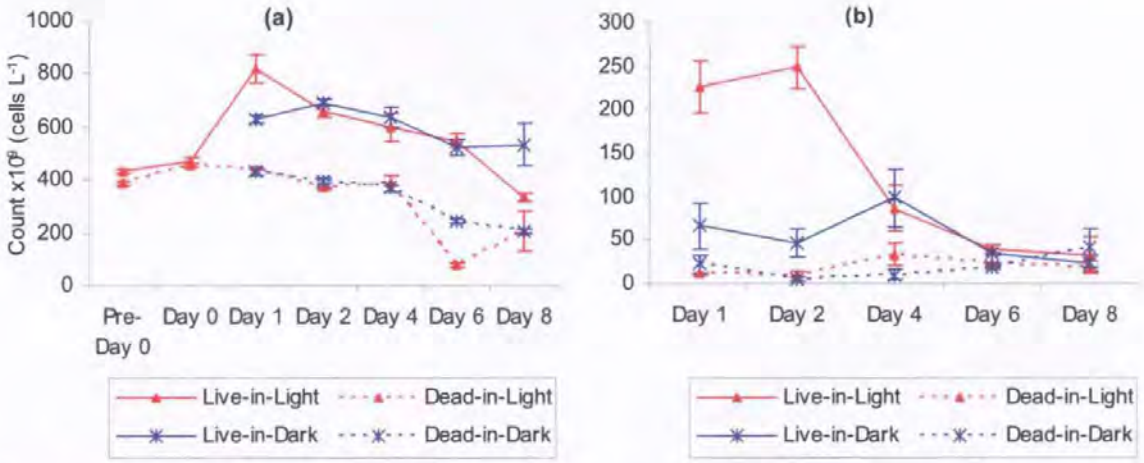

Figure 3.15a-b: A graph showing bacterial cell abundance (cells L^{-1}) of (**a**) free-living and (**b**) aggregate-attached bacteria recorded during Study Two of the aggregation experiment highlighting changes in bacterial abundance in the aggregated samples over the study period.

The results of free-living and aggregate-attached bacteria counts are shown in Figure 3.15a and 3.15b respectively. The summary of total, free-living and aggregate-attached bacterial cell counts for the four different treatments have also been presented in Table 3.3.

Table 3.3: A summary of bacterial cell counts from the four treatments; Live-in-Light, Live-in-Dark, Dead-in-Light and Dead-in-Dark during Study Two of the aggregation experiment.

	Range of bacterial count (x 10^{11} cells L^{-1})		
Treatment	**Total**	**Free-Living**	**Aggregate-Attached**
Live-in-Light	3.6 – 10.0	3.3 – 7.4	0.3 – 2.4
Live-in-Dark	5.6 – 7.4	5.4 – 6.4	0.2 – 1.0
Dead-in-Light	0.9 – 4.5	0.8 – 4.2	0.1 – 0.3
Dead-in-Dark	2.5 – 4.5	2.4 – 4.1	0.1 – 0.4

On the average, aggregate-attached bacteria represents about 37% of the total bacterial cell numbers (Figure 3.16a) over the study period. In the aquatic environment, although attached bacteria are more active than their free-living counterparts (Grossart *et al.*, 2007;

Ghiglione *et al.*, 2007), their abundance is generally lower than the free-living forms. For example in literature, attached-bacteria constitute < 6.8% (Unanue *et al.*, 1992), ≤ 10% (Griffith *et al.*, 1994) and ≤ 22 % (Iriberri *et al.*, 1987) of the total bacterial abundance. Therefore the recorded lower aggregate-attached bacterial abundance reported in this study is not unusual. Since higher production of CH_3I from this study has been attributed to the presence of aggregates, it implies that attached bacteria may be responsible for most of the microbially-mediated CH_3I production making them more metabolically active than the free living bacteria (Turley & Stutt, 2000).

It is important to state that recorded total bacterial abundances from Study Two were at least $x10^3$ L^{-1} higher than those recorded in Study One. This may be due to changes between the first and second field sampling in terms of SPM loading, nutrient availability and bacterial population associated with the TMZ. This may have resulted in the first water sample having a lower bacterial abundance compared to the second water sample.

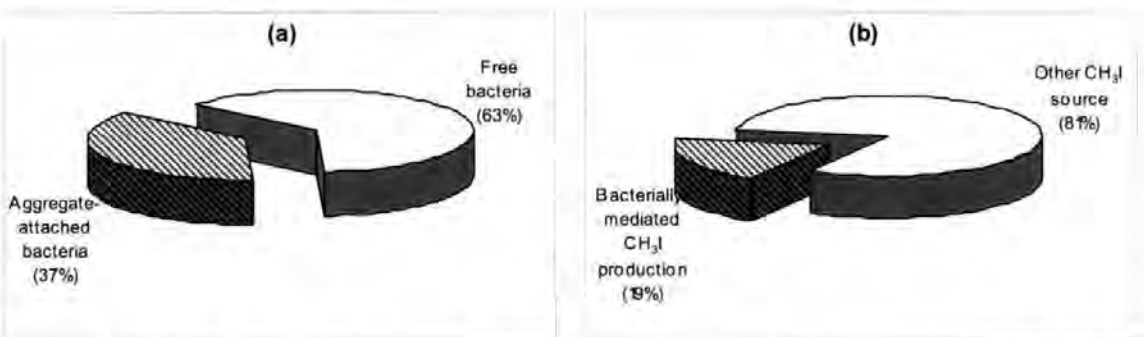

Figure 3.16a-b: A graph showing the percentages in (**a**) attached-bacteria to the total bacteria and (**b**) contribution of bacteria to total observed CH_3I production in the experimental medium (Study Two). The 81% CH_3I from other sources in (b) include background CH_3I concentration at day 0 (part of which may have also been microbially produced).

Observation of cell abundance in both cases (free-living and aggregate-attached bacteria) showed a general decline after a small initial increase with respect to time over the study period. This was not reflected in the pattern of their leucine incorporation rate during the same period. It therefore indicates that bacterial growth during this experiment was not as a

result of increase in cell numbers as observed in the previous experiment. The results of attached bacteria for samples in the dark however conformed to the pattern observed in the first experiment.

3.4.8. Integration of Study Two

As in Study One, analytical results from the measured variables in Study Two were statistically treated using the Pearson's correlation. A summary of the statistical analyses is presented in Table 3.4 whilst the relationships of interest have been graphically presented in Figures 3.17 and 3.18.

Table 3.4: A Pearson correlation matrix of the measured variables for the various experimental treatments during Study Two. Values in bold are p-values of the analyses with those significant at 5% underlined. [CH_3I] = CH_3I concentration (pmol L^{-1}), B.Nos = bacterial abundance (cells L^{-1}), ^3H = bacterial leucine incorporation (pmol L^{-1} h^{-1}), RC[CH_3I] = rate of change of CH_3I (pmol L^{-1}), P/N = bacterial specific activity (pmol $cell^{-1}$ h^{-1}).

Live samples in light (Live-in-Light)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	-0.636			
	0.011			
^3H	0.473	-0.167		
	0.075	**0.551**		
RC[CH_3I]	-0.443	0.677	0.327	
	0.098	**0.006**	**0.234**	
P/N	0.588	-0.335	0.979	0.166
	0.021	**0.222**	**0.000**	**0.555**

Live samples in the dark (Live-in-Dark)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	-0.523			
	0.045			
^3H	0.740	-0.783		
	0.002	**0.001**		
RC[CH_3I]	-0.576	0.521	-0.524	
	0.025	**0.046**	**0.045**	
P/N	0.721	-0.810	0.993	-0.526
	0.002	**0.000**	**0.000**	**0.044**

Dead samples in light (Dead-in-Light)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	-0.731			
	0.002			
^3H	0.781	-0.225		
	0.001	**0.420**		
RC[CH_3I]	-0.524	0.452	-0.332	
	0.045	**0.090**	**0.227**	
P/N	0.866	-0.880	0.599	-0.429
	0.000	**0.000**	**0.018**	**0.110**

Dead samples in the dark (Dead-in-Dark)

	[CH_3I]	B.Nos	^3H	RC[CH_3I]
B.Nos	-0.944			
	0.000			
^3H	0.912	-0.953		
	0.000	**0.000**		
RC[CH_3I]	-0.635	0.629	-0.719	
	0.011	**0.012**	**0.003**	
P/N	0.904	-0.954	0.998	-0.744
	0.000	**0.000**	**0.000**	**0.001**

Since all treatments used in Study Two involved the simulated aggregation procedure (as outlined in section 3.3.2), a comparison between CH_3I concentration and bacterial specific activity in all treatments were all statistically correlated (positive linear relationship) and significant at the 5% level (Figure 3.17a-d) although this relationship was weak for Live-in-Light treatment ($r^2 = 0.346$). This meant that just as expected based on the results of Study One (Live-Rolled treatment), an increase in bacterial activity corresponded to an increase in CH_3I concentrations in all four treatments.

This (independent of temperature) supports the observation made in Study One and hence the elevation in CH_3I concentration could be attributed to bacterial-aggregate relationship in the experimental media. This observation was also true for the two Live treatments (Live-in-Light and Live-in-Dark) based on the correlation between CH_3I concentration and bacterial leucine incorporation rate (Figure 3.18a-b). However, the relationship was statistically significant only in the Rolled samples in the dark (Live-in-Dark).

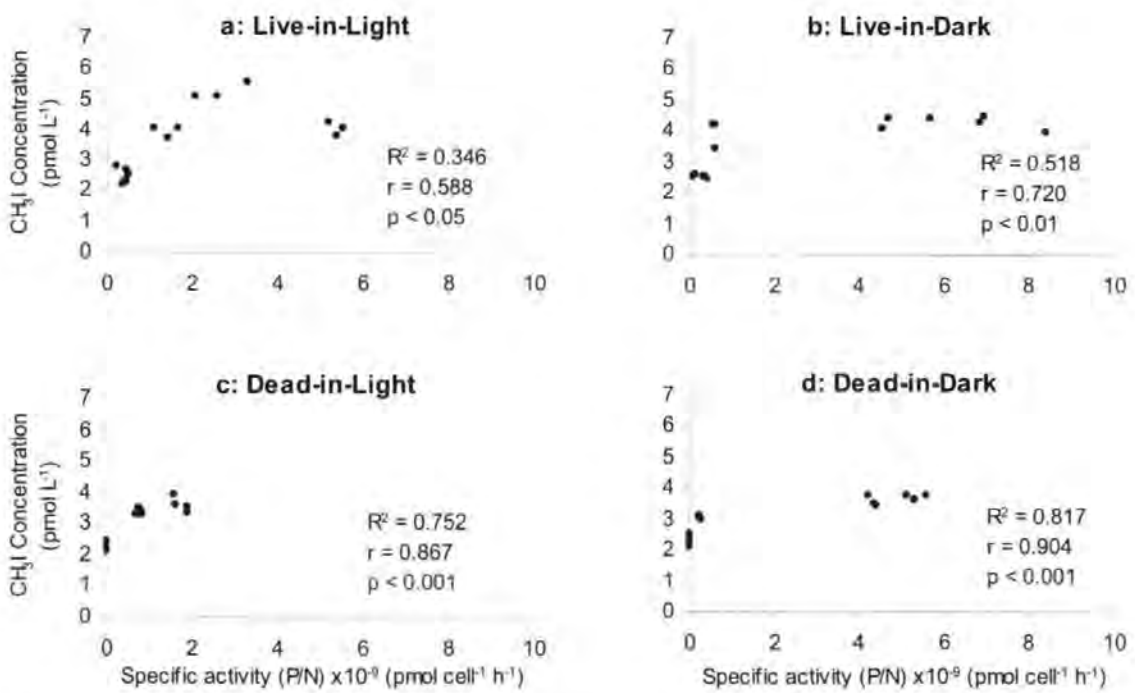

Figure 3.17a-d: Graphs showing the relationships between CH_3I concentrations (pmol L^{-1}) and bacterial specific activity (pmol $cell^{-1}$ h^{-1}) over the study period (Study Two). (a) Live-in-Light, (b) Live-in-Dark, (c) Dead-in-Light, (d) Dead-in-Dark.

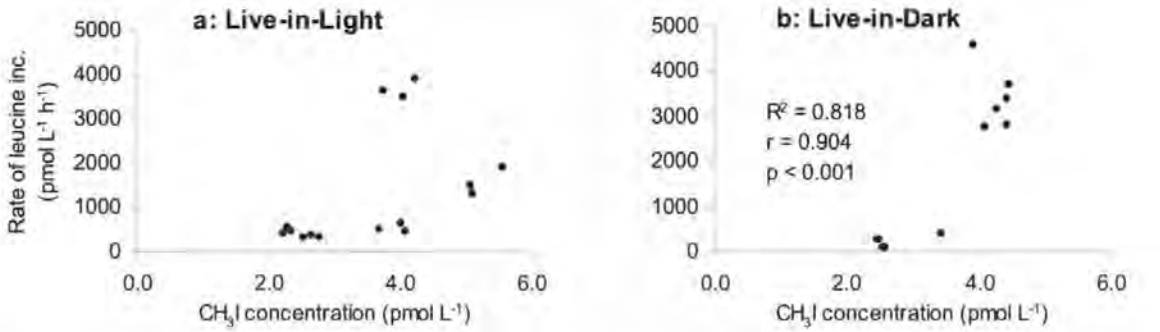

Figure 3.18a-b: Graphs showing the relationships between the rate of bacterial leucine incorporation (pmol L^{-1} h^{-1}) and CH_3I concentrations (pmol L^{-1}) over the study period (Study Two). (a) Live-in-Light, (b) Live-in-Dark.

Beside the Live samples, the significant linear relationship found between CH_3I concentration and bacterial specific activity amongst the two treated sets of samples (see Figure 3.17c and 3.17d) may be (as already mentioned) due to the effect of the antibiotics becoming less effective much earlier than in the first study.

3.4.9. Role of bacterial-aggregate association in CH_3I concentration changes

Bacterial association with aggregates in the aquatic environment until now has never been linked to the production of halocarbons in general and CH_3I in particular. This association however has been shown to be a source of aquatic CH_3I based on the results obtained in both studies. This deduction is based on the comparison between Rolled samples that were antibiotically treated and those that were not treated, yielding a maximum net concentration difference of 1.64 pmol L^{-1} of CH_3I during the course of the study (refer to Figure 3.5).

Even though the dynamics obtained from rolling has been shown to greatly contribute to increases in the rate of bacterial activity, a summation of bacterial leucine incorporation rate for each time point (for both the Rolled and Unrolled Live samples) over the study period of the first study stood at $21.0 \times 10^2 \pm 1.8 \times 10^2$ pmol L^{-1} h^{-1} and $20.3 \times 10^2 \pm 4.7 \times 10^2$ pmol L^{-1} h^{-1} respectively with no statistical difference between them. This suggests that

aggregates in the experimental medium may have enhanced the rate at which CH_3I was produced by bacteria. In the long term however (e.g. the 14 days duration used in this study), the activities of free-living and aggregate-attached bacteria per unit volume of water may not necessarily be different.

From section 3.4.1, the net average rate of microbially-mediated production of CH_3I was estimated at 0.42 ± 0.09 pmol L^{-1} d^{-1} by a mean bacterial abundance of $0.66 \pm 0.32 \times 10^9$ cells L^{-1} (see section 3.4.3). From this, the total global production of CH_3I by bacteria in the marine environment could be estimated. To do this, the total global bacterial abundance in the marine environment is required. With the global bacterial abundance of the upper 200 m of the ocean estimate as approximately 3.6×10^{28} cells (Whitman *et al.*, 1998), the projected net global microbial production of CH_3I in the upper 200 m of the ocean (in excess of the difference between the gross global microbial CH_3I production and removal) will stand at an estimated $1.7 \pm 0.5 \times 10^{12}$ g y^{-1}.

By comparing the rate of bacterial leucine incorporation, the rate of CH_3I concentration changes and bacterial specific activity with CH_3I concentrations revealed that not only did bacterial activity in the experimental media result in the release of CH_3I but also contribute to the removal of CH_3I during the study.

Hypothetically, a number of scenarios could explain this observation: (1) the presence of two different groups of microbial community (one group producing and the other consuming CH_3I) in the same sample; (2) the same microbial community switching between production and consumption depending on changes in physical, chemical and biological conditions in the medium at any given period; (3) grazing (Turley & Mackie, 1994; Zubkov *et al.*, 2000a) by heterotrophic nanoplankton associated with aggregates (Caron *et al.*, 1982) on CH_3I producing bacteria at periods when bacterial abundance and

growth are at their threshold. This makes all the observed concentrations of CH_3I during the experimental period the net concentration between the two different activities.

Attached bacteria from the results obtained during the second study (Figure 3.15b) represented on average, about 37% of the total bacterial population (see Figure 3.16a). Since the activity of aggregate-attached bacteria often has been found to be higher than that of the free-living forms (Alldredge & Youngbluth, 1985; Turley & Stutt, 2000), they may be responsible for the production of most of the observed CH_3I. Bacterial activity accounted for 19% of the total recorded CH_3I concentration (Figure 3.16b) during the study period. Bacterial association with aggregates may therefore result in both the production and consumption of CH_3I in aquatic systems with variations in the net production-consumption attributed to physical, chemical and biological conditions at any given time.

3.4.10. Effect of changes in experimental conditions: Light and Temperature

Changes in physical, chemical and biological conditions affect the activity of bacteria in natural water (e.g. Pomeroy & Deibel, 1986; Turley, 1993). In order to eliminate the possibilities of any such effects resulting from variations in experimental conditions, samples were run both in light and in the dark at an *in situ* temperature during the second experiment. The results (shown in Figure 3.13 and 3.14) indicate that variation in light conditions has an impact on the pattern of bacterial activity, but little effect on their rate of CH_3I production, especially in a short-term period such as during the experiments. These observations were made when maximum bacterial production rates in the light and in the dark ($36.7 \times 10^2 \pm 1.4 \times 10^2$ pmol L^{-1} h^{-1} and $38.9 \times 10^2 \pm 0.9 \times 10^2$ pmol L^{-1} h^{-1} respectively) were statistically found to be the same, but occurred at different periods. This observation implies that bacteria may grow faster in light than in the dark (as explained in section 3.4.6) but that light may have very little effect on their rate of CH_3I production.

On the other hand, since the experimental design involved carrying out the aggregation experiment at the *in situ* temperature, the effect of temperature on bacterial production of CH_3I in natural water could not be discussed. Although changes in temperature (from about 17 °C *in situ* through air temperature of 24 °C and settling down to 15 °C in the culture cabinet) at the early stages of the first study may have contributed to the decrease (Figure 3.5), the effect seems to be more physical than biological. This may be due to the difference in temperature between the field and the laboratory which may have affected the solubility of CH_3I in the water samples thereby leading to CH_3I loss. This assumption was arrived at as a result of the disappearance of this anomaly in the second study which was performed under closely monitored *in situ* temperature environment.

3.5. Summary and conclusions

This study was conducted to investigate two prime questions; (a). Are estuarine aggregates a significant source of CH_3I? (b). Are microbial communities associated with estuarine aggregates significant contributors to the observed CH_3I production in the estuarine environment?

Results from the two studies conducted indicated that bacterial association with estuarine aggregates resulted in a significant production of CH_3I. From the observations made, estuarine aggregates serve as a significant source of CH_3I through bacterial degradation of organic matter. This makes bacteria, potentially important contributors to CH_3I cycling in the environment. Since the rate of production of CH_3I by bacteria was found to be enhanced by the presence of macroscopic aggregates by 7%, it makes an estuary potentially an ideal environment for bacterially-mediated CH_3I production. The effect of light on the rate of bacterially-mediated CH_3I production was found to be insignificant and that of temperature inconclusive. Based on these deductions, it was concluded that;

1. Bacterial association with POM in an estuarine environment results in the net production of CH_3I.

2. The rate of bacterial CH_3I production is enhanced by the process of aggregate formation in an estuarine environment.

3. Microbially-mediated CH_3I production in estuarine systems may be important in the global CH_3I cycle.

CHAPTER FOUR

The development of an analytical procedure for assaying bacterial utilization of methyl iodide in estuarine water

4. The development of an analytical procedure for assaying bacterial utilization of methyl iodide in estuarine water

4.1. Introduction

Previous studies have shown (see Chapter One) that bacteria are capable of using organic compounds including methyl halides as a source of carbon. It is uncertain whether CH_3I could be used as a sole carbon and electron source by bacteria, even though a few papers (e.g. Connell Hancock *et al.*, 1998; Schaefer & Oremland, 1999) have mentioned its effect as a co-metabolite alongside CH_3Br and CH_3Cl. However, no published method for assaying and assessing CH_3I utilization by bacteria in aquatic environments exists. Therefore a method to quantify CH_3I utilization by bacteria was developed.

Bacteria may utilize organic compounds in two different ways; (1), for growth in the form of protein and tissue synthesis and (2), for the production of energy through respiration and oxidation. It was therefore assumed that the CH_3I substrate which is removed by bacteria during an incubation experiment may end up in three locations; (1), in the cells as carbon (C) or (2), respired (oxidized) as CO_2, or (3) unused CH_3I in the experimental medium.

It was therefore imperative to develop an analytical technique which could be used to address these two different pathways. The method to be developed should be capable of determining the end products and quantity of the remaining CH_3I substrate unused by the bacterial community remaining in the experimental medium. This chapter describes the development of such a method.

4.2. Method

4.2.1. Developing a reliable method

4.2.1.1. Bacterial oxidation of substrate

A method to assay methyl bromide utilization by bacteria has been published by Connell *et al.* (1997) and a similar method has been used by Goodwin *et al.* (1998) to estimate bacterial oxidation of dibromomethane (CH_2Br_2) and CH_3Br in natural waters. As CH_3I is more easily oxidized than CH_3Br, these methods could be adapted and modified to determine CH_3I oxidation by bacteria. In this method the main volatile end-product (carbon dioxide) from the oxidation of a [^{14}C] radiolabelled methyl halide is trapped in a solution containing sodium hydroxide and strontium chloride hexahydrate. Dissolved sodium and hydroxyl ions react with carbon dioxide to produce bicarbonate which is then precipitated by strontium chloride hexahydrate as strontium carbonate as shown below.

$$^{14}CO_2 + 2NaOH \longrightarrow Na_2{}^{14}CO_3 + H_2O \quad \textbf{(4.1)}$$

$$Na_2{}^{14}CO_3 + SrCl_2.6H_2O \longrightarrow \mathbf{Sr^{14}CO_3} + 2NaCl + 6H_2O \quad \textbf{(4.2)}$$

4.2.1.2. Bacterial cell incorporation of substrate

Smith & Azam (1992) developed a concise method to determine bacterial protein synthesis using ^3H-leucine, or ^3H-thymidine, as the radiolabelled carbon source. This method has proven to be very effective in tracking the fraction of radiolabelled carbon that ends up in bacterial cells. There is therefore a strong possibility that by adapting this method, the fraction of other radiolabelled carbon sources (e.g. CH_3I) that are incorporated into bacterial cells could also be determined.

The current work developed a method to determine both the bacterial removal and utilization of CH_3I in natural water samples by modifying the methods proposed by Smith & Azam (1992) and Connell *et al.* (1997).

4.2.2. Experimental design one

Undertaking a [^{14}C] CH_3I experiment involving bacterial activity in incubation vials required an initial knowledge of the bacterial response to $^{14}CH_3I$ and the sensitivity of the technique. Since no analytical information had been found in the literature, it was necessary to undertake a preliminary experiment to obtain this important information.

The initial approach (Experiment 1.1) introduced low concentrations of $^{14}CH_3I$ (Amersham Biosciences UK) denoted as $^{14}CH_3I_{(i)}$ into natural water samples and incubated them for two different durations (3 and 6 hours) in order to ascertain the response of the bacterial community present to the added substrate. Three different substrate concentrations were employed in this initial incubation experiment to assess three different end stages (denoted as targets) using the adapted methods. These targets were;

1. The mass of substrate that was incorporated by bacterial cells after incubation denoted as ^{14}C (using Smith & Azam, 1992).
2. The mass of substrate that was converted to carbon dioxide after incubation denoted as $^{14}CO_2$ (using Connell *et al.*, 1997).
3. The mass of substrate that remained unused after incubation denoted as $^{14}CH_3I_{(f)}$.

By assuming that $^{14}CH_3I_{(i)}$ will be equally distributed among the three different targets during processing, each target was expected to generate enough radioactive disintegration to be recorded by the LSC. The minimum concentration to be used for the experiments should be at least three times the detection limit of the instrument. The initial substrate

concentration chosen was 6 nmol L^{-1} in order to ensure detection by the LSC. Natural water samples for the experiment were obtained from the Tamar estuary (on 26th July and 16th August 2004 for Experiments 1.1 and 1.2 respectively), using glass bottles at the turbidity maximum zone at high tide. These were stored at the *in situ* temperature in the laboratory prior to use. The initial concentrations (See Table 4.1 and 4.2) were added into labelled microcentrifuge tubes, 2 mL of water sample added and then incubated. For the control sample tubes, 2 mL of an autoclaved water sample was used instead.

As discussed in the results section, the substrate concentration used in Experiment 1.1 (presented in Table 4.1) did not provide any statistically significant difference between the proportions used by bacteria from the three different added concentrations. Therefore higher concentrations (presented in Table 4.2) chosen to yield distinguishable results were used for Experiment 1.2.

Table 4.1: Different substrate concentrations and incubation durations used in Experiment 1.1 (ctls = control samples, repls = replicate samples).

Duration (Hours)	Added $^{14}CH_3I$ substrate concentration (nmol L^{-1})		
	6	**17**	**43**
3	3 ctls + 5 repls	3 ctls + 5 repls	3 ctls + 5 repls
6	3 ctls + 5 repls	3 ctls + 5 repls	3 ctls + 5 repls

Table 4.2: Different substrate concentrations and incubation durations applied in Experiment 1.2 (ctls = control samples, repls = replicate samples).

Duration (Hours)	Added $^{14}CH_3I$ substrate concentration (nmol L^{-1})		
	1853	**4475**	**8110**
3	3 ctls + 5 repls	3 ctls + 5 repls	3 ctls + 5 repls
6	3 ctls + 5 repls	3 ctls + 5 repls	3 ctls + 5 repls

Each analysis was divided into three separate but continuous stages as shown in the schematic diagram (Figure 4.1).

NB:

The use of the term **Complex** henceforth denotes a solution of ($NaOH + Na_2CO_3 + SrCl_2.6H_2O$)

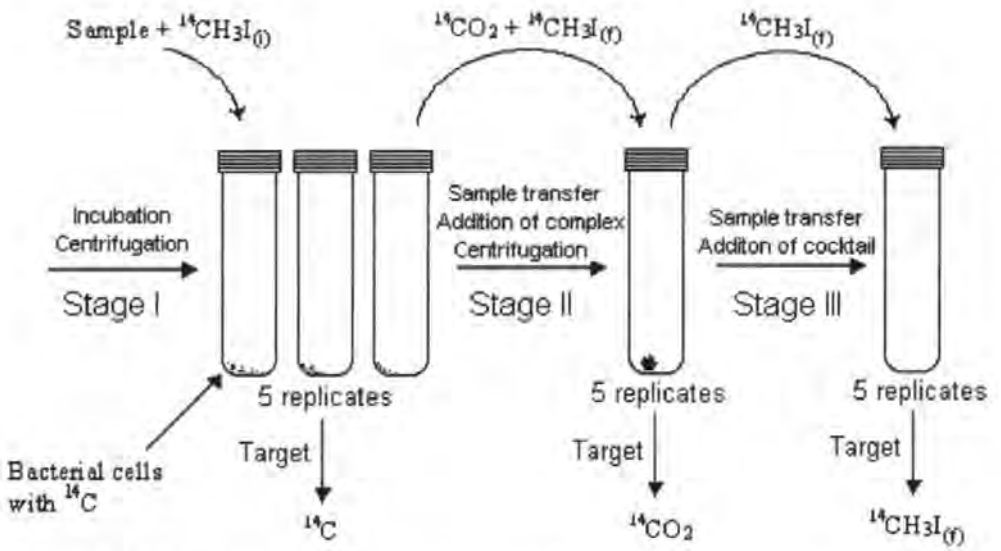

Figure 4.1: A schematic diagram showing the stage by stage assaying of radiolabelled CH_3I by bacteria present in replicate experimental media.

4.2.2.1. Practical procedure one

STAGE I

Water samples were added to microcentrifuge tubes containing $^{14}CH_3I_{(i)}$ substrate which was followed by vortex mixing to homogenize the samples and incubation for the specified duration at the *in situ* temperature (13.1 and 13.7 °C for Experiments 1.1 and 1.2 respectively) in the dark (incubator). At the end of the incubation period, the samples were centrifuged at 12000 rpm for a period of ten minutes in order to obtain the proportion of $^{14}CH_3I_{(i)}$ that is incorporated into bacterial cells.

STAGE II

After centrifugation, 1 mL of supernatant from each replicate sample tube was immediately pipetted into a second set of labelled microcentrifuge tubes already containing a mixture of 20 µl of NaOH, 100 µl of Na_2CO_3 and 500 µl of $SrCl_2.6H_2O$. The sample tubes from this stage were then vortex mixed and centrifuged as described for stage 1 in order to target the proportion of $^{14}CH_3I_{(i)}$ converted by the bacteria to $^{14}CO_2$.

STAGE III

From each tube, 1 mL of supernatant was pipetted into a third set of microcentrifuge tubes. Optiphase "HiSafe" 3 scintillation cocktail (1 mL) was added to each samples and vortex mixed to create a gel. This was to estimate the amount of added substrate that remained unused in the experimental medium ($^{14}CH_3I_{(f)}$) after the incubation period. All the remaining supernatants in stages I and II were aspirated after the centrifugation process and the tubes washed by the addition of 1.5 mL of Milli-Q water to remove any excess substrate remaining in the tubes. These were vortex mixed, centrifuged and aspirated again.

Each sample tube was then filled with 1 mL of scintillation cocktail and together with the other samples, kept in the dark for at least thirty minutes before being analysed with the aid of a LSC. As a measure of quality control, three replicates for each concentration used during incubations were pipetted from the stock into clean microcentrifuge tubes and 1 mL of scintillation cocktail was added and analysed together with the experimental samples. The total amount of radiolabelled substrate degraded by the bacterial community was estimated from results of stage I and II samples and the balance checked against the total added amount.

4.2.3. Experimental design two

After the application of the first experimental design (Experiments 1.1 and 1.2), lower than expected percentage substrate recoveries were obtained (a maximum of 23% during a 3-hour incubation). It was therefore necessary to revise the experimental procedure in order to improve the substrate recovery. This resulted in a second experimental approach (see Figure 4.2), the purpose of which was to identify the sink for the unaccounted substrate henceforth denoted as $[^{14}CH_3I_{(i)}]$Lost. This section summarizes the changes made to the first experimental design.

Table 4.3: Experimental conditions for the second experimental design (Experiments 2.1 and 2.2)

Experimental Conditions	
Incubation duration used	one hour
Incubation temperature (*in situ*) for Experiment 2.1	18.4 °C
Incubation temperature (*in situ*) for Experiment 2.2	11.6 °C
Initial substrate concentration (Experiment 2.1)	2186 nmol L^{-1}
Initial substrate concentration (Experiment 2.2)	492 nmol L^{-1}
Volume of water sample used	2 mL
Number of replicates	4
Number of controls	3

There were three substrate distribution targets involved just as in experimental design one;

1. Amount of substrate that ended up in bacterial cells after incubation = ^{14}C
2. Amount of substrate that ended up as gaseous carbon after incubation = $^{14}CO_2$
3. Amount of substrate that remained unused after incubation = $^{14}CH_3I_{(f)}$

4.2.3.1. Practical procedure two

The technique applied in the new experimental design involved the use of three sets of samples (A, B, and C), each designed to estimate one of the substrate targets (instead of one sample set for all three substrate targets used in experimental design one). This

approach eliminated the extensive time spent on the same sample to analyze all the substrate distribution targets.

NB:

1. Addition of substrate and incubation of A, B, and C took place simultaneously.
2. After the end of each stage (I, II, and III) in A, B, and C as shown in the experimental design (see Figure 4.2), there was the addition of scintillation cocktail to the samples, which were then left in the dark for at least thirty minutes and then counted for C-14 disintegration.

With sample set (A), replicate samples were analysed from Stage I to III as in experimental design one.

With sample set (B), replicate samples were analysed only up to Stage II. At Stage I, there was the addition of the complex and centrifugation was carried out immediately after incubation to obtain both the $^{14}CO_2$ precipitate and bacterial cells with ^{14}C in one step. At Stage II, scintillation cocktail was added to the supernatant from Stage I to obtain $^{14}CH_3I_{(f)}$.

With sample set (C), replicate samples were analysed at a single Stage. Scintillation cocktail was added immediately after incubation to obtain all three substrate targets together.

The estimation of the three substrate distribution targets was achieved using results from all sample sets. ^{14}C was estimated from sample set A, $^{14}CO_2$ from sample set (B) and $^{14}CH_3I_{(f)}$ from sample set (C) using the following eduations;

$[^{14}C] = [^{14}C]$ **(A)**

$[^{14}CO_2] = [^{14}C + {}^{14}CO_2]$ **(B)** $- [^{14}C]$ **(A)**

$[^{14}CH_3I_{(f)}] = [^{14}C + {}^{14}CO_2 + {}^{14}CH_3I_{(f)}]$ **(C)** $- [^{14}C + {}^{14}CO_2]$ **(B)**

Hence;

$[^{14}C] + [^{14}CO_2] + [^{14}CH_3I_{(f)}]$ is expected to be equal to $[^{14}CH_3I_{(i)}]$

And;

$[^{14}CH_3I_{(i)}] - \{[^{14}C] + [^{14}CO_2] + [^{14}CH_3I_{(f)}]\} = [^{14}CH_3I_{(i)}]$Lost.

Conducting this experiment requires that the analytical instrument involved (LSC) is capable of providing consistent readings from the samples run. This was achieved by performing a system calibration using a set of known concentrations of CH_3I. The result of this is provided below together with calculated values of the detection limit and the relative standard deviation (RSD) of the instrument.

Mean Blank Conc. = 0.095 ± 0.005 nmol L^{-1}

System Detection Limit = Mean Blank Conc. + (3 x SD)

= $0.095 + (3 \times 0.005) = 0.11$ nmol L^{-1}

Instrumental RSD = $(0.005/0.095) \times 100 = 5.2\%$

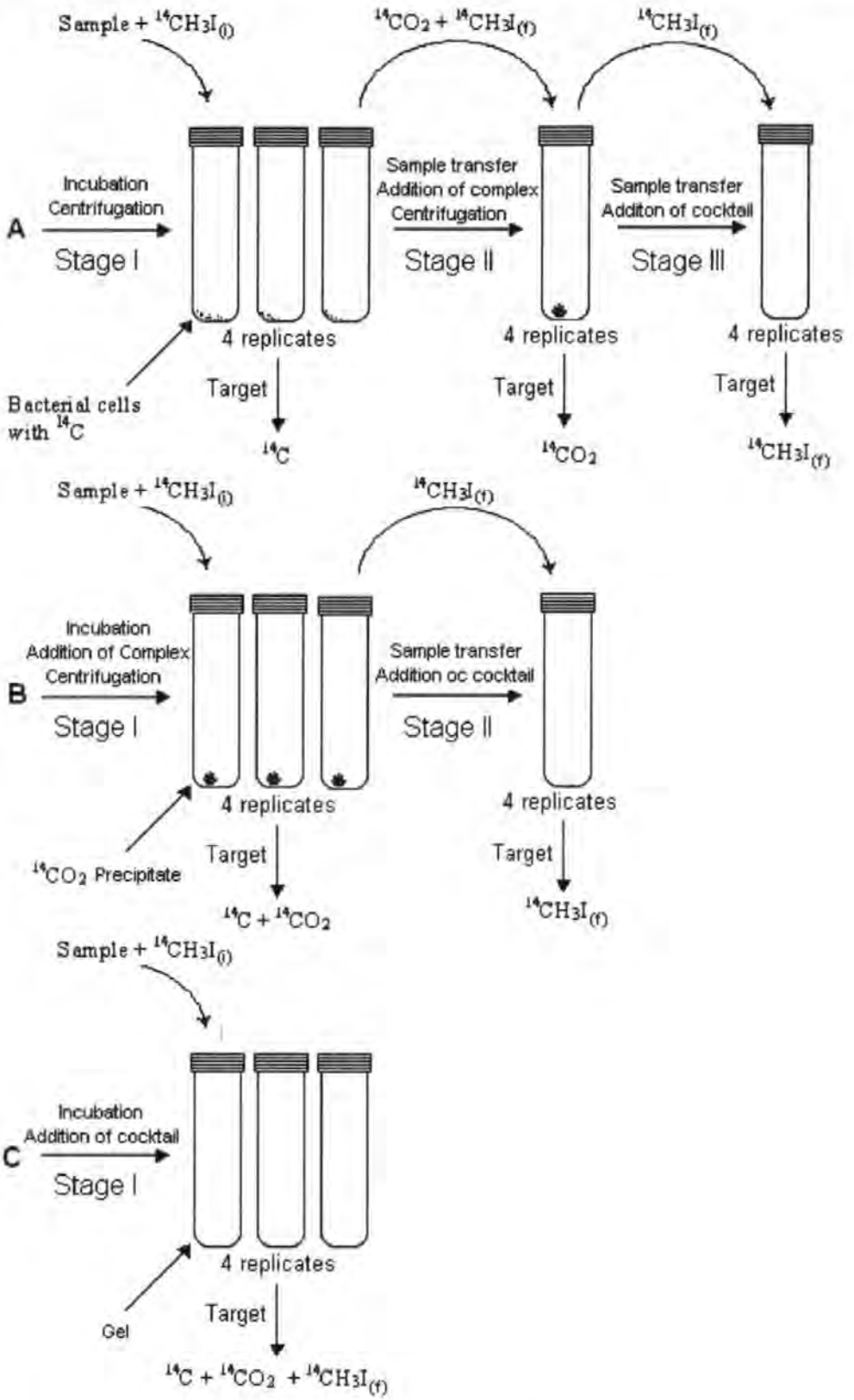

Figure 4.2: A schematic diagram showing the sequence and stage by stage assaying of utilized $^{14}CH_3I$ by bacteria present in replicate experimental media after experimental modifications.

4.3. Results and Discussion

In this section, the results and discussion from the analytical development using both experimental approaches are presented. These include findings from both Experiments 1.1 and 1.2 carried out using the first experimental approach and Experiments 2.1 and 2.2 carried out using the second experimental approach.

4.3.1. Bacterial methyl iodide substrate (nmol L^{-1}) uptake (Experiment 1.1)

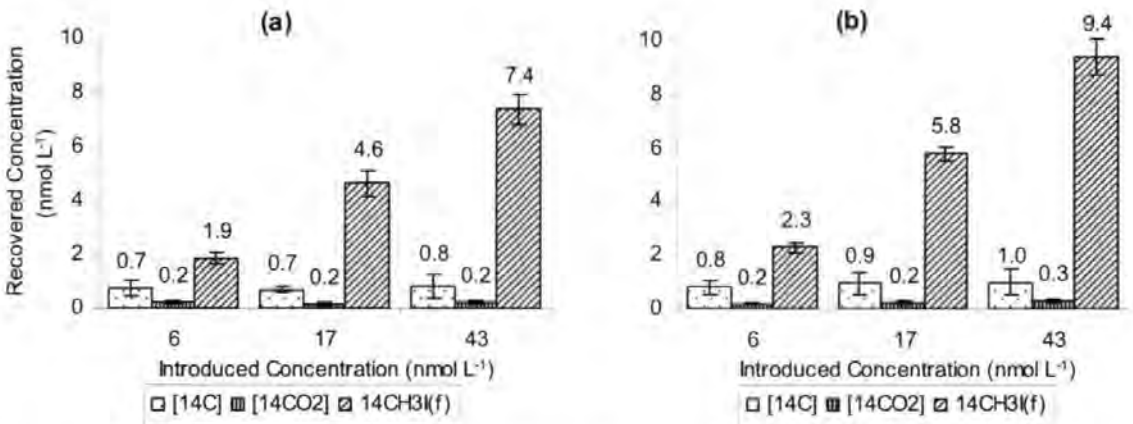

Figure 4.3a-b: Bacterial CH_3I utilization during (a) three hours and (b) six hours incubation period using the first experimental approach (Experiment 1.1) in order to investigate bacterial response to radiolabelled CH_3I as a substrate upon introduction.

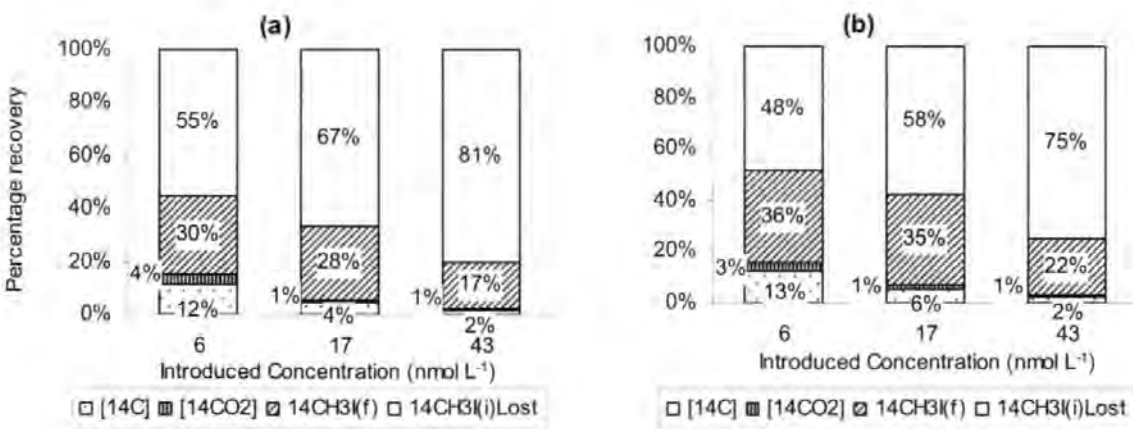

Figure 4.4a-b: Percentage recoveries of the various substrate targets of bacterial CH_3I utilization during (a) three hours and (b) six hours incubation period and with three different substrate concentrations using the first experimental approach (Experiment 1.1)

From the results of experiment 1.1, an unusually large portion of the $^{14}CH_3I_{(i)}$ (up to 81%) was unaccounted for (see Figure 4.4a-b). However, the results for all the three different $^{14}CH_3I_{(i)}$ substrate concentration levels used (presented in Figure 4.3a-b) indicated bacterial response to the addition of ^{14}C-radiolabelled CH_3I. During the experiment, it was expected that results would show an increase in concentration of ^{14}C and $^{14}CO_2$ with an increase in $^{14}CH_3I_{(i)}$. However, there were no statistically distinguishable differences between bacterial responses from the three different concentrations used in both the 3-hour and 6-hour durations of incubation (see Appendix 7). From these observations, it could be deduced that due to the low substrate concentrations used in Experiment 1.1, there were no detectable differences in bacterial response. Therefore higher substrate concentrations are required to proceed with the experiment.

4.3.2. Bacterial methyl iodide substrate (nmol L^{-1}) uptake (Experiment 1.2)

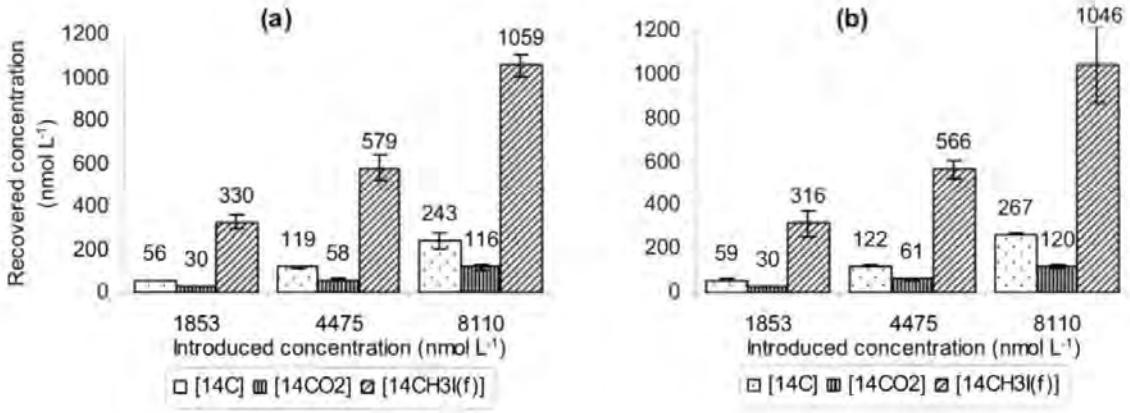

Figure 4.5a-b: Bacterial response to the three different concentrations of radiolabelled CH_3I substrate for (a) three hours, (b) six hours shown as the recorded concentration for the various substrate targets (Experiment 1.2).

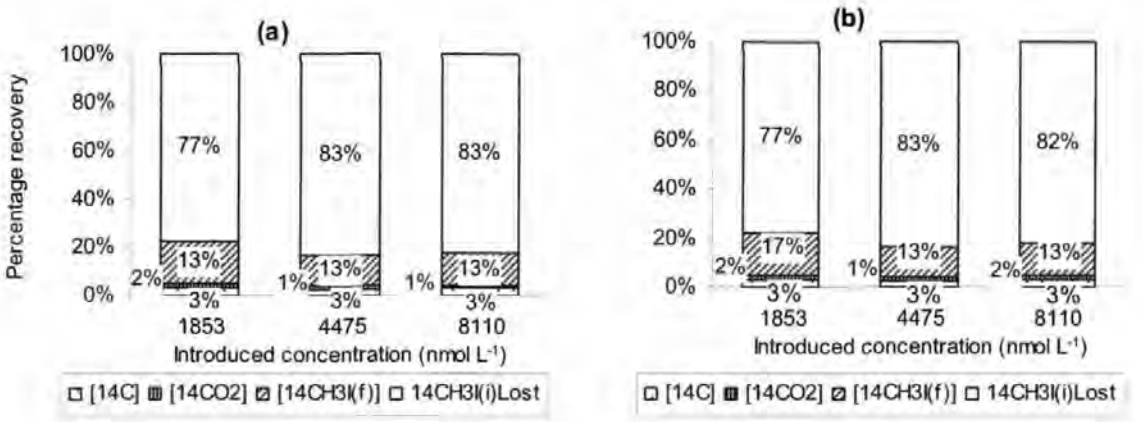

Figure 4.6a-b: Percentage recoveries of the various substrate targets of bacterial CH_3I utilization during (a) three hours, (b) six hours incubation period with three different substrate concentrations using the first experimental approach (Experiment 1.2).

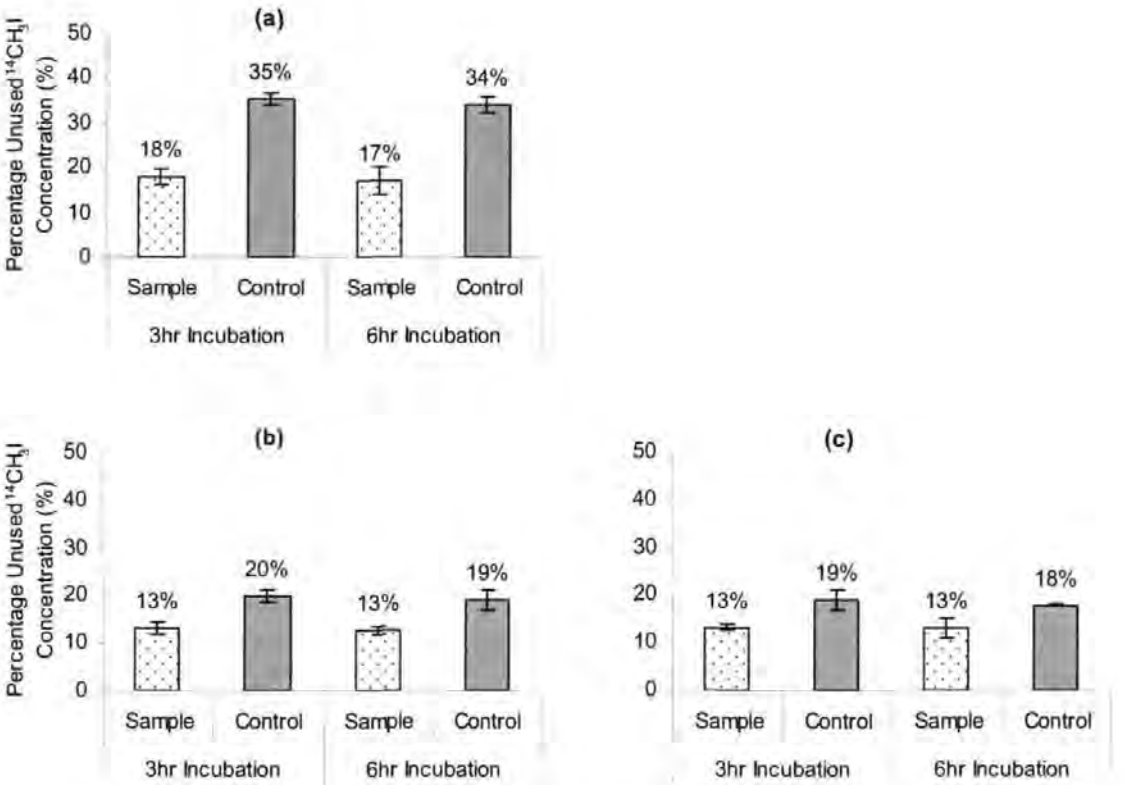

Figure 4.7a-c: Comparisons of the recorded percentages of unused $^{14}CH_3I$ concentration (sample and control) for three different added substrate concentrations (a) 1853 nmol L^{-1}, (b) 4475 nmol L^{-1} and (c) 8110 nmol L^{-1} at two different incubation times (three and six hours) using the first experimental approach (Experiment 1.2).

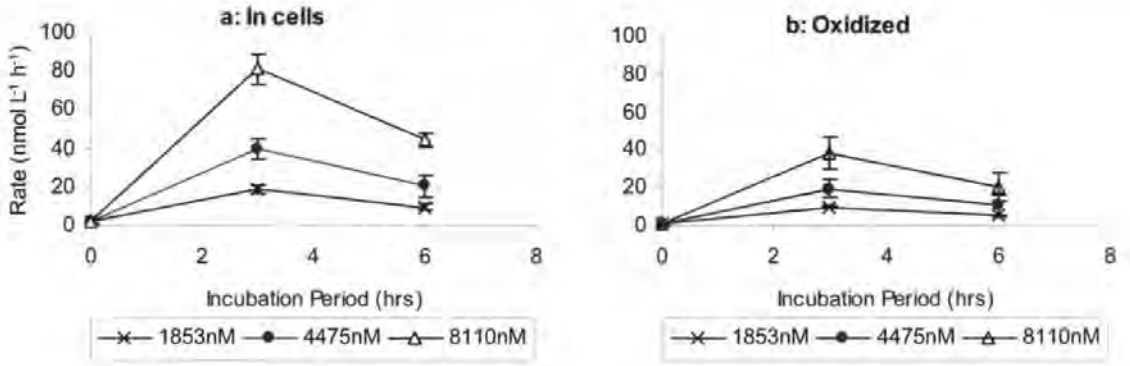

Figure 4.8a-b: Comparison of the rates of bacterial removal of CH_3I (a) recorded in cells (b) recorded as oxidized in the experimental media upon the introduction of three different CH_3I substrate concentrations (1853, 4475, 8110 nmol L^{-1} (denoted as nM)) and incubated for three different time periods (t = 0, 3, 6 hours respectively) using the first experimental approach (Experiment 1.2).

From the results of Experiment 1.2 (Figure 4.5a-b), recorded $^{14}CH_3I_{(i)}$ in bacterial cells ranged between 56 – 267 nmol L^{-1} representing 3% of each of the three different substrate concentrations introduced and incubation periods of three and six hours (Figure 4.6a-b). The recorded $^{14}CH_3I_{(i)}$ as $^{14}CO_2$ from the same sets of samples ranged between 30 – 120 nmol L^{-1} (see Figure 4.5a-b) representing between 1 – 2% (Figure 4.6a-b) of the introduced concentrations. Unlike the results from Experiment 1.1, there were statistical significant differences between the recorded concentrations of each target from the three different introduced concentrations during each incubation period of Experiment 1.2 (see Appendix 8). By comparison, the estimated rate of removal (shown in Figure 4.8a-b) between the two different time periods (3 and 6 hours and inaddition to time t = 0,) suggest that optimum bacterial removal of CH_3I substrate occur between t = 0 and t = 3 hours. It was therefore decided to perform subsequent incubations at one hour duration.

The total recovered $^{14}CH_3I$ (the amount recorded in bacterial cells + the amount recorded as $^{14}CO_2$) together with the amount that remained unused ($^{14}CH_3I_{(f)}$) in the culture medium (See Figure 4.6a-b) however represents less than 25% of the added substrate concentration showing that similar to Experiment 1.1, a largest fraction of the $^{14}CH_3I_{(i)}$ (over 75%) was

unaccounted for. This is most likely due to the loss of $^{14}CH_3I_{(i)}$ and $^{14}CO_2$ during sample transfer between the various stages.

4.3.3. Bacterial methyl iodide substrate (nmol L^{-1}) uptake (Experiment 2.1)

Based on the initial results (Experiments 1.1 and 1.2), changes in experimental procedures were necessary to minimize the loss of the added substrate (as shown in Figures 4.4a-b and 4.6a-b). These adjustments have been described in section 4.2.3.1. The results from adopting the new experimental approach (Experimental design two) are described in this section.

Table 4.4: Estimated concentrations (nmol L^{-1}) of the various substrate targets from Experiment 2.1.

	Controls	SD	Samples	SD	Sample - Control	SD
[^{14}C]**A**	11.9	0.5	159	30.1	148	29.6
[^{14}C+$^{14}CO_2$]**B**	10.9	0.4	548	6.6	538	6.1
[^{14}C+$^{14}CO_2$+$^{14}CH_3I_{(i)}$]**C**			1873	17.8	1873	17.8

Table 4.5: Recoveries of the introduced concentration (nmol L^{-1}) through the various substrate Targets from Experiment 2.1.

Target	Intro. Conc. (nmol L^{-1})	SD	Recovered Conc. (nmol L^{-1})	SD	Percentage (%)	%SD
[^{14}C]	2186	42.3	148	29.6	6.7	1.4
[$^{14}CO_2$]	2186	42.3	390	23.5	17.8	1.1
[$^{14}CH_3I_{(i)}$]	2186	42.3	1336	18.6	61.1	0.8
[$^{14}CH_3I_{(i)}$]Lost	2186	42.3	313	0.0	14.3	0.0

With the new approach, the recovery of $^{14}CH_3I_{(i)}$ was improved to 86% (see Figure 4.9) as compared to < 30% observed for the initial procedure. Therefore there was an enhancement in the recovery of around 55% although around 14% (see Figure 4.9) was still unaccounted for.

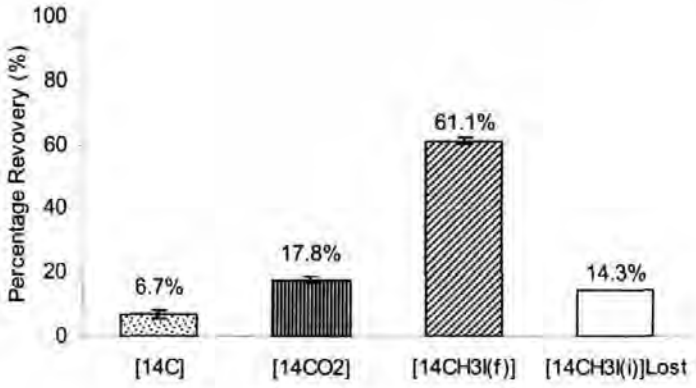

Figure 4.9: Comparison of the percentage recoveries between the various CH_3I substrate targets of bacteria in Experiment 2.1.

The loss of $^{14}CO_2$ accounted for a significant portion of the total concentration lost (up to 83%) during Experiments 1.1 and 1.2. The application of the second experimental design resulted in the reduction of substrate losses. Percentage recovery of $^{14}CO_2$ in Experiment 2.1 increased to about 18% as compared to only about 2% in Experiment 1.2 (Figure 4.9). With respect to the unused portion of the substrate ($^{14}CH_3I_{(f)}$), the percentage recovery was 61% during Experiment 2.1 as compared to 17% during Experiments 1.2. These findings imply that around 16% of the 70% concentration loss during the application of Experimental design one was as a result of $^{14}CO_2$ loss. This loss is likely to have occurred during the centrifugation step in Stage I and substrate volatility during transfer of sample in Stage II. There was an additional loss of about 43% of $^{14}CH_3I_{(f)}$ during the application of the first procedure due to CH_3I volatility coupled with sample transfer, vortex mixing and centrifugation in Stage I, II and III.

The new approach yielded a significant improvement and hence may be applied in assessing bacterial utilization of CH_3I in the aquatic environment.

4.3.4. Bacterial methyl iodide substrate (nmol L^{-1}) uptake (Experiment 2.2)

From the results of experiment 2.2 (Figure 4.10), the accrued experimental loss estimated from Stage I to III of the experimental set (A) reveals a further reduction of approximately 8% compared to Experiment 2.1. This may be due to further improvement in personal handling from one step of the experiment to the other. It may also be as a result of a lower incubation temperature (11.6 °C) based on the *in situ* temperature of the water sample as compared to 18.4 °C in the previous experiment and hence a reduced volatility. This suggests that by reducing the experimental stress such as vortex mixing and centrifugation on the volatile substrate (CH_3I) as shown by the revised experimental procedure, losses could be reduced significantly.

Table 4.6: Estimated mean concentrations (nmol L^{-1}) of the various substrate targets from Experiment 2.2.

	Controls	SD	Samples	SD	Sample - Control	SD
$[^{14}C]$**A**	11.8	0.8	31.8	2.4	20.1	1.6
$[^{14}C+^{14}CO_2]$**B**	9.7	0.4	56.0	1.0	46.2	0.6
$[^{14}C+^{14}CO_2+^{14}CH_3I_{(l)}]$**C**			479	5.2	479	5.2

Table 4.7: Recovered concentrations (nmol L^{-1}) through the various substrate targets from Experiment 2.2.

Target	Intro. Conc. (nmol L^{-1})	SD	Recovered Conc. (nmol L^{-1})	SD	Percentage (%)	%SD
$[^{14}C]$	492	3.2	20.1	1.6	4.1	0.3
$[^{14}CO_2]$	492	3.2	26.2	1.0	5.3	0.2
$[^{14}CH_3I_{(l)}]$	492	3.2	432	4.7	87.9	0.9
$[^{14}CH_3I_{(l)}]$**Lost**	492	3.2	13.3	4.1	2.7	0.8

A comparison of percentage recoveries between Experiment 2.1 and 2.2 indicate a lower bacterial removal of substrate in the latter experiment (16% lower). This difference may be as a result of differences in bacterial community structure of the water samples used in the two different experiments as well as seasonal variation in temperature.

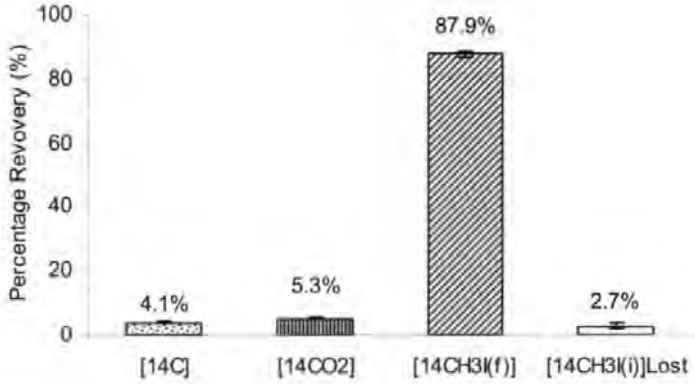

Figure 4.10: Comparison of the percentage recoveries between the various CH_3I substrate targets of bacteria in Experimental 2.2.

Since the level of CH_3I substrate concentration that was observed to produce significant and reproducible bacterial responses far exceed (about one hundred thousand folds higher) the *in situ* concentration (3.6 pmol L^{-1}) at the sampling location in the Tamar estuary, results derived from the application of this method could only represent potential bacterial CH_3I removal in the natural aquatic environment. The developed analytical procedure could therefore be applicable only in the qualitative interpretation of microbial CH_3I removal in aquatic systems where the influence of spatial and temporal variations in physicochemical variables on microbial activity is taken into account.

4.4. Summary and conclusions

Bacteria associated with estuarine aggregates have been found to contribute to the production of CH_3I in the aquatic environment through the degradation of particulate organic matter (presented in Chapter Three). It has also been noted that as well as the production of CH_3I, there was removal of CH_3I by bacteria present in the experimental medium although the actual mechanism and pathway of this methylotrophic bacterial process remain uncertain. From the literature, there is evidence (e.g. Goodwin *et al.*, 1997b; Vannelli *et al.*, 1999; McDonald *et al.*, 2002) which supports the hypothesis that certain bacteria (methylotrophs) are capable of oxidizing CH_3Br and CH_3Cl. It has

however not been mentioned in the literature whether CH_3I is utilized by methylotrophic bacteria as a sole carbon and energy source.

It was therefore necessary to ascertain the possible removal and utilization of CH_3I by methylotrophs and to develop a rigorous analytical method that could be used to effectively investigate and assay the process of CH_3I removal by methylotrophs in natural water samples. A reliable and reproducible method was developed through adaptations and modifications of existing methods (Smith & Azam, 1992; Connell *et al.*, 1997). In this method a [^{14}C] radiolabelled CH_3I was used as the substrate to identify and estimate bacterial utilization of CH_3I. From the findings, the following conclusions were drawn;

1. The development of an analytical procedure to investigate the removal of CH_3I by bacteria was successful and was shown to be effective and reproducible.

2. It was shown that bacteria are capable of removal and degradation of CH_3I in aquatic systems but the removal rates only represent the potential bacterial CH_3I utilization.

CHAPTER FIVE

The dynamics of bacterial activity on methyl iodide production and removal in the Tamar estuary (Plymouth, UK)

5. The dynamics of bacterial activity on methyl iodide production and removal in the Tamar estuary (Plymouth, UK)

5.1. Background

The Tamar estuary is a highly dynamic system that undergoes physical, chemical and biological changes during the transition from the freshwater to the marine environment (e.g. Morris *et al.*, 1982; Jackson *et al.*, 1987; West *et al.*, 1990; Uncles *et al.*, 1994; Uncles & Lewis, 2001) and hence represents a suitable system in which to study environmentally important processes. Although several studies conducted in estuarine systems (e.g. Goosen *et al.*, 1999; Eiler *et al.*, 2003; Langenheder *et al.*, 2004) including the Tamar estuary (e.g. Clarke & Joint, 1986; Readman *et al.*, 1982) have involved bacterial activity, none are known that addresses either bacterial removal of CH_3I or the measurement of CH_3I concentration.

5.2. Aims and objectives

The aims and objectives of the current investigation are;

1. To investigate spatial and seasonal changes in CH_3I concentration in the Tamar estuary.
2. To investigate spatial and seasonal variations in the rate of bacterial CH_3I removal in the Tamar estuary.
3. To investigate various physico-chemical variables that play a role in the variations in bacterial activity over a period of time (nine months) in the Tamar estuary.

5.3. Description of the Tamar estuary

The Tamar estuary (Figure 5.1) is a coastal plain partially mixed estuary located to the south-west of Plymouth, UK (Grabemann *et al.*, 1997). The Tamar extends from North Cornwall flowing for approximately 100 km towards the south, while amassing water discharge from its two major tributaries, the river Tavy and the river Lynher (Miller, 1999). It has a catchment area of approximately 1700 km^2 (Evans *et al.*, 1993). The estuary is tidal for only about 31 km from the Weir head to the mouth where it discharges into Plymouth Sound (Grabemann *et al.*, 1997).

The Tamar estuary experiences a semi-diurnal tidal effect (Uncles & Stephens, 2001) with tidal ranges of 6.5m at springs and 1.5m at neaps (Miller, 1999). At the mouth, it has mean spring and neap tidal ranges of 4.7 and 2.2m (Uncles & Stephens, 2001). It is also known to exhibit asymmetric tidal currents (Grabemann *et al.*, 1997) with peak flood velocities exceeding those of the ebb, especially in the upper reaches (Bale *et al.*, 1985).

The tidal asymmetry persists but becomes weaker down the estuary (Uncles *et al.*, 1985). During spring tides, there is the occurrence of strong tidal pumping of freshwater in the central reaches and also in the lower reaches of the estuary (Uncles & Lewis, 2001) resulting in a flux of suspended sediment to the estuarine bed (Uncles *et al.*, 1985).

The presence of the TMZ in the Tamar estuary provides a suitable niche and nutrient source for particle-attached bacteria which have been reported (Crump & Baross, 1996; Crump *et al.*, 1998) to be 10 to 100 times more active than free living bacteria and therefore could account for up to 90% of heterotrophic bacterial activity (Wright, 1978) in estuarine systems.

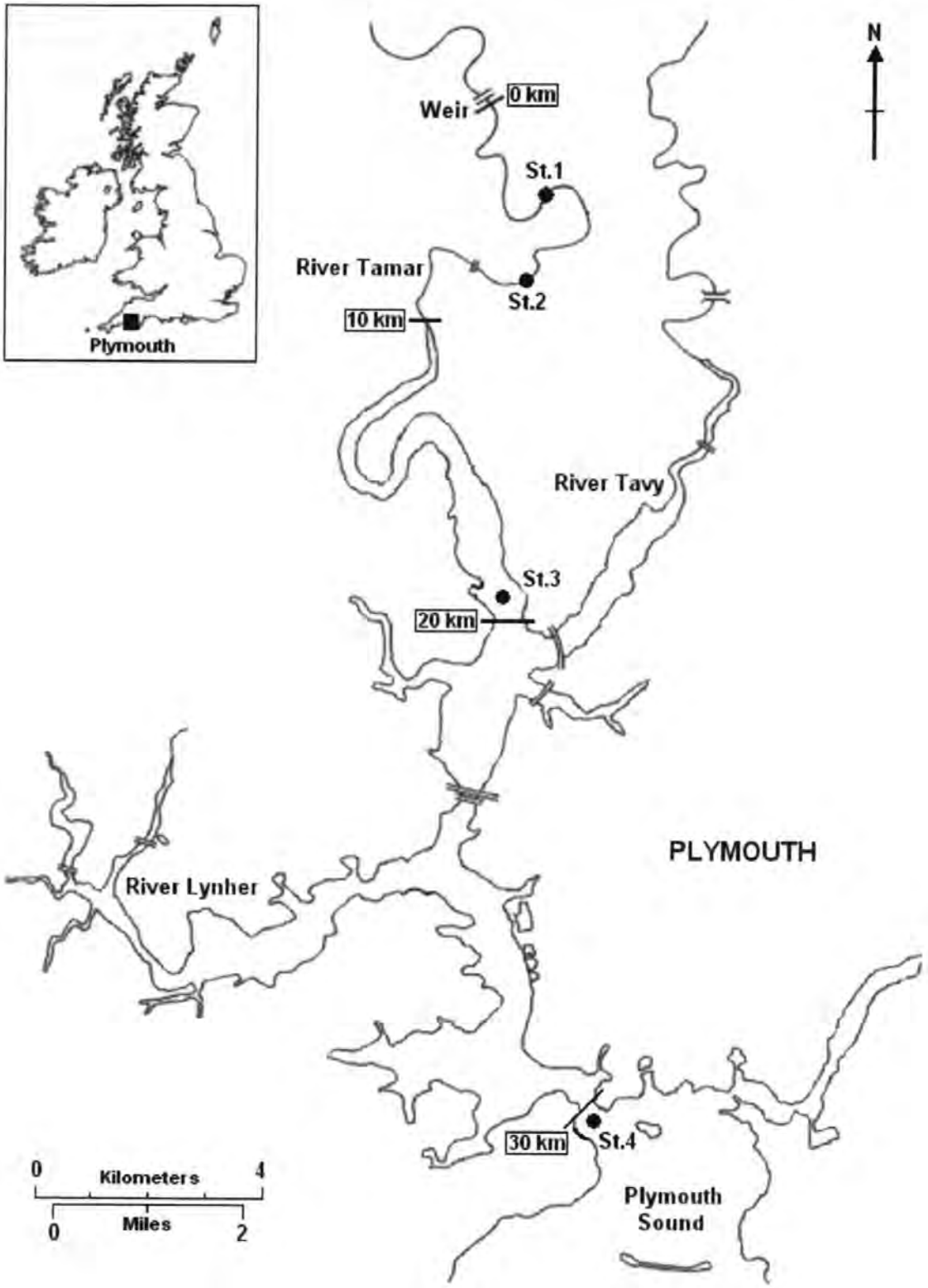

Figure 5.1: A map of the Tamar estuary showing the selected sampling locations from the freshwater region to the saline region of the estuary (Plymouth Sound); St.1 (Morwellham), St.2 (Tuckermarsh), St.3 (Cargreen) and St.4 (The Narrows).

5.4. Method

5.4.1. Field and laboratory protocols

Direct measurement of physico-chemical variables and water samples were made at four stations along the Tamar estuary whilst other physico-chemical variables in addition to biological parameters were measured in the laboratory. A summary of the measured variables, sampling stations and number of replicates are presented in Tables 5.1 and 5.2. Details of sampling and analytical methods used are presented in Chapter Two.

Table 5.1: A summary of field and laboratory work carried out during the study of the Tamar estuary (all measurements and samples were from surface water <1m depth).

Sampling Dates (2005)	Sampling Period	Sampling Stations	Field Measurements	Laboratory Measurements
16th March	8:30am -12:40pm	Morwellham (3.5 km from weir) Tuckermarsh (7.5 km from weir) Cargreen (20 km from weir) The Narrows (30 km from weir)	Temperature Salinity Dissolved oxygen concentration pH Surface water samples	Bacterial enumeration Bacterial CH_3I removal rate CH_3I concentration Bacterial leucine incorporation rate SPM (Dry weight)
4th April	8:40am -12:45pm			
29th April	8:00am – 12:00pm			
12th May	7:50am – 11:40am			
3rd June	11:00am - 2:30pm			
13th June	8:50am – 12:35pm			
30th June	8:00am – 12:05pm			
26th July	8:15am – 12:10pm			
9th August	7:00am – 11:10am			
25th August	8:30am – 12:30pm			
12th September	9:50am – 1:35pm			
26th September	9:35am – 1:40pm			
25th November	9:40am – 1:20pm			

Table 5.2: Number of replicate samples carried out in the laboratory analyses.

Stations where water samples were collected	Bacterial CH_3I removal analysis	Bacterial leucine incorporation analysis	Bacterial enumeration	$[CH_3I]$ analysis	SPM analysis
	No. of live & (control) replicates	No. of live & (control) replicates	No. of live & (control) replicates	No. of replicates	No. of replicates
Morwellham	4 (3)	3 (3)	3 (3)	1	1
Tuckermarsh	4 (3)	3 (3)	3 (3)	1	1
Cargreen	4 (3)	3 (3)	3 (3)	1	1
The Narrows	4 (3)	3 (3)	3 (3)	1	1

5.4.1.1. Spatial and temporal resolution of sampling

Four sampling locations along the Tamar estuary were chosen to (i) cover the interface between the freshwater and seawater reaches of the upper estuary, (ii) to include the TMZ, (iii) cover the longitudinal section of the estuary. The sampling locations are presented in Figure 5.1. Only four locations were chosen due to the time required to conduct the field work activities as well as preparative laboratory work for each batch of samples within the same day. For the same reason, the temporal resolution of the sampling exercise was fortnightly to allow adequate time to perform all sample analyses and data processing.

5.4.1.2. Tides

Based on the geographical positions of the sampling locations, it was crucial to sample when the upper reaches of the estuary were easily accessible by boat (MBA Sepia). However, the water depth at the upper reaches during low water was less than 2 m, making it impossible for the boat to reach two of the sampling locations in the upper reaches of the

estuary. Therefore the boat moved upstream between mid-flood tide and high water (whilst salinity and turbidity were continuously monitored). All sample and data collection was then conducted from the station farther upstream (Morwellham) to the station farther downstream (The Narrows).

5.4.2. Collection of water samples

Samples were collected using a sampling bucket from the top 30 cm of the water column. Samples for CH_3I analysis were kept in 30 mL glass reagent bottles with glass stoppers stored in the dark to prevent the loss of CH_3I. Samples for the remaining laboratory work (i.e. bacterial abundance analysis, bacterial leucine incorporation, bacterial CH_3I removal and the analysis of SPM dry weight) were kept in 500 mL solvent bottles with polyethylene caps. All the water samples (for laboratory analyses presented in this chapter) were immediately stored at *in situ* temperature in a Thermo-Tote Incubator (Scientific Device Lab Inc.) which was also used to transport the samples from the field to the laboratory (see section 5.5.2).

5.4.3. Collection of physico-chemical data

The collection of physico-chemical data (namely surface water temperature, salinity, pH and dissolved oxygen concentration) in the Tamar estuary was carried out using a Multiparameter Water Quality Monitor 6600-M (YSI Inc.). Detailed descriptions of the sampling protocols in the Tamar are outlined in Section 2.4. Wind speed data for the sampling dates and times were obtained from the local wind station at the Plymouth Marine Laboratory situated about 400 m from the mouth of the Tamar estuary. This was used for the purpose of estimating water to air flux of CH_3I over the Tamar estuary.

5.4.4. Application of statistical methods

With the aid of Minitab statistical software, a range of statistical approaches were employed to analyse the resulting (raw) data from field survey and laboratory work. Many of the data analyses (e.g. Pearson's correlation, analyses of variance (ANOVA) and regression) depend on the assumption that data sets are normally distributed. However, environmental data sets are often non-normally distributed, although with an appropriate transformation, a dataset can approximate a normal distribution (Miller & Miller, 2005).

One of the most appropriate tests for verifying normality is the Kolmogorov-Smirnov test. It works by comparing the cumulative distribution of the data set with an expected cumulative Gaussian distribution and provides a p-value based on the largest discrepancy. In the event that the dataset is non-normally distributed, the Box-Cox transformation could be applied. The Box-Cox transformation is a family of transformations particularly useful for correcting non-normality (Miller & Miller, 2005). It is defined as

$$T(Y) = (Y^{\lambda} - 1)/\lambda \qquad \textbf{(5.1)}$$

Where

Y = the response variable and

λ = the transformation parameter.

However when $\lambda = 0$, or near zero, the natural logarithm of the data is taken instead of using equation (5.1).

For the present study, Pearson's correlation coefficient was used to analyse the significance of the relationship between two continuous variables. This was conducted after each dataset was checked for normality using the Kolmogorov-Smirnov test and after application of the Box-Cox transformation where necessary.

Significant differences between the means of different populations were tested to identify spatial and seasonal variations. Since this study consists of multiparametric datasets, the analysis of variances (ANOVA) was employed to serve this purpose. ANOVA functions by testing if the means of the groups formed by values of an independent variable or combination of values for multiple independent variables are significantly different. In addition to ANOVA, regression analyses were used to establish a model of the relationships between variables that were significantly related.

5.5. Results and Discussion

5.5.1. Variations in physical and chemical variables

This section presents the results and discussion for the measured physico-chemical variables along the Tamar estuary over the study period. Variables presented here are (i) salinity, (ii) surface water temperature, (iii) dissolved oxygen concentration, (iv) suspended particulate matter concentration, (v) pH, (vi) CH_3I concentration.

5.5.1.1. Salinity

Among the variables measured along the estuary, salinity was the most spatially varied between the selected sampling locations along the Tamar estuary (refer to Appendix 10i). Through conservative mixing, salinity is determined by the strength of freshwater and saltwater intrusions. In this respect, the maximum salinity of 35.1 was recorded at the station farthest downstream (The Narrows) whilst the minimum salinity of 0.1 was recorded at the station farthest upstream (Morwellham).

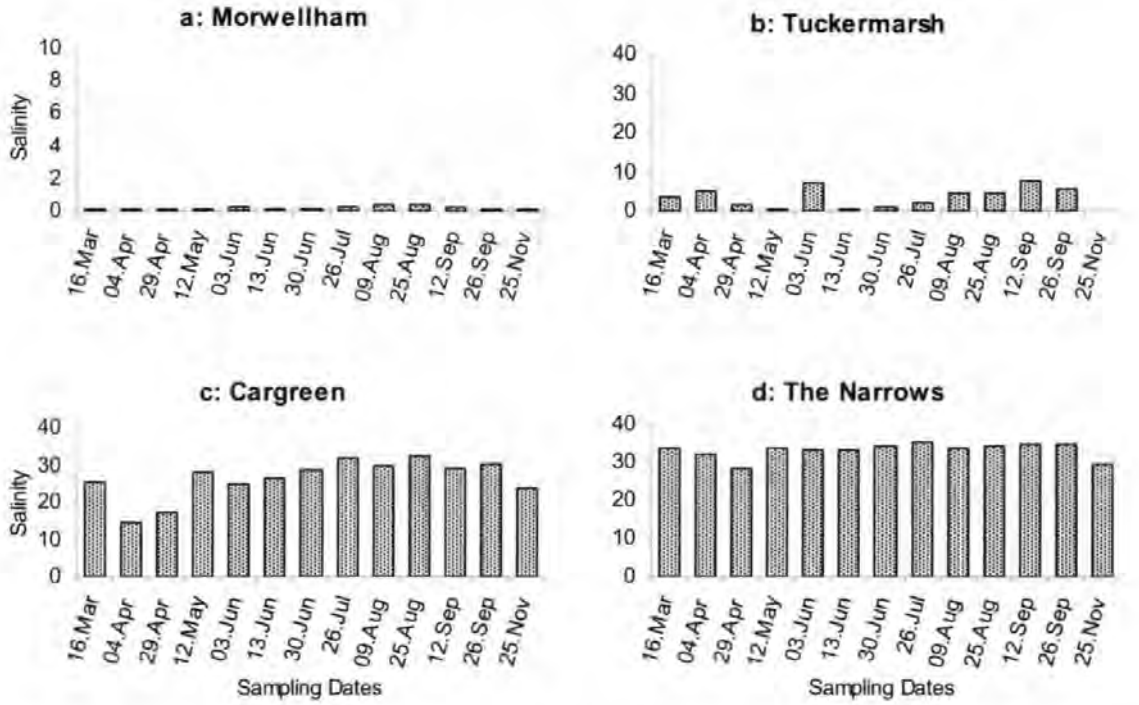

Figure 5.2a-d: Salinity of surface waters at the four selected sampling stations along the Tamar Estuary (2005) (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows. Note change in the scale of the y-axis for Morwellham.

From Figure 5.2a-d, the chosen sample sites fall within two distinctive salinity regions in the Tamar estuary. Morwellham and Tuckermarsh form a low salinity region (typically < 7) whilst Cargreen and The Narrows form a high salinity region (typically > 25) of the estuary. Seasonally, the lowest values of salinity at each sampling station were observed during the autumn and spring when freshwater discharge into the estuary was at its maximum. This is as a result of high freshwater runoff brought into the estuary by the River Tamar and its two tributaries, Tavy and Lynher (Uncles *et al.*, 1983; Miller, 1999; Uncles & Stephens, 2001) during these periods. An increase in salinity was observed at the low salinity region during the warm season (Figure 5.2a-b) which is likely to be as a result of lower river discharge of freshwater into the estuary during the summer thereby increasing the effect of seawater incursion in the freshwater reaches of the estuary (Miller, 1999). Similarly, maximum salinities were observed at the high salinity region during the warm season (Figure 5.2c-d).

5.5.1.2. Surface water temperature

Surface water temperature from the four sampling stations along the Tamar estuary between February and November 2005, varied between 6.0-19.6 °C (Figure 5.3a-d), which was within the same measured temperature range reported in the literature (e.g. 3 – 21 °C by Morris *et al.*, 1982; and 3.1-19.6 °C by Uncles & Stephens, 2001).

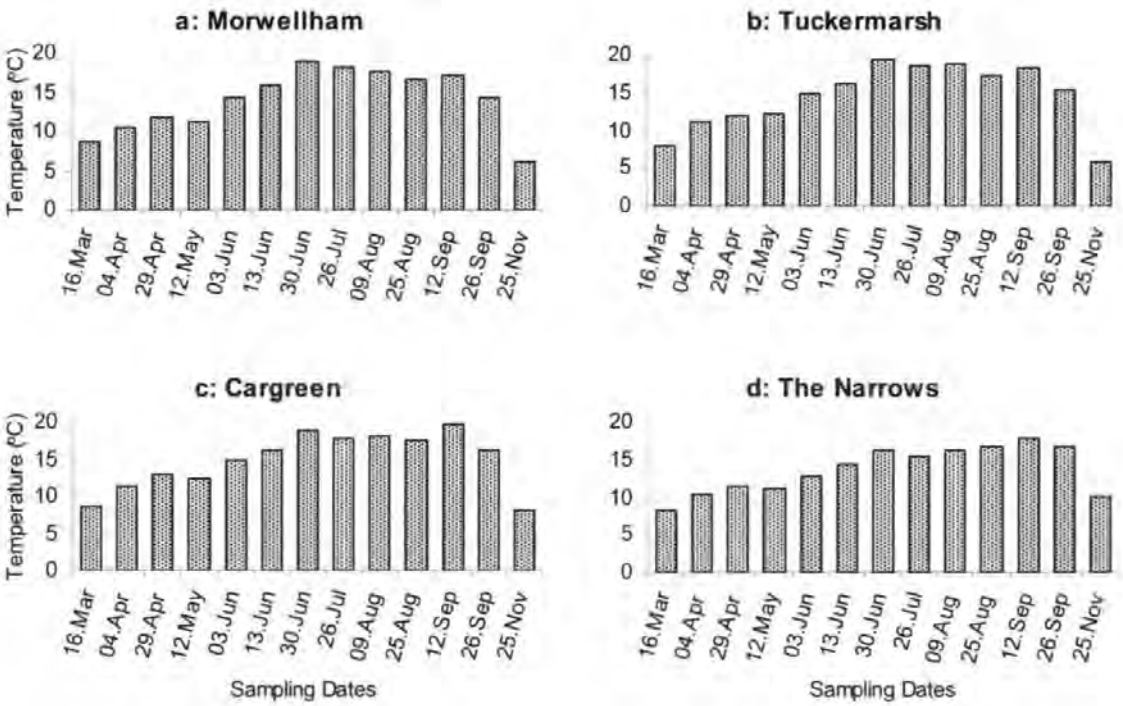

Figure 5.3a-d: Recorded surface water temperature (°C) at the four selected sampling stations along the Tamar estuary (2005) (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

The maximum temperature was recorded in early September at Cargreen whilst the minimum temperature at all sites was recorded during the latter part of November. In general, three peaks of temperature were observed at all stations during the course of the surveys in April, June and September. Surface water temperature during the study period generally decreased from the upper estuary (low salinity region) towards the lower estuary (high salinity region) (see Figure 5.4) especially during the warm season with Morwellham and Tuckermarsh recording the highest temperature on each sampling date. According to Morris *et al.* (1982) the general pattern of spatial variation in temperature in the estuary

may be predominantly controlled by mixing between freshwater and seawater. However the scatter distribution of Figure 5.4 appear to be slightly convex and this according to Uncles & Stephens (2001), illustrate heat gain from the atmosphere by the estuarine water.

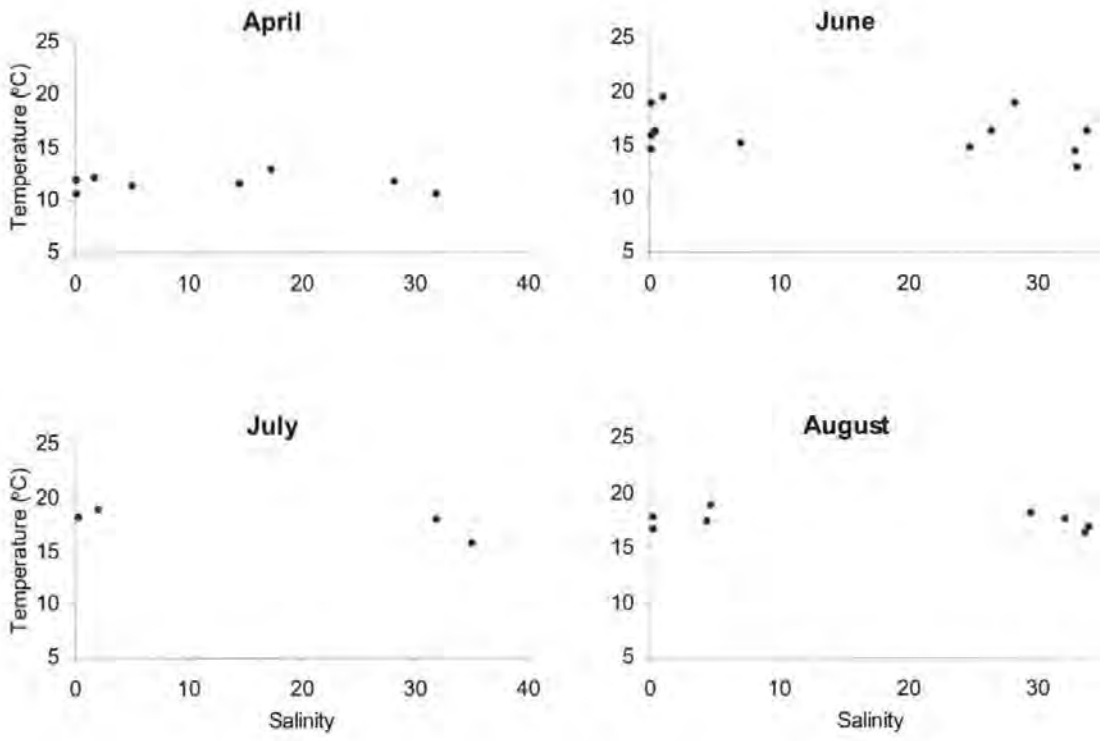

Figure 5.4: Scatter plots of surface water temperature (°C) versus salinity for the months of April, June, July and August 2005 along the Tamar estuary. Data used are from the four selected sampling locations during sampling dates within each of the months presented.

Recorded temperature was >15 °C between the months of June and September whilst values <15 °C were observed before June and after September thereby forming an annual cycle of alternating warm and cold temperatures. This seasonal trend in temperature in the Tamar estuary conforms to that reported by Morris *et al.* (1982), Uncles & Stephens (2001). This seasonal variation is therefore likely to have important impacts on the ecological variation in estuarine biological activities (Beukema, 1990).

5.5.1.3. Dissolved Oxygen

During the cold period (before June and after September), there were no distinctive variations in percentage dissolved oxygen saturation (%DO) between the sampling stations for each sampling date (see Figure 5.5a-d). However during the warm period (i.e. between the months of July and August) two distinctive populations of %DO readings were apparent.

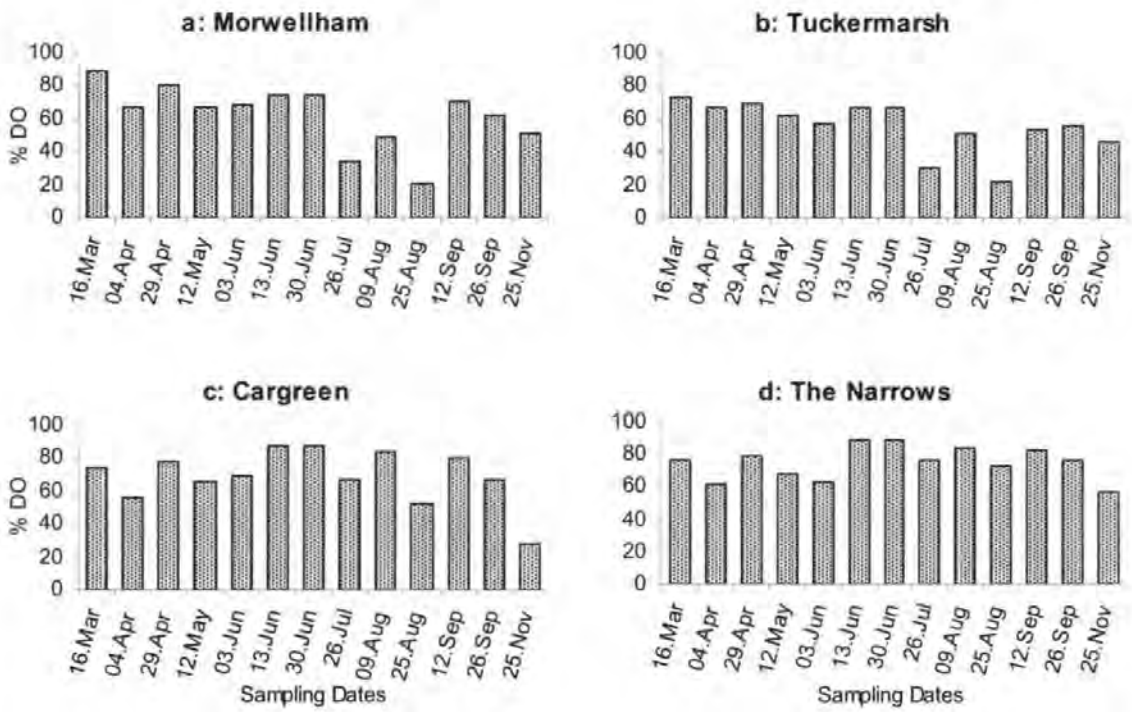

Figure 5.5a-d: Percentage dissolved oxygen saturation (%) in surface water at the four selected sampling stations along the Tamar estuary (2005) (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows

Morwellham and Tuckermarsh recorded the minimum readings during this period whilst Cargreen and The Narrows recorded the relatively high readings during the same period. This may have been as a result of the high oxygen demand exerted in the very low salinity region (Morwellham and Tuckermarsh) of the estuary during the warm season (Morris *et al.*, 1982) owing to a higher microbial activity. Higher heterotrophic microbial respiration in the low salinity region of the estuary is as a result of the availability of POC in addition to dissolved organic carbon (DOC) derived from allochthonous inputs of organic material

(Coffin *et al.*, 1989; Hullar *et al.*, 1996; Schultz Jr. *et al.*, 2003). Through heterotrophic remineralization (Goosen *et al.*, 1997), the utilization of DOC by bacteria may therefore affect the mass balance of DO (Tipping, *et al.*, 1997) in the Tamar estuary.

Over the survey period (especially during the warm period), there was evidence of %DO oscillation at each sampling station (high %DO followed by low %DO readings). This may be attributed to either competition or succession or both in microbial communities (Bouvier & del Giorgio, 2002) during the warm period. In addition, phytoplankton activity will occur particularly at the lower reaches of the estuary during the warm season since photosynthesis is light limited in the upper reaches due to high turbidity (Goosen *et al.*, 1999) which might explain the observed higher %DO at Cargreen and The Narrows.

5.5.1.4. Suspended particulate matter (SPM)

The concentration of suspended particulate matter (SPM) obtained from the four sampling stations indicated substantial spatial and seasonal variations (see Figure 5.6a-d). There was a general decrease in SPM load from the upper reaches towards the lower reaches of the estuary. In this respect, SPM load was generally very low in the high salinity region which conforms to the observations made by Stephens *et al.* (1992); Uncles & Stephens (1993a); Tattersall *et al.* (2003). However the greatest temporal variation occurred within the low salinity region with mean values of 174.6 ± 133.1 mg L^{-1} and 78.4 ± 58.0 mg L^{-1} at Morwellham and Tuckermarsh respectively. This may be as a result of high sediment input through river discharge especially during periods of intense flooding but quickly settles out during transportation down the estuary (Tattersall *et al.*, 2003).

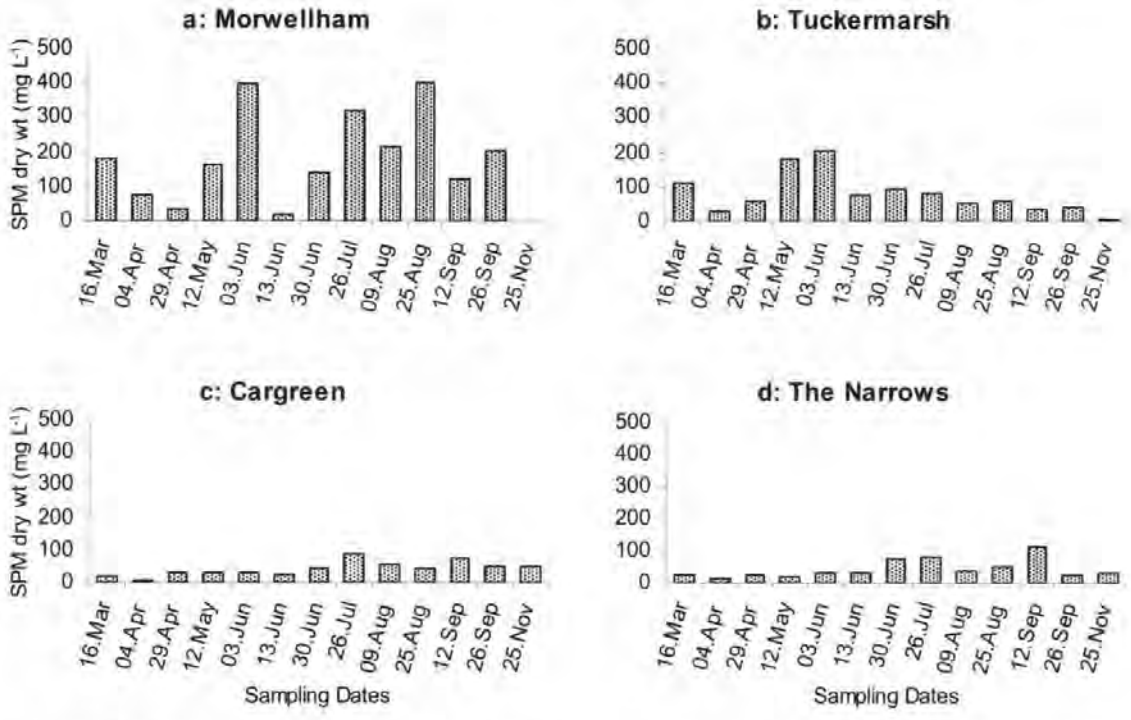

Figure 5.6a-d: Concentration of suspended particulate matter (mg L^{-1}) of surface water at the four selected sampling stations along the Tamar Estuary (2005) (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

The maximum SPM concentration of 403 mg L^{-1} was recorded at Morwellham. This is less than half the maximum concentration of 950 mg L^{-1} measured in the Tamar estuary by Morris *et al.*, (1987) but similar to that (400 mg L^{-1}) reported by Miller (1999). Oscillations in SPM loads were prevalent in the estuary (especially at Morwellham). This may be due to periodic resuspension of bed sediments through periodic increase in tidal range (Uncles *et al.*, 1984; Bale *et al.*, 1985; Uncles & Stephens, 1993b; Uncles *et al.*, 1994) especially during low runoff conditions (Uncles & Stephens, 1989; Grabemann *et al.*, 1997).

5.5.1.5. pH

During the course of the survey, recorded pH along the Tamar estuary ranged from 7.4 to 9.0 (see Figure 5.7a-d). According to Morris *et al.* (1982) there is generally a pattern of increasing pH with increasing salinity in estuarine systems (6.8 – 8.5); which was

prominent from Tuckermarsh through to The Narrows. However, Morwellham with the lowest salinity exhibited the highest pH recorded (an average of 8.3) in the Tamar estuary during the study. Within the months of May and November, Morwellham recorded a pH of 9. Periods of elevated pH were also observed at Tuckermarsh. Observed discrepancies in pH of natural waters occur as a response to adjustments in inorganic carbon system through *in situ* production and respiration (Morris *et al.*, 1982). It could therefore be assumed that the relatively high pH fluctuation within the freshwater region of the Tamar estuary (Morwellham and Tuckermarsh) may be as a result of weakly buffered carbonate system (Jackson *et al.*, 1987) coupled with pronounced temporal pCO_2 variation through heterotrophic respiration of organic carbon (Heip *et al.*, 1995; Gattuso *et al.*, 1998; Cai *et al.*, 1999) and the periodic removal of CO_2 through primary production within the region (Jackson *et al.*, 1987).

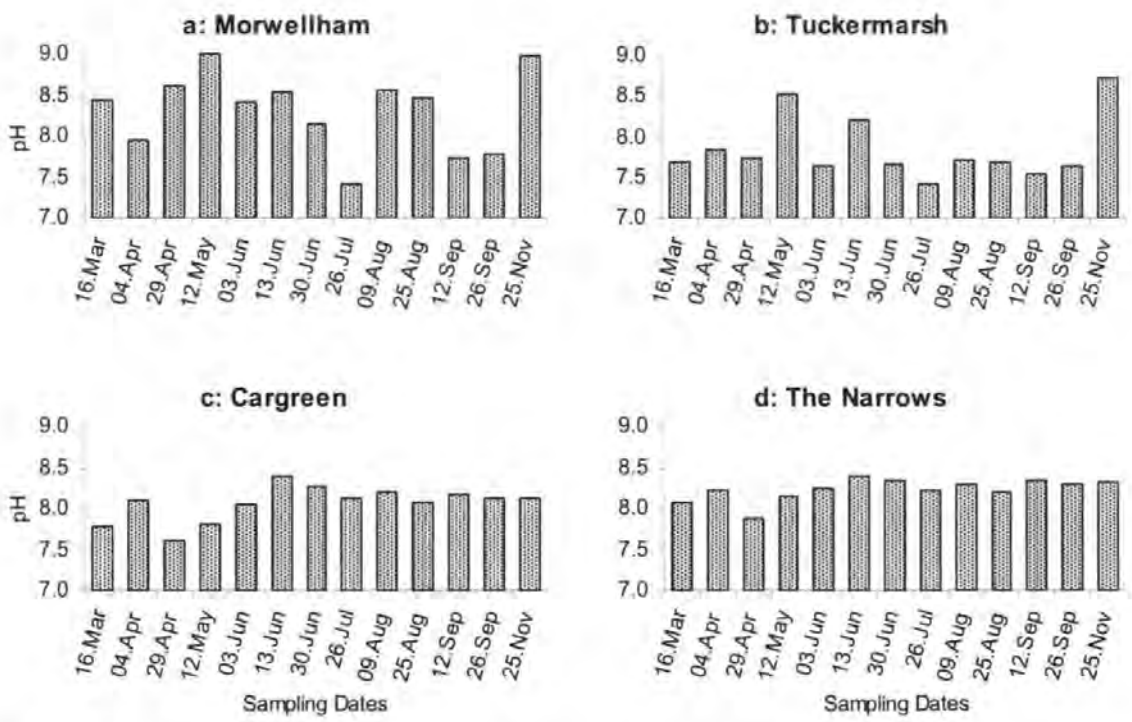

Figure 5.7a-d: pH of surface waters at the four selected sampling stations along the Tamar Estuary (2005) (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

Seasonal variation in pH was less prominent within the high salinity region of the estuary. This could be explained by the high buffering capacity of seawater as a result of high salinity which maintains the consistency of pH in this region (Pytkowicz & Atlas, 1975).

5.5.1.6. Methyl iodide concentration

During the course of the survey, observed CH_3I concentrations varied between 0.06 – 2.04 pmol L^{-1} along the estuary. Spatially there occurred two distinctive regions of high (Cargreen and The Narrows) and low (Morwellham and Tuckermarsh) CH_3I concentrations in the Tamar estuary (see Figure 5.8).

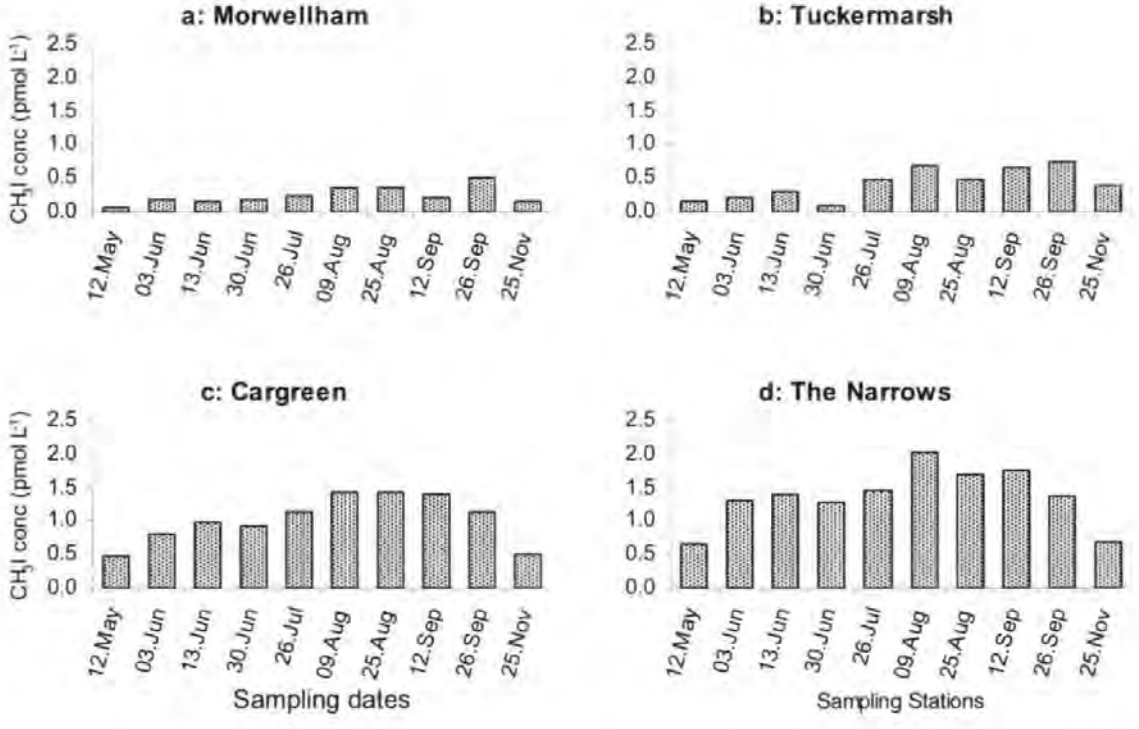

Figure 5.8a-d: CH_3I concentration (pmol L^{-1}) at the four selected sampling locations along the Tamar estuary (2005) (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

The Narrows with a mean CH_3I concentration of 1.36 ± 0.44 pmol L^{-1} had the highest recorded concentration. However, this mean concentration in comparison with reported CH_3I concentrations in coastal waters and the open ocean (presented in Table 2.3 in Chapter Two), is about two to five times lower. Morwellham with a mean CH_3I

concentration of 0.24 ± 0.13 pmol L^{-1} had the lowest recorded concentration over the survey period. Spatially, recorded CH_3I concentrations at the two stations within the lower reaches of the Tamar estuary were much higher than that from the stations within the upper reaches. With mean CH_3I concentration during the warm season equal to about twice that of the cold season, there appeared to be a seasonal trend at each of the sampling station. A summary has been provided in Table 5.3.

Table 5.3: A summary of recorded CH_3I concentrations at the selected sampling stations along the Tamar Estuary (2005) during the cold season (<15 °C) and warm season (>15 °C).

Sampling Stations	All Data (pmol L^{-1})		Cold Season (C) (pmol L^{-1})		Warm Season (W) (pmol L^{-1})		Ratio (W/C)
	mean ± sd	Range	mean ± sd	Range	mean ± sd	Range	
Morwellham	0.24 ± 0.13	0.06 – 0.49	0.12 ± 0.06	0.06 – 0.17	0.29 ± 0.12	0.16 – 0.49	2.4
Tuckermarsh	0.41 ± 0.23	0.09 – 0.73	0.24 ± 0.12	0.14 – 0.38	0.49 ± 0.23	0.09 – 0.73	2.0
Cargreen	1.02 ± 0.35	0.46 – 1.43	0.60 ± 0.20	0.46 – 0.82	1.20 ± 0.22	0.91 – 1.43	2.0
The Narrows	1.36 ± 0.44	0.65 – 2.04	0.88 ± 0.38	0.65 – 1.31	1.57 ± 0.27	1.28 – 2.04	1.8

According to Cook *et al.* (2000), iodide concentration in estuarine systems has been found to be higher at the seawater region compared to the freshwater region of an estuary which supports the observed spatial distribution of CH_3I concentration in the Tamar estuary during this study. They also suggested that this pattern of spatial distribution is more biologically-mediated than physically-mediated since no correlation was found between iodide and salinity hence ruling out mixing process as the reason. Since the formation of CH_3I (whether biological, physical or chemical) requires iodide as a precursor, it may imply that CH_3I may follow a similar trend of spatial distribution of iodide in an estuary such as the Tamar. This coupled with spatial variation in CH_3I consumption (e.g. microbially-mediated removal) may explain the pattern of spatial distribution of CH_3I in the estuary.

Maximum CH_3I concentrations were typically recorded between the months of June and September. Since biological activity (including that of bacteria) is optimal during the warm season (e.g. Felip *et al.*, 1996; Isaksen & Teske, 1996; Pomeroy & Wiebe, 2001; Current work, 2005), the observed CH_3I concentration peak between spring and summer is likely to have occurred as a result of increased biological production of CH_3I in the estuary during this period.

Seasonal variation in CH_3I concentration occurred at all sampling stations. The ratio of concentration recorded during the warm and cold seasons at each sampling site (see Table 5.3) revealed that twice as much CH_3I was present during the warm season. Generally, the warm/cold seasonal concentration ratio between the selected sampling sites decreases from the freshwater to seawater region of the Tamar estuary. This may suggest a more pronounced seasonal variation in CH_3I dynamics in the freshwater reaches as compared to the seawater reaches of the estuaries.

Recorded average CH_3I concentrations as well as the spatial and temporal variations from the Tamar estuary during this study could not be compared with other estuarine systems around the world since no published work regarding CH_3I concentration studies in estuarine systems was found. This therefore highlights the novelty of the current work. However, CH_3I concentration recorded at the high salinity region (mouth) of the Tamar estuary (The Narrows) could be compared with that of Plymouth's L4, a coastal sampling station about 12 km from the mouth of the Tamar estuary. At L4, the reported CH_3I concentration (Archer *et al.*, 2007) over a two-year period (2002-2004) ranged between 0.6 – 14.6 pmol L^{-1} with a mean concentration of 6.1 pmol L^{-1}. During the present study, recorded mean CH_3I concentration at The Narrows (1.36 pmol L^{-1}) is 4.5 times lower than that of L4. Considering the fact that the production of halocarbons is predominant in

offshore coastal marine waters (e.g Nightingale *et al.*, 1995), compared to inshore waters, the lower CH_3I concentration at The Narrows relative to L4 was expected.

5.5.2. Rate of methyl iodide flux in the Tamar Estuary

The flux of gases between environmental reservoirs is an important process in their biogeochemical cycling. Transfer of gases across the water-air interface is driven by a concentration gradient between water and air and a transfer velocity (e.g. Liss, 1983; Wanninkhof, 1992; McGillis *et al.*, 2001; Frost & Upstill-Goddard, 2002). Factors such as wind speed, surface films and temperature all may affect the transfer velocity (e.g. Liss, 1983; Nightingale *et al.*, 2000; Tsai & Liu, 2003). Amongst these physical factors, wind speed and temperature affect transfer velocity the most.

The calculation of water to air flux of CH_3I in the Tamar estuary involved the equation;

$$F_{CH3I} = k_{CH3I} * \Delta C_{CH3I} \quad \textbf{(5.2)}$$

Where F_{CH3I} = flux of methyl iodide (nmol m^{-2} d^{-1})

k_{CH3I} = transfer velocity (cm h^{-1}) of methyl iodide

ΔC_{CH3I} = air-water concentration difference of CH_3I (pmol L^{-1})

Since no direct wind speed was recorded during the study, wind speed data (with a resolution of one minute) recorded at PML (close to the Tamar estuary) at a height of 15 m has been used for this water to air CH_3I flux calculation. Since the exact times of the field sampling of this study are known, mean wind speeds from PML taken during the time window of each sampling period have been used in the estimation of the transfer velocities in order to minimize the error of not using direct wind speed data over the Tamar estuary. The wind speeds have also been corrected to a common reference height of 10 m for the

estimation of transfer velocity using equation (5.3) which is based on relationships for logarithmic wind profile (Donelan, 1990) and a unitless neutral drag coefficient C_D (Garratt, 1977).

$$u_{10} = \frac{u_x * \frac{K}{\sqrt{C_D}}}{\frac{K}{\sqrt{C_D}} + \ln \frac{z_x}{10}} \quad \textbf{(5.3)}$$

$$C_D = 0.75 \times 10^{-3} + 0.067 \times 10^{-3} * u_{10} \quad \textbf{(5.4)}$$

Where u_{10} = wind speed at 10 m height

u_x = measure wind speed (m s^{-1})

z_x = height (m) at which u_x is measured

K = von Karman's constant = 0.41

C_D = unitless drag coefficient

Since u_{10} appears in both equations (5.3) and (5.4), it was calculated through iteration.

From literature, different relationships between transfer velocity of CO_2 and wind speed have been parameterized by several authors. In this thesis, four of the parameterized relationships (Liss & Merlivat, 1986; Wanninkhof, 1992; Clark *et al.*, 1995 and Nightingale *et al.*, 2000) have been employed in estimating the transfer velocity for water to air flux of CH_3I calculation in the Tamar estuary for comparison.

The relationship between wind speed and transfer velocity described by Liss & Merlivat, 1986 (referred to as LM86 hereafter) was based on wind tunnel experiments using a data set from a gas exchange experiment in a lake (Wanninkhof *et al.*, 1985) with sulphur hexafluoride (SF_6) as a deliberate tracer and for different wind speed ranges. Linear

relationships for different wind speeds are expressed (for wind speeds measured at PML during periods of sampling and applied in this thesis) as follows;

$$k_{LM86\,(660)} = 1.17u \qquad \text{for } u \leq 3.6 \text{ ms}^{-1} \qquad \textbf{(5.5)}$$

$$k_{LM86\,(660)} = (2.85u - 9.65) \qquad \text{for } 3.6 < u \leq 13 \text{ ms}^{-1} \qquad \textbf{(5.6)}$$

Wanninkhof, 1992 (referred to as W92 hereafter) described the relationship between transfer velocity and wind speed to be a quadratic curve based on a transfer velocity estimated from bomb-derived radiocarbon [^{14}C] in the ocean (Broecker *et al.*, 1985). This relationship is expressed as;

$$k_{W92\,(660)} = 0.31u^2 \qquad \textbf{(5.7)}$$

Since the application of different wind speed ranges by LM86 and the relationship based on bomb-derived radiocarbon lead to large differences in CO_2 fluxes (Nightingale *et al.*, 2000), a relationship useful for water to air flux estimation of gases in both regional budget studies and global modelling exercises has been provided by Nightingale *et al.*, 2000 (referred to as NG00 hereafter). This parameterization of transfer velocity as a function of wind speed was based on two deliberate (conservative and volatile) tracer experiments involving bacterium spores (conservative tracer) and $^3He/SF_6$. The NG00 relationship is provided as;

$$k_{NG00\,(660)} = (0.222u^2 + 0.333u) \qquad \textbf{(5.8)}$$

The relationship between the transfer velocity and wind speed described by Clark *et al.*, 1995 (referred to as CL95 hereafter) was also based on a dual tracer technique employing the use of ^3He and SF_6. Since the CL95 tracer experiment was carried out in a tidal river

(Hudson river, Poughkeepsie, USA), the relationship between the transfer velocity of CO_2 and wind speed may be the most suitable for water to air flux calculation in an estuarine environment such as the Tamar estuary. The CL95 relationship is given as;

$$k_{CL95\,(600)} = 2 + 0.24u^2 \quad \textbf{(5.9)}$$

In all the four relationships described, u is the wind speed (m s^{-1}) whilst 660 and 600 are the Schmidt numbers (Sc) of CO_2 at 20 °C in seawater and freshwater respectively.

The transfer velocity of CH_3I could be estimated from that of CO_2 using the equation;

$$k_{CH3I} = k_{CO2\,(600)} \cdot (Sc_{CH3I}/600)^{0.5} \quad \text{(based on CL95)} \quad \textbf{(5.10)}$$

Where Sc_{CH3I} = Schmidt number of CH_3I.

When equation (5.9) is substituted into equation (5.10), it becomes;

$$k_{CH3I} = (2 + 0.24u^2) \cdot (Sc_{CH3I}/600)^{-0.5} \quad \textbf{(5.11)}$$

The Schmidt number of any gas depends on the molecular diffusivity of that gas. However, for methyl halides, the molecular diffusivity of only CH_3Br has been measured (De Bruyn & Saltzman, 1997) which is expressed as;

$$Sc_{CH3Br} = 2004 - 99.5T + 1.39T^2 \quad \textbf{(5.12)}$$

Where T = temperature (°C) for a range of 5 – 35 °C.
NB: Recorded temperature of this study range between 6.0 and 19.6 °C

Also, a relationship between the molecular diffusivity of CH_3I and that of CH_3Br is provided by Wilke & Chang (1955);

$$D_c\,(CH_3I)/D_c\,(CH_3Br) = ((V_b\,(CH_3Br)/(V_b\,(CH_3I))^{0.6} \quad \textbf{(5.13)}$$

Where D_c = diffusion coefficient ($cm^2\ s^{-1}$)

V_b = molar volume ($cm^3\ mol^{-1}$) of each gas at its normal boiling point.

With the molar volumes of CH_3Br and CH_3I estimated as 52.9 and 62.9 $cm^3\ mol^{-1}$ respectively (Reid *et al.*, 1987), the Schmidt number of CH_3I can be estimated from equations (5.12) and (5.13) as follows;

$$Sc_{CH3I} = (62.9/52.9)^{0.6} \cdot (2004 - 99.5T + 1.39T^2) \quad \textbf{(5.14)}$$

Where T = water temperature (°C)

$$\Delta C_{CH3I} = (C_a/H) - C_w \quad \textbf{(5.15)}$$

Where C_a = concentration of CH_3I in air (pmol L^{-1})

C_w = concentration of CH_3I in water (pmol L^{-1})

H = Henry's law constant

The solubility of a gas in water is a function of temperature and salinity. Since the value of H is based on the solubility of the gas in question, the value of H is also a function of temperature and salinity. According to Moore *et al.* (1995) the value of H for CH_3I could be estimated using the equation;

$$H = e^{13.32 - 4338/T} \quad \textbf{(5.16)}$$

Where T = Absolute water temperature (Kelvin)

Equation (5.16) was tested by the authors through the measurement of H in salinities of 25.4 and 30.4 in order to address the effect of salinity on H. The authors found no statistically significant difference between the H for the two different salinities therefore stated that it could be used for a salinity range of 25 and 35 without any further correction. In this study the application of equation (5.16) was sufficient for estimating H for CH_3I at Cargreen and The Narrows with high salinities ranging between 25 and 35 (except two

data points at Cargreen). To the best of my ability, no relationship that addresses the effect of temperature as well as salinity on H at low salinities was found in the literature for the other two sampling stations within the freshwater reaches of the estuary. For this reason, flux estimates at Morwellham and Tuckermarsh with salinities of ≤ 7 which are also based on the use of equation (5.16) in estimating H are likely to be overestimated.

According to Oram & Penkett (1994), there is a significant seasonal cycle in air concentration of CH_3I from a maximum of 43.1 pptv during the summer and dropping to 1 pptv or less during the autumn and winter months. Also, according to Carpenter (2003), there is general agreement that the concentration of CH_3I over the open ocean are between 0.5 and 2 ppt(v) with coastal regions recording higher amounts. To estimate the water to air flux of CH_3I over the Tamar estuary, the air concentration (C_a) of CH_3I has been assumed to range between the agreed minimum value of 0.5 pptv (Carpenter, 2003) and 1 pptv (Oram & Penkett, 1994). This assumption has been made because no atmospheric concentration of CH_3I over the Tamar estuary was measured during the study. In this sense, two air concentrations of 0.5 pptv and 1 pptv have been used in the flux calculation of this thesis and their mean flux rates compared. The values have been converted to 0.0204 and 0.0408 pmol L^{-1} respectively using the ideal gas equation.

As a summary of the water-air flux calculations, the Schmidt number of CH_3I (Sc_{CH3I}) was estimated using equation 5.14 (corrected for temperature). This was then used in equation 5.11 to estimate the transfer velocity of CH_3I (k_{CH3I}) which is also corrected for wind speed. Together with the concentration gradient of CH_3I (ΔC_{CH3I}) estimated using equations 5.15 and 5.16, the water to air flux of CH_3I (F_{CH3I}) over the Tamar estuary was calculated using equation 5.2.

Table 5.4: A table summarizing the estimation of water-air CH_3I flux in the Tamar estuary based on the estimated transfer velocity (k) from wind speed (u) using a number of approaches. NB: * is based on C_a = 1 pptv and ** is based on C_a = 0.5 pptv.

Method used in estimating the transfer velocity (k)	Estimated mean (sd) water to air CH_3I flux (nmol m^{-2} d^{-1})				
	Morwellham	Tuckermarsh	Cargreen	The Narrows	Mean Flux
Liss & Merlivat (1986)*	0.08 (0.12)	0.10 (0.18)	0.41 (0.84)	0.52 (0.98)	**0.28** (0.66)
Wanninkhof (1992)*	0.11 (0.11)	0.16 (0.18)	0.61 (0.92)	0.77 (1.01)	**0.41** (0.73)
Clark et al (1995)*	0.13 (0.11)	0.22 (0.22)	0.80 (0.82)	1.03 (0.87)	**0.54** (0.70)
Clark *et al* (1995)**	0.15 (0.18)	0.28 (0.28)	0.91 (0.86)	1.14 (0.91)	**0.62** (0.75)
Nightingale *et al* (2000)*	0.10 (0.11)	0.15 (0.21)	0.60 (0.81)	0.77 (0.88)	**0.41** (0.65)

By estimating the transfer velocity (k) of CH_3I in the Tamar estuary based on the four different approaches (i.e. LM86, W92, CL95 and NG00), the results of the water-air CH_3I flux rates in the estuary has been summarized in Table 5.4. From the mean flux rates (based on 1 pptv air concentration), the highest estimated values (with a mean of 0.5 ± 0.7 nmol m^{-2} d^{-1}) for all stations were obtained using the CL95 transfer velocity relationship whilst the least estimated values (with a mean of 0.3 ± 0.7 nmol m^{-2} d^{-1}) were obtained using the LM86 relationship. On average, estimated water-air flux rates of CH_3I in the Tamar estuary using the W92 and NG00 relationships yielded the same mean flux rate of 0.4 ± 0.7 nmol m^{-2} d^{-1} which is 24% lower than the mean flux derived from CL95 relationship. Also a comparison between the mean flux rates of 0.5 ± 0.7 nmol m^{-2} d^{-1} (based on air concentration of 1 pptv) and 0.6 ± 0.8 nmol m^{-2} d^{-1} (based on air concentration of 0.5 pptv) using the CL95 relationship resulted in a difference of 13%. This indicate that if the lower air concentration of 0.5 pptv is applied in this study for the calculation of water to air flux of CH_3I in the Tamar estuary may result in an overestimation.

The LM86 transfer velocity relationship has failed to show any obvious correlation with wind speed (Nightingale *et al.*, 2000). The described W92 approach was also simply a quadratic fit based on the oceanic uptake of bomb derived ^{14}C and is only in agreement

with the global average transfer velocity when the mean global wind speeds are applied (Nightingale *et al.*, 2000). Although the NG00 relationship which was based on Southern Ocean and coastal seas at different wind speeds has addressed the drawbacks of the LM86 and W92 approaches, it does not take into account estuarine environments. The CL95 relationship is the only one amongst the four which was based on estuarine environment making it the most appropriate at predicting water to air flux in the Tamar estuary. Therefore all subsequent CH_3I water to air flux calculation in the Tamar estuary (including global estimate) are carried out using the CL95 relationship and C_a of 1 pptv.

Table 5.5: A table showing the estimated transfer velocity (k) of CH_3I and the measured concentration of CH_3I in water (C_w) over the selected sampling stations along the Tamar estuary. The transfer velocity (k) is based on normalized wind speed (u) for 10 m height and the estimated Schmidt number of CH_3I using the CL95 relationship.

Date	u (m s^{-1})	k_{CH3I} (cm h^{-1}) and C_w (pmol L^{-1})							
		Morwellham		Tuckermarsh		Cargreen		The Narrows	
		k_{CH3I}	C_w	k_{CH3I}	C_w	k_{CH3I}	C_w	k_{CH3I}	C_w
12-May	3.0	5.88	0.06	5.70	0.14	5.65	0.46	5.87	0.65
03-Jun	2.8	4.84	0.17	4.74	0.21	4.79	0.82	5.11	1.31
13-Jun	3.6	6.19	0.16	6.14	0.29	6.12	0.97	6.53	1.41
30-Jun	2.1	3.28	0.18	3.21	0.09	3.29	0.91	3.63	1.28
26-Jul	1.9	3.13	0.24	3.05	0.48	3.16	1.13	3.43	1.46
09-Aug	2.6	3.98	0.35	3.83	0.68	3.93	1.43	4.21	2.04
25-Aug	6.1	12.6	0.37	12.2	0.47	12.2	1.43	12.5	1.70
12-Sep	0.7	2.40	0.22	2.30	0.66	2.20	1.40	2.35	1.75
26-Sep	4.2	7.86	0.49	7.57	0.73	7.36	1.13	7.25	1.37
25-Nov	4.1	10.03	0.14	10.08	0.38	9.49	0.51	8.91	0.67

Table 5.6: Estimated spatial and temporal rates of water to air flux of CH_3I over the Tamar estuary (based on 1 pptv air concentration) with Henry's constant (*H*) estimated at *in situ* surface water temperatures. NB: The unit of ΔC_{CH3I} presented in this table is pmol L^{-1}.

	Water to air flux of CH_3I in the Tamar (nmol m^{-2} d^{-1})								**Mean Flux** (sd)
	Morwellham		Tuckermarsh		Cargreen		The Narrows		
Date	ΔC_{CH3I}	F_{CH3I}	ΔC_{CH3I}	F_{CH3I}	ΔC_{CH3I}	F_{CH3I}	ΔC_{CH3I}	F_{CH3I}	
12-May	0.265	0.19	0.117	0.09	0.174	0.13	0.363	0.26	**0.2** (0.1)
03-Jun	0.787	0.06	0.037	0.03	0.571	0.42	1.046	0.73	**0.3** (0.3)
13-Jun	0.073	0.08	0.061	0.06	0.724	0.76	1.181	1.16	**0.5** (0.5)
30-Jun	0.007	0.01	0.109	0.08	0.716	0.49	1.065	0.66	**0.3** (0.3)
26-Jul	0.017	0.01	0.263	0.17	0.907	0.56	1.238	0.70	**0.4** (0.3)
09-Aug	0.129	0.10	0.467	0.37	1.210	0.94	1.688	1.23	**0.7** (0.5)
25-Aug	0.140	0.31	0.253	0.58	1.294	2.97	1.481	3.30	**1.8** (1.6)
12-Sep	0.004	0.00	0.427	0.20	1.189	0.58	1.455	0.66	**0.4** (0.3)
26-Sep	0.230	0.27	0.462	0.57	0.814	1.03	0.997	1.26	**0.8** (0.5)
25-Nov	0.256	0.23	0.018	0.02	0.154	0.15	0.339	0.34	**0.2** (0.1)
Mean Flux sd		**0.1** (0.1)		**0.2** (0.2)		**0.8** (0.8)		**1.0** (0.9)	

From the estimated flux rates (Table 5.6) during this study, the Tamar estuary was marginally a source (water to air flux) of atmospheric CH_3I. During the study period, the water to air transfer of CH_3I ranged between 0.01 – 3.30 nmol m^{-2} d^{-1}. Spatially, with an average value of 1.0 ± 0.9 nmol m^{-2} d^{-1} (which is over 10 times lower than that at the coastal station L4), the highest water to air flux of CH_3I occurred at The Narrows where observed surface water concentrations of CH_3I were the highest. The rate decreased from the seawater reaches towards the freshwater reaches of the estuary (i.e. with decreasing CH_3I concentration in surface water) with Morwellham recording the lowest water to air flux rate of 0.1 ± 0.1 nmol m^{-2} d^{-1} (see Table 5.6). The potential of the Tamar estuary as a CH_3I source therefore increases from the low salinity region towards the high salinity region with the net exchange depending on the air/water concentration gradient.

Temporal variation in water to air flux of CH_3I was also observed in the estuary. With respect to the sampling dates, the highest mean rate of CH_3I water to air flux in the Tamar (1.8 ± 1.6 nmol m^{-2} d^{-1}) was recorded in the month of August whilst the lowest mean flux rate of 0.2 ± 0.1 nmol m^{-2} d^{-1} was recorded during the month of May and November (see Table 5.6). With a mean rate of 0.7 ± 0.8 nmol m^{-2} d^{-1}, the observed temporal variation of water to air flux was highest during the warm season and this is three times as high as the mean recorded rate during the cold season (0.2 ± 0.2 nmol m^{-2} d^{-1}). The temporal variability in the estimated water to air CH_3I flux rates in the Tamar estuary may be driven by the variability in water concentration and the reliability of the transfer velocity on varying wind speeds during the study period.

The estimated water to air flux rate of CH_3I over the Tamar estuary could not be compared to other estuarine systems since no information regarding the flux of CH_3I in estuaries was found in the literature. However, estimated values from other marine regions summarized in Table 5.7 could be compared with that of Tamar estuary.

Table 5.7: Comparing the estimated water to air flux rate of CH_3I in the Tamar estuary with other marine regions.

Region	Mean Flux Rate (nmol m^{-2} d^{-1})	Source
Southern North Sea	9.5	Campos *et al.* (1996)
Arctic Ocean	8.1	Schall & Heumann (1993)
Pacific Ocean	16	Moore & Groszko (1999)
Eastern Atlantic Ocean	25	Moore & Groszko (1999)
Labrador Sea	11.9	Moore & Groszko (1999)
Plymouth L4 (UK)	11.9	Archer *et al.* (2007)
Tamar Estuary (UK)	0.54	Present study

The closest region for comparison with Tamar estuary is L4. The Tamar estuary recorded an estimated mean flux rate of 0.5 ± 0.7 nmol m^{-2} d^{-1} over the study period. At L4, the estimated daily water to air flux rate using the W92 relationship is 11.9 nmol m^{-2} d^{-1}

(Archer *et al.*, 2007). This compared to the estimated mean water to air flux rate of CH_3I over the Tamar estuary is over twenty times higher. Considering the fact that surface water concentration of CH_3I at L4 (i.e. 6.1 pmol L^{-1}) is only four and half times higher than the mean concentration at the mouth of the Tamar estuary (i.e. 1.36 pmol L^{-1}), the difference in the mean daily water-air flux rate of CH_3I between the Tamar estuary and L4 suggest a massive net production rate of CH_3I at L4 which drives the water to air flux. Similarly, the mean flux rate of CH_3I in the Tamar estuary compared to the other marine regions in Table 5.7 was far lower ranging from fifteen to fifty times lower than that of the Arctic Ocean and Eastern Atlantic Ocean respectively. This may imply that estuarine systems are the least contributors to atmospheric CH_3I from the aquatic environment.

From the estimated mean daily water to air flux, the Tamar estuary (with an estimated surface area of 11 x 10^6 m^2 (Law, 1989)) has a total water to air CH_3I flux rate of 5.9 x 10^6 nmol d^{-1} and a total annual water to air CH_3I flux rate of 2.2 x 10^9 nmol y^{-1} (0.31 x 10^3 g y^{-1}). These estimates are however limited by the use of wind speed measurements which were not obtained directly from the Tamar estuary, the assumed air concentration (40.8 x 10^{-3} pmol L^{-1}) of CH_3I and salinity effect on transfer velocity in the freshwater region of the estuary. According to Moore & Groszko (1999), the total global flux of CH_3I is estimated to be between 128 and 355 x 10^9 g y^{-1}. From the total water to air flux of CH_3I in the Tamar estuary, estuarine systems with a tentative estimated global surface area of 1.4 x 10^6 km^2 (Gattuso *et al.*, 1998) will have an estimated total global water to air flux of 3.9 x 10^7 g y^{-1}. This estimated total global water to air flux by estuarine systems represents only 0.03% of the minimum total global flux (128 x 10^9 g y^{-1}) therefore could be considered as negligible. However by comparing this amount (3.9 x 10^7g y^{-1}) to the estimated total global bacterial production (1.7 x 10^{12} g y^{-1}) suggest that much of the produced CH_3I in the estuarine environment is consumed by other processes (e.g. methylotrophic bacterial CH_3I removal) also within estuarine environment.

5.5.3. Spatial and seasonal variations in bacterial properties along the Tamar estuary

This section presents and discusses bacterial properties of interest i.e. (i) bacterial abundance, (ii) bacterial leucine incorporation and (iii) bacterial CH_3I removal. Prior to these analyses, the data from the four selected sampling locations were grouped into low salinity (<8) (i.e. Morwellham and Tuckermarsh) and high salinity (>8) regions of the estuary (i.e. Cargreen and The Narrows) as well as cold (<15 °C) and warm (>15 °C) seasons during the sampling period.

The recorded data from the estuary were statistically analysed in the following manner in order to evaluate spatial and seasonal differences. These were;

(a) Spatial variation between the selected sampling locations (summarized in Table 5.8).

(b) Overall variation in the estuary (also summarized in Table 5.8).

(c) Seasonal variation at each sampling location (summarized in Table 5.9).

(d) Seasonal variation ratio in the estuary (presented in Table 5.9).

(e) Spatial variation between low and high salinity regions (summarized in Table 5.10).

Table 5.8: A summary of bacterial parameters (spatial variation at the selected sampling locations) along the Tamar estuary (2005) with recorded mean values and ranges of the measured parameters.

Sampling Locations	Bacterial Abundance ($x10^9$ cells L^{-1})		Rate of Bacterial Leucine Incorporation (pmol L^{-1} h^{-1})		Rate of Bacterial CH_3I Removal (nmol L^{-1} h^{-1})	
	mean ± sd	Range	mean ± sd	Range	mean ± sd	Range
Morwellham	0.68 ± 0.54	0.09 – 1.64	58.3 ± 60.7	4.70 – 188	48.9 ± 45.6	5.40 – 124
Tuckermarsh	1.03 ± 1.01	0.11 – 3.22	143 ± 179	6.40 – 583	50.4 ± 39.3	4.10 – 100
Cargreen	1.25 ± 1.28	0.06 – 3.61	126 ± 144	11.7 – 420	40.2 ± 41.9	3.70 – 149
The Narrows	0.71 ± 0.69	0.03 – 1.82	29.7 ± 22.7	7.60 – 70.6	43.5 ± 43.7	2.00 – 129
All Stations	0.92 ± 0.93	0.03 – 3.61	89.2 ± 125	4.70 – 583	48.9 ± 45.6	2.00 – 149

Table 5.9: A summary of bacterial parameters at each selected sampling location (variation between cold and warm seasons) along the Tamar estuary (2005). A ratio between warm (W) and cold (C) seasons is included to illustrate the seasonal variations at each sampling location.

Sampling Locations	Bacterial Activity	Cold Season (<15°C)		Warm Season (>15°C)		Ratio (W/C)
		mean ± sd	Range	mean ± sd	Range	
Morwellham	Bacterial Abundance ($x10^9$ cells L^{-1})	0.20 ± 0.08	0.09 – 0.31	1.01 ± 0.45	0.15 – 1.64	5.1
	Rate of Bacterial Leu. Inc. (pmol L^{-1} h^{-1})	16.2 ± 15.6	4.70 – 45.5	94.6 ± 62.1	28.0 – 188	5.8
	Total Bacterial CH_3I Removal (nmol L^{-1} h^{-1})	9.50 ± 3.90	5.40 – 15.4	82.7 ± 35.4	35.2 – 124	8.7
Tuckermarsh	Bacterial Abundance ($x10^9$ cells L^{-1})	0.22 ± 0.07	0.12 – 0.30	1.60 ± 0.97	0.17 – 3.22	7.3
	Rate of Bacterial Leu. Inc. (pmol L^{-1} h^{-1})	44.8 ± 63.7	6.40 – 173.8	227 ± 207	44.2 – 583	5.1
	Total Bacterial CH_3I Removal (nmol L^{-1} h^{-1})	12.5 ± 6.00	4.10 – 20.3	83.1 ± 19.2	50.4 – 100	6.6
Cargreen	Bacterial Abundance ($x10^9$ cells L^{-1})	0.17 ± 0.13	0.06 – 0.38	2.02 ± 1.15	0.07 – 3.61	11.9
	Rate of Bacterial Leu. Inc. (pmol L^{-1} h^{-1})	17.7 ± 5.00	11.7 – 23.6	215 ± 144	53.7 – 420	12.1
	Total Bacterial CH_3I Removal (nmol L^{-1} h^{-1})	14.8 ± 15.9	3.70 – 46.0	65.3 ± 43.3	8.90 – 149	4.4
The Narrows	Bacterial Abundance ($x10^9$ cells L^{-1})	0.10 ± 0.09	0.03 – 0.26	1.14 ± 0.57	0.05 – 1.82	11.4
	Rate of Bacterial Leu. Inc. (pmol L^{-1} h^{-1})	9.20 ± 1.60	7.60 – 12.3	42.3 ± 20.7	12.0 – 70.6	4.6
	Total Bacterial CH_3I Removal (nmol L^{-1} h^{-1})	7.60 ± 5.90	2.00 – 18.3	75.2 ± 35.6	31.3 – 129	9.9

Table 5.10: A summary of bacterial activity (spatial variation between low/high salinity regions and seasonal variation between cold/warm seasons) along the Tamar estuary (2005) with recorded mean values and ranges of the measured parameters.

	Bacterial Abundance ($x10^9$ cells L^{-1})		**Rate of Bacterial Leucine Incorporation (pmol L^{-1} h^{-1})**		**Rate of Bacterial CH_3I Removal (nmol L^{-1} h^{-1})**	
	mean ± sd	Range	mean ± sd	Range	mean ± sd	Range
Low Salinity Region	0.85 ± 0.81	0.09 – 3.22	101 ± 138	4.70 – 583	49.7 ± 41.7	4.10 – 124
High Salinity	0.98 ± 1.04	0.03 – 3.61	75.7 ± 113	7.60 – 420	43.0 ± 41.6	2.10 – 149

Table 5.11: Summary of spatial and seasonal statistical analyses of bacterial activities (refer to the following Appendices for actual statistical analyses. For (i) 10a-c, (ii) 11a-c, (iii) 12a-b, 13a-b, 14a-b, (iv) 15a-d, 16a-d, 17a-d, (v) 18a-c).

Analysed Variations in the Tamar estuary	**Statistically significant ($p<0.05$)**		
	Bacterial CH_3I removal rate	**Bacterial leucine incorporation**	**Bacterial abundance**
(i) Spatial variation between the selected sampling locations	No	No	No
(ii) Spatial variation between high and low salinity regions	No	No	No
(iii) Spatial variation during cold/warm seasons	No (Cold) No (Warm)	No (Cold) Yes (Warm)	No (Cold) No (Warm)
(iv) Seasonal variation at each selected sampling location	Yes (All)	No (Tuckermarsh) Yes (All others)	Yes (All)
(v) Overall seasonal variation in the estuary	Yes	Yes	Yes

5.5.3.1. Bacterial abundance

Throughout the sampling period, recorded bacterial abundance in the Tamar estuary varied between 0.03 x 10^9 and 3.6 x 10^9 cells L^{-1} (Figure 5.9a-d). The maximum recorded abundance during the survey period was however over ten times less than that recorded in some estuarine systems (e.g. 45.4 x 10^9 cells L^{-1} for the Humber estuary, reported by Bent & Goulder, 1981; see Table 1.1 in Chapter One).

The mean bacterial abundance in the Tamar estuary over the study period was estimated as $0.97 \pm 0.95 \times 10^9$ cells L^{-1}. On average, Morwellham (with mean abundance of 0.7×10^9 cells L^{-1}) recorded the lowest bacterial abundance during the survey period. Although higher bacterial abundance was observed at the mid-estuary (Tuckermarsh and Cargreen) compared to Morwellham and The Narrows, no significant spatial variation was observed between the sampling locations.

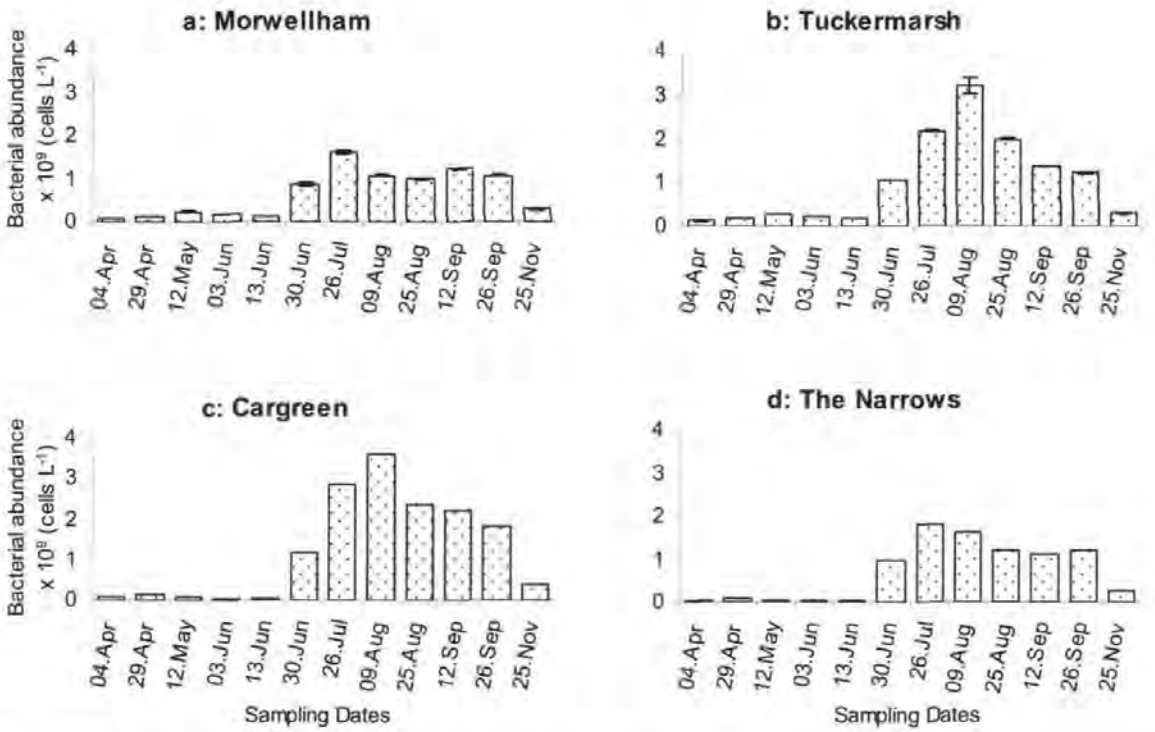

Figure 5.9a-d: Temporal bacterial abundance (cells L^{-1}) at the four selected sampling stations along the Tamar Estuary (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

Also, between all the four sampling locations in the Tamar estuary, there was no significant spatial variation during the cold ($p = 0.212$; $r^2 = 0.081$) and warm ($p = 0.122$; $r^2 = 0.086$) seasons. The higher abundance at the mid-estuary (compared to the extreme ends of the estuary) could be as a result of the prevalence of resuspended particles (e.g. Bent & Goulder, 1981; Joint & Pomroy, 1982; Painchaud & Therriault, 1989) with high organic matter constituent to provide a suitable niche and nutrients for bacterial colonization and growth (Crump *et al.* 1998).

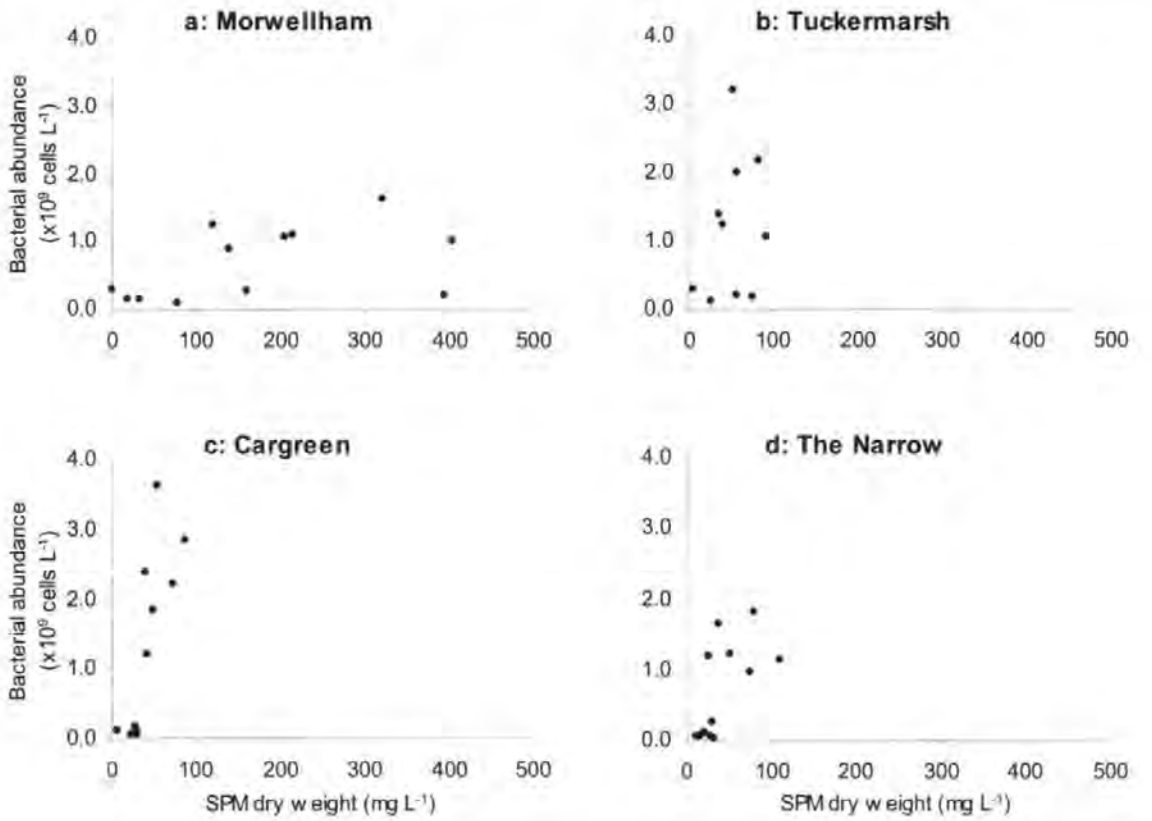

Figure 5.10a-d: Scatter plots showing the correlation between bacterial abundance (cells L^{-1}) and SPM dry weight (mg L^{-1}) at the four selected sampling stations along the Tamar Estuary (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

This is supported by the relationship presented in Figure 5.10a-d which indicates that an increase in SPM concentration corresponded to an increase in bacterial abundance at all the selected sampling locations along the Tamar estuary (even though this relationship was less prominent at Morwellham where SPM concentration was highest). Bacteria population within the mid-estuary is likely to be more diverse (a mix of freshwater and seawater species) due to the continuously changing physico-chemical conditions (especially pronounced salinity and SPM changes associated with tidal movements) within this part of the estuary (e.g. Crump *et al.*, 1999; Troussellier *et al.* 2002).

Based on the correlation between bacterial abundance and SPM concentration in the Tamar (Figure 5.10a-d), much higher increases in bacterial abundance with respect to SPM concentration increases was observed within the mid-estuary (Tuckermarsh and Cargreen).

This compared to the other two sampling stations (especially Morwellham) suggest that, bacterial growth in the estuary was also dependent on other forms of substrates in the POM associated with SPM prevalent in high concentration at the upper estuary (including Morwellham). EPS produced by benthic diatoms inhabiting intertidal mudflats (Happey-Wood & Jones, 1988; Smith & Underwood, 1998; Smith & Underwood, 2000) may have provided this additional substrate to support bacteria growth (Morris *et al.*, 1985; Owens, 1986) within the mid-estuarine region.

A significant seasonal variation ($p < 0.001$; $r^2 = 0.448$) of bacterial abundance in the entire Tamar estuary as well as within each sampling location was observed during the study. From June, there was a rapid increase in bacterial abundance peaking in August at all selected sampling stations (except Morwellham) (see Figure 5.9a-d). Prior to the commencement of the warm season (before June), recorded bacterial abundance at all sampling stations were just up to 0.3×10^9 cells L^{-1}. This is also evidence that growth of bacteria in the Tamar estuary during the course of the present study was very temperature dependent (i.e. p-values < 0.05; r^2-values > 0.42 for bacterial abundance versus temperature at every station). An assessment of the correlation between bacterial abundance and temperature has been provided in section 5.5.4.5.

5.5.3.2. Bacterial leucine incorporation

This section will explore the spatial and seasonal variations in the rate of bacterial leucine incorporation at the selected sampling locations along the Tamar estuary during the study period.

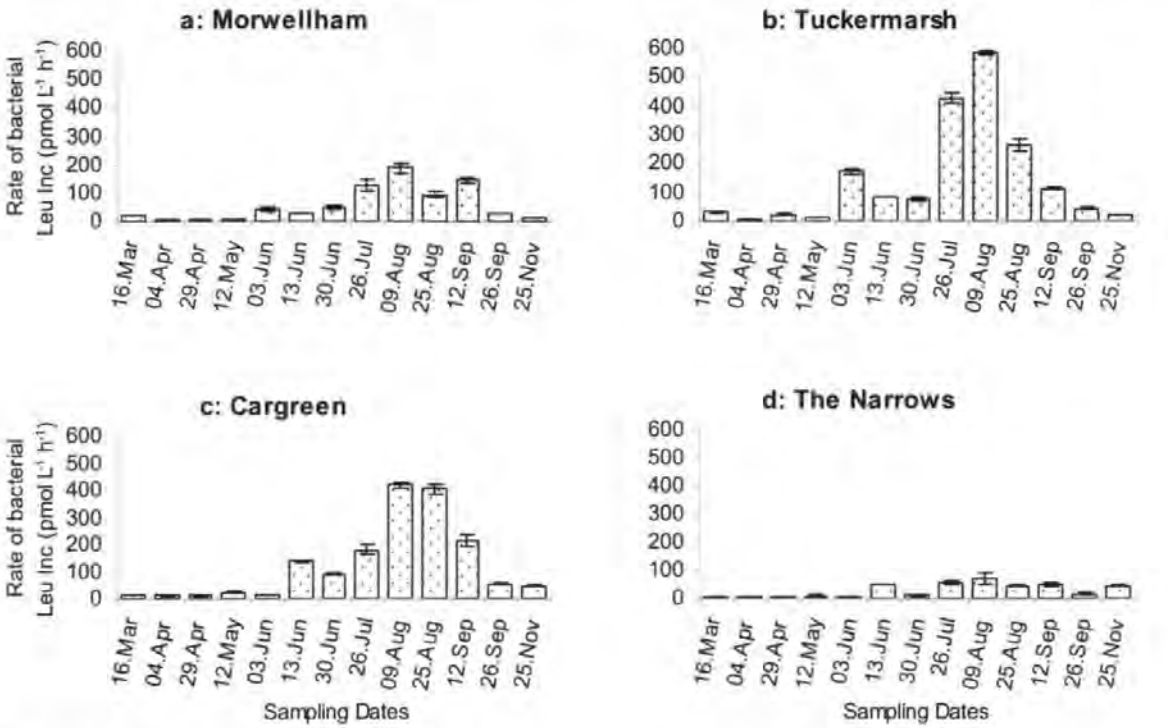

Figure 5.11a-d: Temporal variation in rate of bacterial leucine incorporation (pmol L^{-1} h^{-1}) at the four selected sampling stations along the Tamar Estuary (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

Bacterial production was investigated through the rate of leucine incorporation into bacterial protein (Figure 5.11). Similar to the pattern of spatial distribution of bacterial abundance, the highest bacterial leucine incorporation rate was recorded in the mid-estuary (mean of 143 pmol L^{-1} h^{-1}at Tuckermarsh and 126 pmol L^{-1} at Cargreen; see Table 5.8) whilst the lowest was recorded at The Narrows (mean of 30 pmol L^{-1} h^{-1}). Spatial variation between the selected sampling locations was pronounced between The Narrows and the rest of the selected sampling stations but less prominent in the mid-estuary (i.e. between Tuckermarsh and Cargreen). There was also no statistically significant variation between the low and high salinity regions of the estuary and between the sampling locations during the cold season.

As presented in Figure 5.12a-d, the relationship between bacterial leucine uptake and abundance was found to be significantly correlated (p-values < 0.01; r^2-values > 0.6) at all the selected sampling stations. Since increased bacterial production corresponds to

increased bacterial abundance and vice versa (e.g. Iriberri *et al.*, 1987; Unanue *et al.*, 1992), the relationship between bacterial abundance and leucine incorporation exhibited pronounced bacterial activity in the mid-estuary (Figure 5.12b and c).

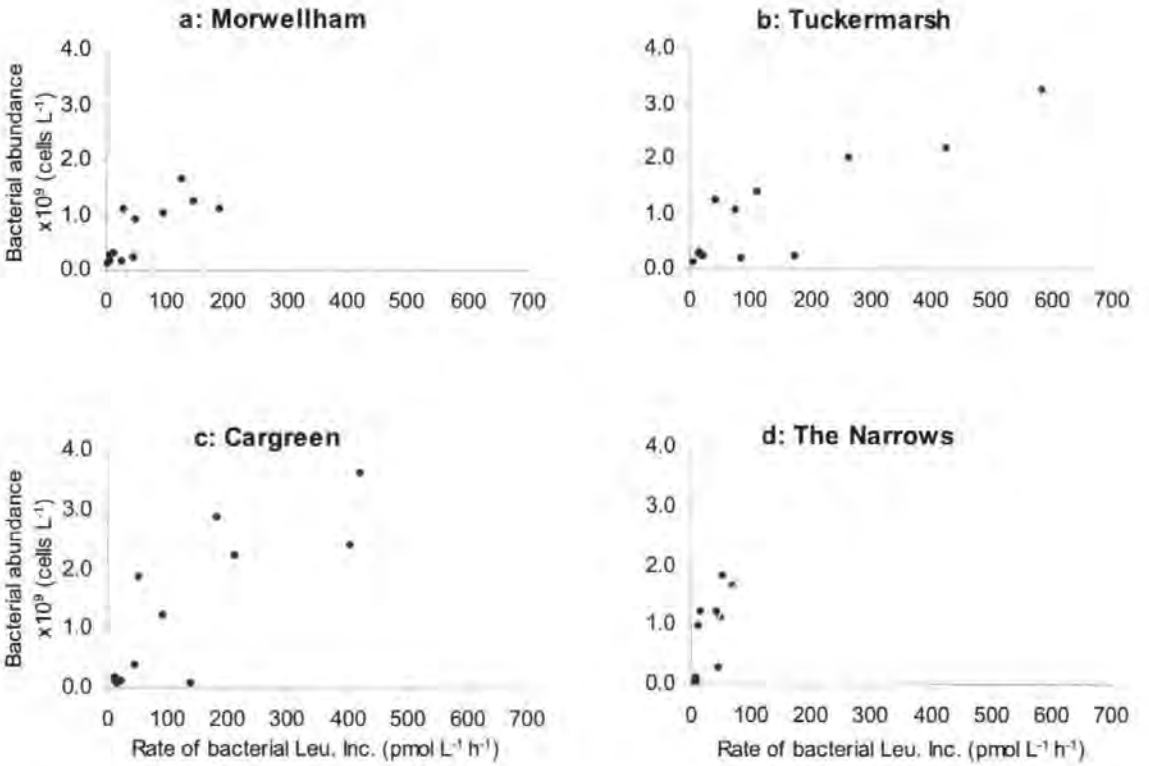

Figure 5.12a-d: Scatter plots showing the correlation between the rate of bacterial leucine incorporation (pmol L^{-1} h^{-1}) and bacterial abundance (cells L^{-1}) at the four selected sampling stations along the Tamar Estuary (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

From Figure 5.11, all the selected sampling locations exhibited strong seasonality in bacterial leucine incorporation. Two peaks of the bacterial leucine incorporation rate were observed during the warm season with the maximum peak occurring in August at all sampling locations indicating strong seasonal variation in bacterial activity in the estuary. The rate of bacterial leucine incorporation rate in the Tamar estuary during the warm season was from five times (at Tuckermarsh and The Narrows) to twelve times (at Cargreen) higher than that of the cold season (see Table 5.9). Seasonal variation in bacterial leucine incorporation was therefore experienced in the entire estuary.

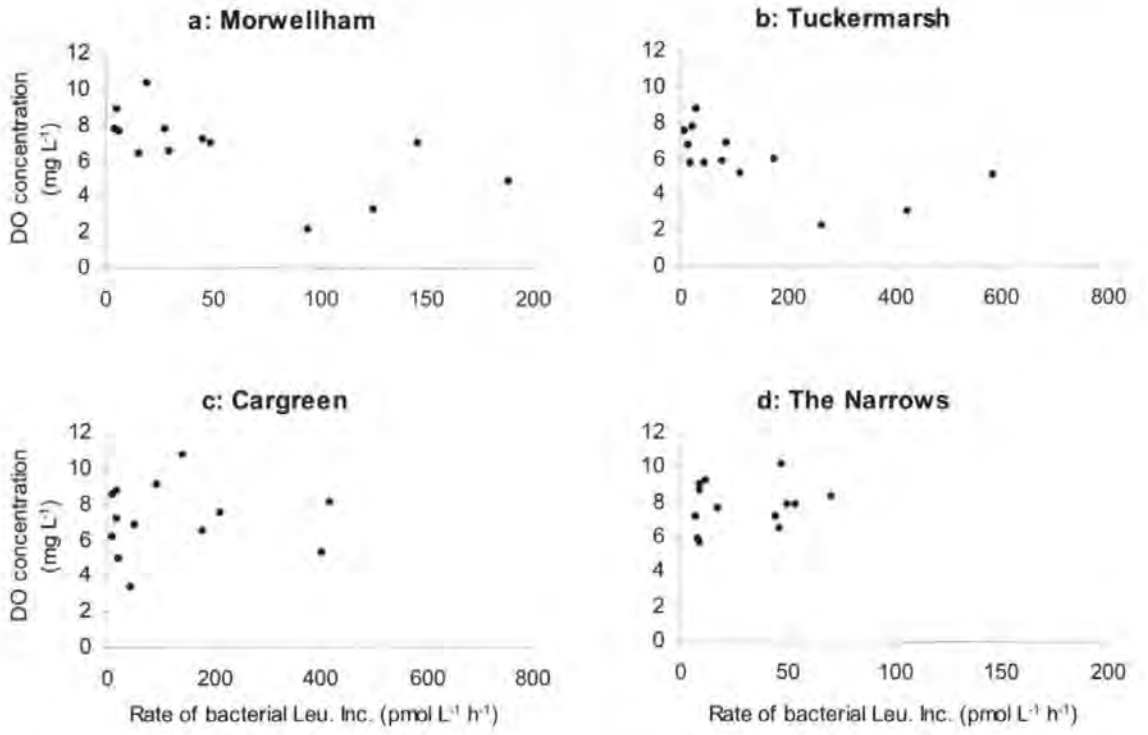

Figure 5.13a-d: Scatter plots showing the correlation between the rate of bacterial leucine incorporation (pmol L^{-1} h^{-1}) and DO concentration (mg L^{-1}) at the four selected sampling stations along the Tamar Estuary (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows. Note the difference in scale of the x-axis.

From the scatter plots (Figure 5.13a-d), there existed a weak statistically significant correlation between bacterial activity and DO concentration at Morwellham ($p < 0.05$, $r^2 = 0.396$) and Tuckermarsh ($p < 0.02$, $r^2 = 0.419$) but non-existing statistically significant correlation at Cargreen ($p > 0.10$, $r^2 = 0.001$) and The Narrows ($p > 0.10$, $r^2 = 0.041$) in the Tamar estuary.

The relationship between bacterial leucine incorporation rates and DO concentration in the Tamar estuary exhibited a negative correlation within the upper region (Morwellham and Tuckermarsh) and a positive correlation within the lower reaches (Cargreen and The Narrows) of the estuary. The absence of primary productivity (Joint & Pomroy, 1981; Jackson *et al.*, 1987) due to high SPM concentration within the upper reaches (Uncles *et al.*, 1994; Tattersall *et al.*, 2003) coupled with high heterotrophic microbial activity

(Goosen *et al.*, 1997) (with high oxygen demand; Morris *et al.*, 1982) may explain the negative correlation between bacterial activities and DO concentration.

However, due to the presence of photosynthetic activity within the lower reaches of the estuary where SPM concentration is very low (see Figure 5.6c-d), primary productivity through phytoplankton activity in surface water (Goosen *et al.*, 1999) may contribute to the DO concentration in this region and hence may also explain the positive correlation between bacterial activities and DO concentration.

By comparison with other temperate estuaries (presented in Table 1.1 of Chapter One), the range of bacterial productivity from the Tamar estuary (0.3 – 43.2 mg C m^{-3} d^{-1}) is the lowest amongst the listed estuaries (ranging from 0.5 – 480 mg C m^{-3} d^{-1}). This is likely due to the Tamar estuary also having the lowest bacterial abundance (0.03 – 3.6 x 10^9 cells L^{-1}) compared to the other estuaries (ranging from 0.4 – 45.4 x 10^9 cells L^{-1}). However, by assuming all bacterial cells are contributing equally to the total productivity, the specific bacterial activity (productivity per cell) in the Tamar estuary (2.1- 41.5 fg C d^{-1} $cell^{-1}$), with a mean bacterial specific activity of 11.0 fg C d^{-1} $cell^{-1}$, is amongst the estuaries with high bacterial specific activities.

5.5.3.3. Bacterial methyl iodide removal

This section highlights the spatial and seasonal variations in the rate of bacterial CH_3I removal at the selected sampling locations along the Tamar estuary during the study period.

The rate of bacteria CH_3I removal in water samples from the four sampling locations along the Tamar estuary is presented in Figure 5.14a-d and summarized in Table 5.8. Unlike bacterial leucine incorporation, the exhibited spatial and seasonal variation was dissimilar

to that found for bacterial abundance (section 5.5.3.1). Overall, CH_3I removal rate during the survey period ranged between 2 – 149 nmol L^{-1} h^{-1} with a mean rate of 45.8 ± 41.6 nmol L^{-1} h^{-1}. Tuckermarsh with a mean removal rate of 50.4 ± 39.3 nmol L^{-1} h^{-1} recorded the highest rate of bacterial CH_3I removal whilst Cargreen with a mean removal rate of 40.2 ± 41.9 nmol L^{-1} h^{-1} however recorded the lowest removal rate over the survey period. This may be because unlike the general activity of bacteria which is dependent on the spatial availability and distribution of substrate (POC and DOC) in the estuary, microbial CH_3I removal is carried out by a specific group of bacteria (methylotrophs). It could therefore be hypothesized that the pattern of methylotrophic bacterial activity in the estuary is dependent on the community structure of the methylotrophs and the spatial availability of CH_3I (as substrate) along the estuary.

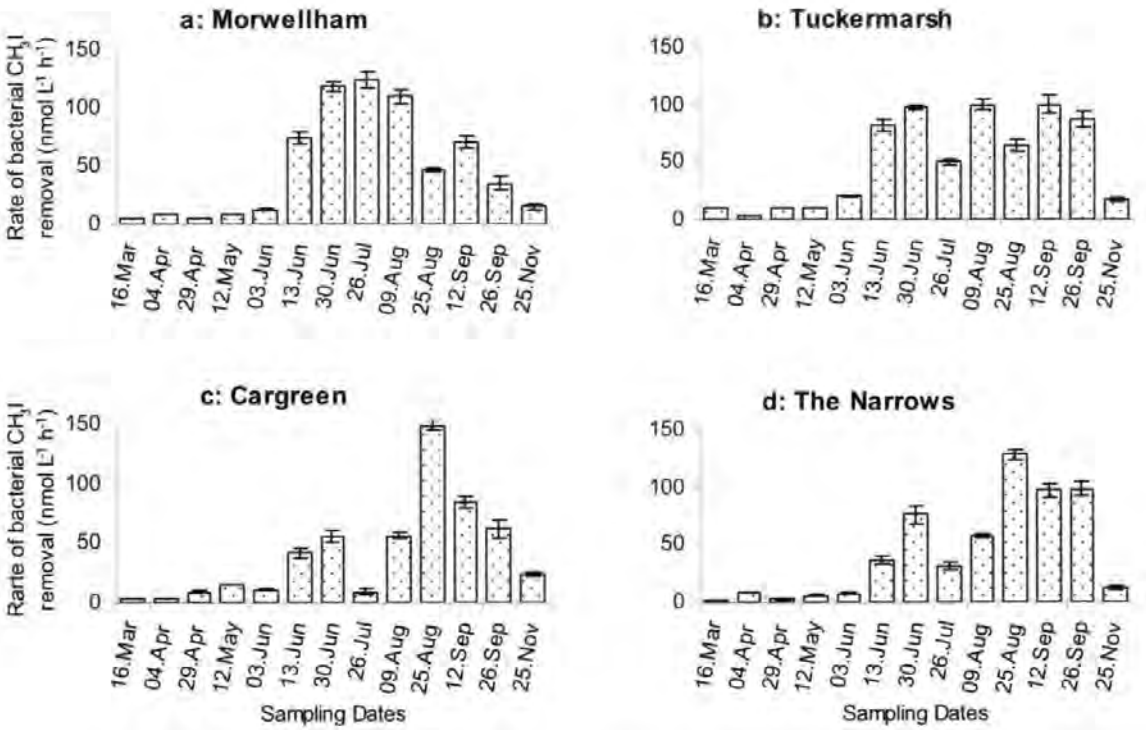

Figure 5.14a-d: Temporal variation in bacterial CH_3I removal (nmol L^{-1} h^{-1}) at the four selected sampling stations along the Tamar Estuary (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

Nutrient availability will always be necessary for bacterial growth but seasonal variations in bacterial CH_3I removal may be more closely coupled with seasonal pattern of

temperature in the estuary since temperature variation in estuaries is known to affect the interactions between the physical and biological activities (Uncles & Stephens, 2001). A detailed discussion of the correlation between bacterial CH_3I removal and temperature is provided in section 5.5.4.1. Seasonally, a significant variation was prominent in the estuary. Between cold (<15 °C) and warm (>15 °C) seasons, there was strong seasonality at all sampling locations (refer to Table 5.9 and Appendix 9a-d). Within the low salinity region of the estuary, the highest CH_3I removal rate was observed in August. On the other hand, the highest CH_3I removal rate at Morwellham was observed in July whilst that of Tuckermarsh was observed in September. The lowest rates at all selected sampling stations were however recorded between March and April during the study period.

The results (see Figure 5.14a-d) would suggest that there were successions of cycles in bacterial CH_3I removal during the period. This is indicated by the presence of a number of peaks of high CH_3I removal rate by methylotrophic bacteria observed mainly within the warm season (between June and September).

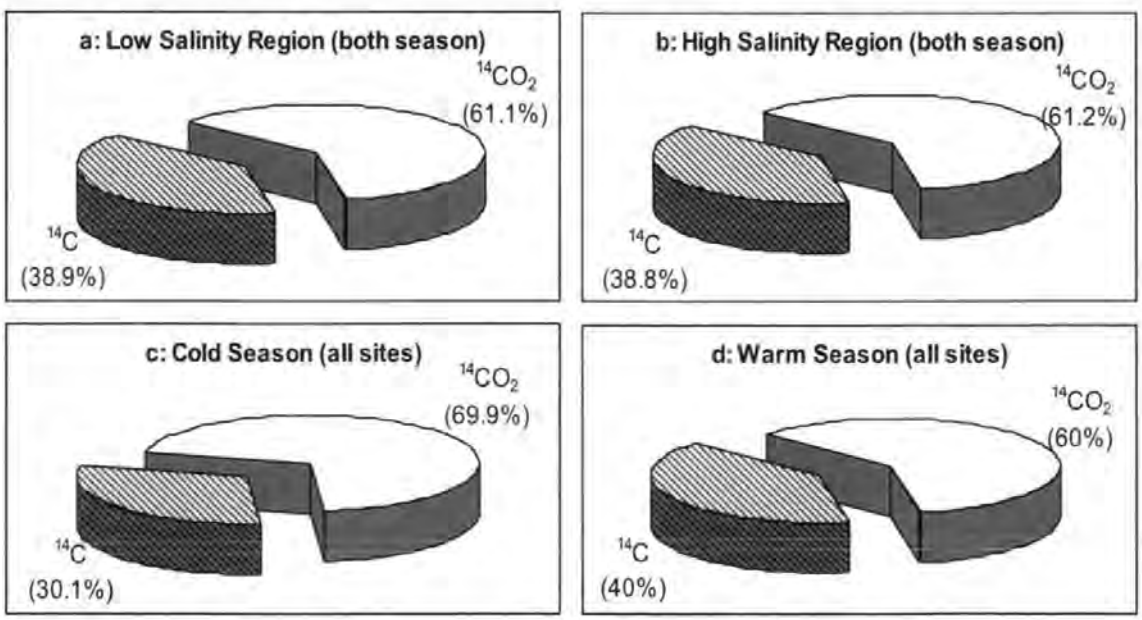

Figure 5.15a-d: Percentage bacterial CH_3I utilization (^{14}C represents CH_3I that ended up in bacterial cells, $^{14}CO_2$ represents oxidized CH_3I recorded as CO_2) in the Tamar estuary. **(a)** in the low salinity region, **(b)** in the high salinity region, **(c)** during the cold season, **(d)** during the warm season.

These successive cycles (an increase followed by a decrease) in the rate of bacterial CH_3I removal were found to be almost identical within the high salinity region (Cargreen and The Narrows) of the Tamar estuary during the study period (Figure 5.14a-d). Within the low salinity region (Morwellham and Tuckermarsh) the cycles were different especially during August when there appeared to be a trend reversal when compared to the lower reaches of the estuary. This could be as result of changes or shift in bacteria community structure between the upper and lower reaches of the estuary (e.g. Crump *et al.*, 2004; Troussellier *et al.*, 2002) leading to differences in methylotrophic bacterial growth pattern.

Of the total recorded CH_3I removal by bacteria in the Tamar estuary, between 26 – 41% was incorporated into bacterial cells whilst between 59 – 74% was oxidized to CO_2 by the bacteria via respiration. There was no change in the mean percentage C from CH_3I incorporated into bacterial cells (39%) and that used in oxidation (61%) between the low salinity and high salinity regions of the estuary (Figure 5.15a-b). However, comparing the mean percentages, there was about 10% variation between the cold and warm seasons (see Figure 5.15c-d) indicating a seasonal shift in the mean proportional utilization of CH_3I by methylotrophic bacteria in the Tamar estuary. It could be hypothesized that the observed seasonal shift in percentage substrate utilization is indicative of the methylotrophic bacteria spending more energy for protein synthesis and reproduction purpose during the warm season when substrate availability is relatively high whilst spending more nutrient in energy generation through respiration during the cold season when environmental factors such as temperature are not conducive. This shift could therefore serve as a vital strategy (Iriberri *et al.*, 1987) of methylotrophic bacteria for the warm/cold seasonality in the estuary.

5.5.4. Spatial and seasonal relationship between bacterial properties and physico-chemical variables in the Tamar estuary

Biological activity is influenced by variations in environmental physical and chemical variables (refer to Chapter One, section 1.2.3 for review). During the course of the study several of the physico-chemical variables were surveyed along the Tamar estuary (see section 5.5.1). The relationship of the measured physico-chemical variables with each other and with bacterial activity, especially the rate of leucine incorporation and CH_3I removal may vary spatially and seasonally. It is therefore important to assess which of these physical and chemical factors are closely related and then determine the statistical significance of these relationships. This should facilitate a better understanding of the microbial processes taking place in the Tamar estuary and how they might impact upon CH_3I removal. To achieve this, a number of statistical analyses were conducted and presented in this section. A Pearson's correlation coefficient analysis was conducted for all parameters for each location as well as for all data sets for all sampling locations (see Appendices 19 to 23) at 5% level of significance.

General observations made from all correlation coefficient analyses (see Appendix 19 to 23) show that the most closely related variables (based on statistical significance) to bacterial CH_3I removal (as the main response factor) are temperature, bacterial abundance and bacterial leucine incorporation. This is followed by DO and SPM. Salinity, CH_3I concentration and pH are however only poorly correlated to bacterial CH_3I removal. These trends are also seen from the cluster analysis (see Figures 5.16 and 5.17) which highlights the close similarities between bacterial CH_3I removal and temperature, bacterial abundance, bacterial activity and SPM.

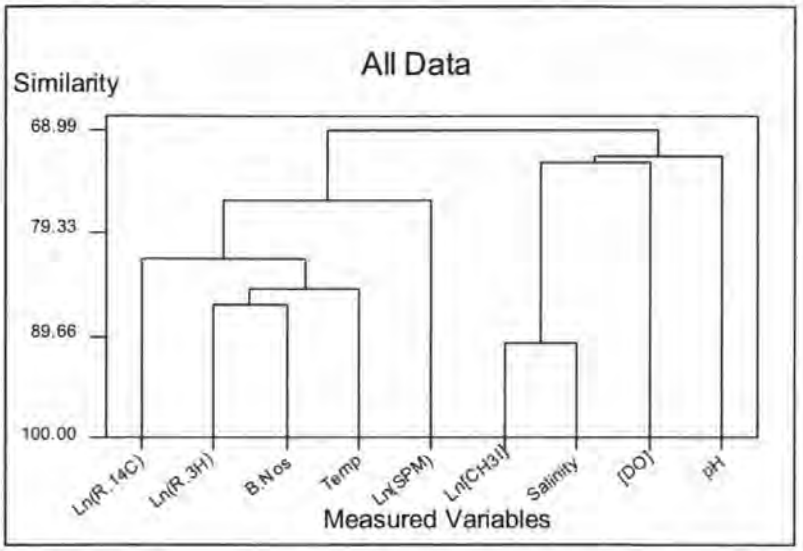

Figure 5.16: Similarities between observed variables for all sampling locations along the Tamar estuary together. Ln = natural logarithm, R.^{14}C = rate of CH_3I removal, R.^{3}H = rate of bacterial leucine incorporation, B.Nos = bacterial abundance, [CH_3I] = CH_3I concentration, Temp = temperature, [DO] = dissolved oxygen concentration, SPM = suspended particulate matter.

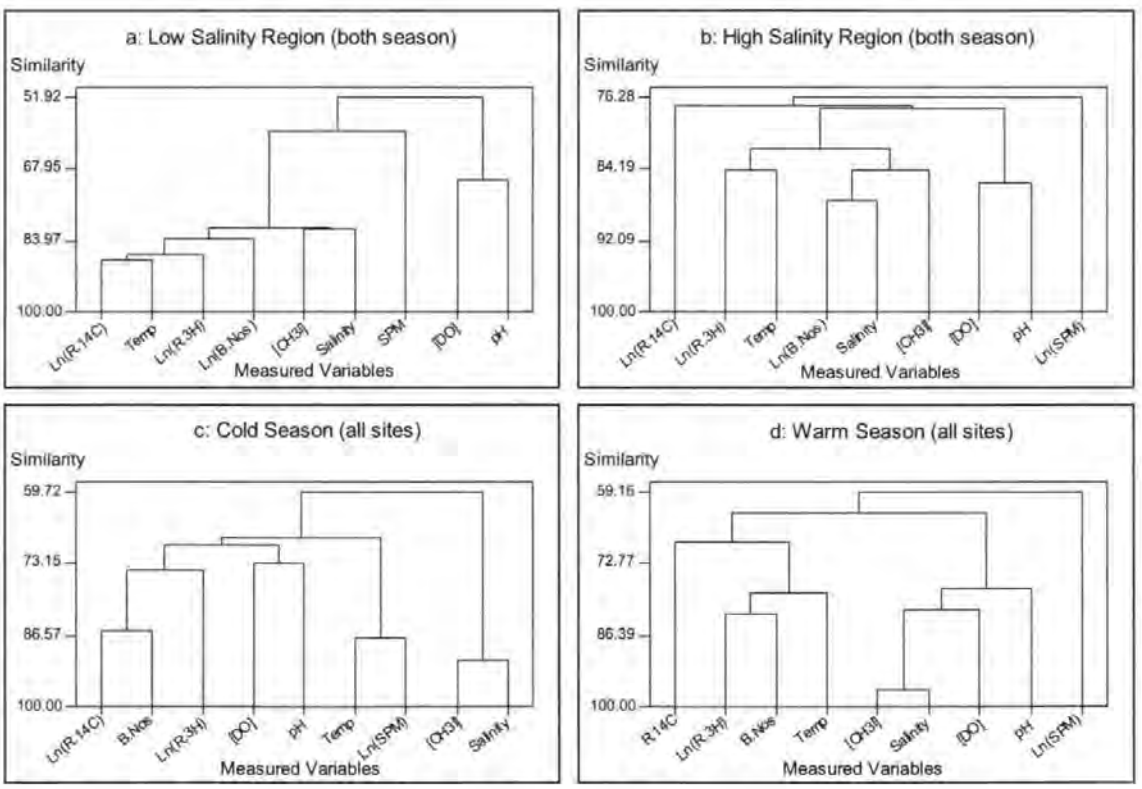

Figure 5.17a-d: Similarities between observed variables in the Tamar estuary; **(a)** at the low salinity region, **(b)** at the high salinity region, **(c)** during the cold season, **(d)** during the warm season (see Figure 5.16 for key to abbreviations).

Based on statistical significance of the p-values of the correlation coefficient analyses, some of the closely related variables are not statistically related to bacterial CH_3I removal at every sampling location (e.g. DO at Cargreen and The Narrows). However, it is

important to mention that the correlation matrix only tests the significance of linear relationships between the variables even though other forms of statistically significant relationships might exist between some of the variables. Therefore the relationship between each pair of variables of importance must be separately considered in order to understand better how they are related. For this reason, variables that are closely related to bacterial CH_3I removal were separately analysed for each sampling station.

5.5.4.1. Rate of methyl iodide removal vs. temperature

Based on a best fit curve (Figure 5.18a), the relationship between bacterial CH_3I removal and temperature is exponential. At the 5% significance level this relationship is highly significant in the Tamar estuary ($p< 0.0001$; $r^2 = 0.651$). Although the exponential relationship between bacterial CH_3I removal and temperature at the four sampling locations were all statistically significant, a comparison shows that Morwellham and The Narrows had the best fit curve (p-values of < 0.001; r^2-values > 0.7) whilst Cargreen had the least fit curve ($p = 0.003$; $r^2 = 0.599$).

According to Griffith *et al.* (1994), unlike free-living bacteria, attached bacteria are less influenced by changing temperature in the estuarine environment. Since SPM concentration is very low at The Narrows, it may imply that methylotrophic bacterial community at The Narrows are free-living forms therefore responding faster to increase in temperature at the onset of the warm season (Figure 5.18e). On the other hand, the SPM concentration at the three remaining sampling stations (especially within the low salinity region) is relatively high therefore could be speculated that the methylotrophic bacterial community here are mostly attached which explains their relatively less rapid response to temperature increase (Figure 5.18b-d).

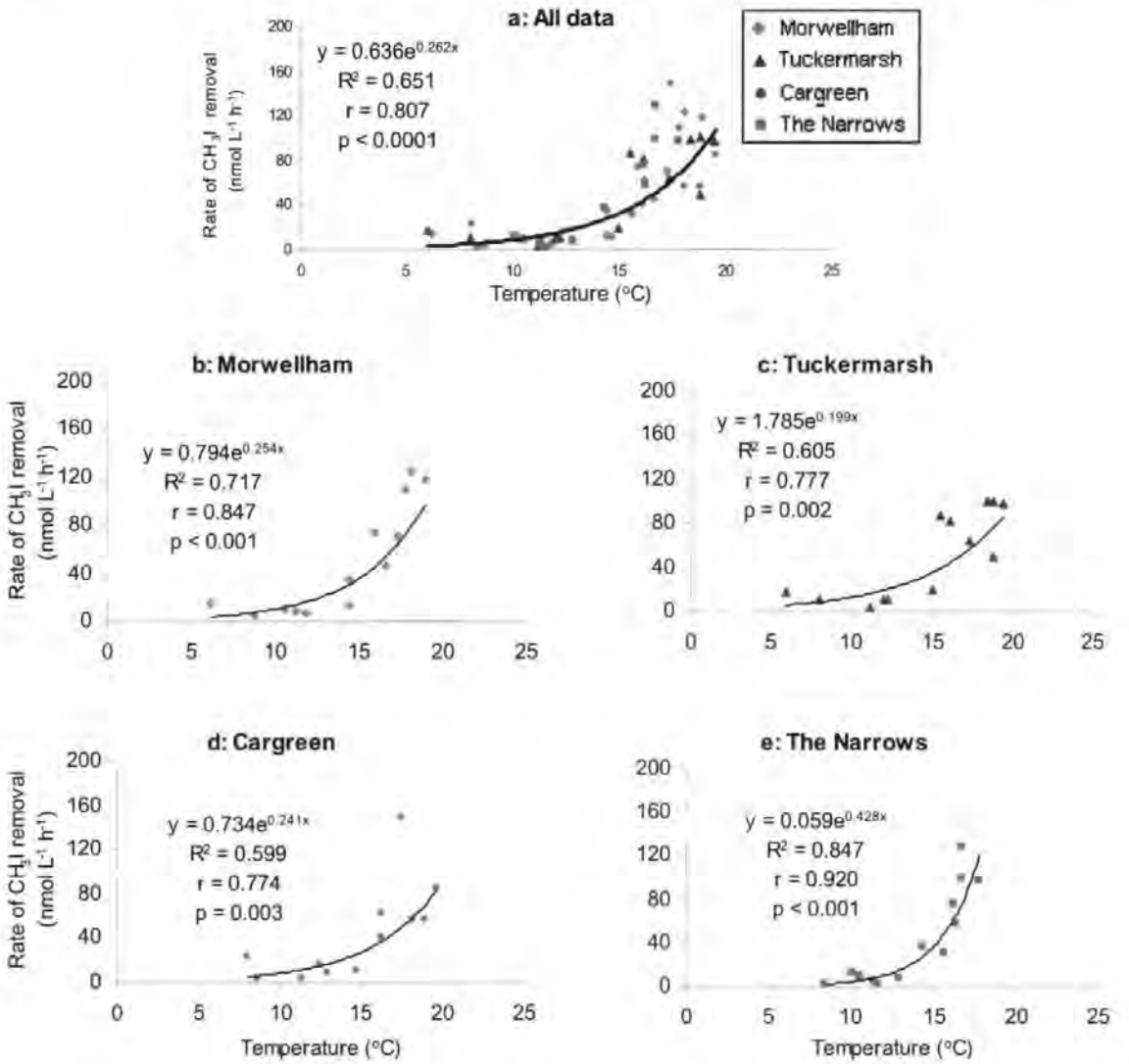

Figure 5.18a-e: Relationship between the rate of bacterial CH_3I removal (nmol $L^{-1}h^{-1}$) and surface water temperature (^{o}C) along the Tamar Estuary. **(a)** All data, **(b)** Morwellham, **(c)** Tuckermarsh, **(d)** Cargreen, **(e)** The Narrows.

5.5.4.2. Rate of methyl iodide removal vs. bacterial abundance

From the scatter plots presented by Figure 5.19a-d, a general increase in bacterial abundance corresponds to an increase in the rate of bacterial CH_3I removal. However, the positive correlation between the rate of bacterial CH_3I removal and bacterial abundance at all selected sampling stations along the Tamar estuary (except Morwellham) were not statistically significant. Since the rate of bacterial CH_3I removal is plotted against the total recorded bacterial abundance but only the methylotrophic bacterial communities amongst them are responsible for the CH_3I removal that could explain the resulting poor correlation.

The mean rate of bacterial CH_3I removal has been estimated as 45.8 ± 41.6 nmol L^{-1} h^{-1} (see section 5.5.3.3) by a mean bacterial abundance of 0.97 ± 0.95 x 10^9 cells L^{-1} (see section 5.5.3.1).

According to Whitman *et al.* (1998), the total global bacterial abundance in the upper 200 m of the ocean has been estimated as 3.6 x 10^{28} cells. However no information regarding the estimated abundance of methylotrophic bacteria was found in literature. If it is assumed that 1% (3.6 x 10^{26} cells) of the reported total global bacterial abundance is capable of CH_3I removal, then the potential gross global removal of CH_3I could be estimated.

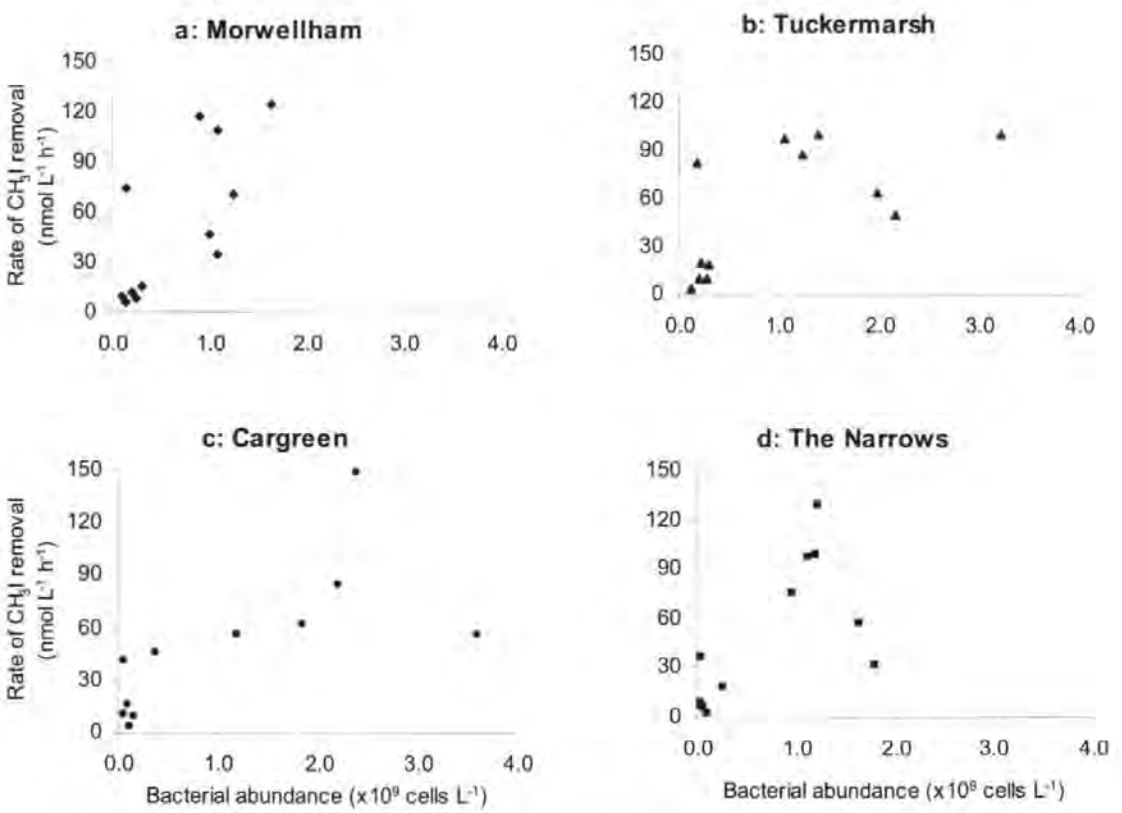

Figure 5.19a-d: Scatter plots of the relationship between the rate of bacterial CH_3I removal (nmol $L^{-1}h^{-1}$) and abundance (cells L^{-1}) along the Tamar Estuary. **(a)** Morwellham, **(b)** Tuckermarsh, **(c)** Cargreen, **(d)** The Narrows.

Therefore the potential gross global microbially-mediated removal of CH_3I in the upper 200 m of the ocean is approximately $2.1 \pm 1.9 \times 10^{16}$ g y^{-1}. This makes bacterial activity not only a source but also a potential sink of CH_3I in the aquatic environment.

The global estimate of microbially-mediated CH_3I production from this study (details of estimation provided in section 3.4.9 of Chapter Three) stands at $1.7 \pm 0.5 \times 10^{12}$ g y^{-1}. This is over 10^4 g y^{-1} lower than the estimated global microbially-mediated CH_3I removal. This however does not imply that the estimated global microbial CH_3I removal is higher than production since that of production is a net value (from a coupled microbial production/removal). It would also be important to compare the estimated gross global microbially-mediated CH_3I removal to the gross global production to understand the accurate significance of microbial removal on the global CH_3I cycle. However limited information with regards to global estimates of CH_3I production is available. Reported estimates from some authors are from single sources of production such as macroalgae (e.g. between $10^7 - 10^8$ g y^{-1} reported by Manley *et al.*, 1992; Nightingale *et al.*, 1995; Giese *et al.*, 1999). With the high estimated potential CH_3I removal (in order of 10^{16} g y^{-1}), it could be speculated that microbially-mediated removal plays a significant role in the global cycle of CH_3I.

Estimation assumptions

Estimation of the total global microbially-mediated removal of CH_3I was based on the assumptions that (i) the conditions used in bacteria CH_3I removal studied during the laboratory incubation experiment could potentially prevail in the natural environment (ii) 1% of all bacteria cells in the aquatic environment are capable of removing and utilizing CH_3I and (iii) the rate of removal of CH_3I at every region of the aquatic environment is the same.

Limitations

Limitations to this global estimate are (a) the substrate concentrations used in the laboratory experiment to achieve reliable and reproducible results are far above the natural CH_3I concentration levels, (b) only a portion of the total global bacterial abundance (methylotrophs) are responsible for the removal of CH_3I in the aquatic environment, (c) the rate of bacterial CH_3I removal in the aquatic environment varies from region to region, and (d) estimated total bacterial abundance used in the estimation of CH_3I removal was based only on the upper 200 m of the ocean. The highlighted assumptions and their limitations therefore render the application of the estimated global microbially-mediated CH_3I removal rate only as a guide in the discussion of the potential role of methylotrophs in the global CH_3I cycle.

5.5.4.3. Rate of methyl iodide removal vs. rate of leucine incorporation

The existence of any significant relationship between bacterial leucine incorporation and CH_3I removal could provide a way of estimating the potential rate of bacterial CH_3I removal in any system from the rate of bacterial leucine incorporation if the influences of environmental physico-chemical variables are constant. The relationship between these two biological activities (shown in Figure 5.20a) suggests a linear relationship with an increase of 1 pmol L^{-1} h^{-1} in the rate of bacterial leucine incorporation corresponding to about 1000 fold increase in the rate of bacterial CH_3I removal.

Based on the extensive spread of the scatter plots (Figure 5.20a-e), the relationship between bacterial leucine incorporation and CH_3I removals at the four sampling sites were all poorly correlated. The general observation shown in Figure 5.20a, indicate that an increase in bacterial leucine incorporation rate also results in an increase in bacterial CH_3I removal. However, due to the weakness of the correlation, the relationship between these

two variables may possibly be influenced by other conditions. These may include change in bacterial choice of carbon substrate with changing environmental conditions in the estuary (Schultz *et al.*, 2003), shift in the composition (Crump *et al.*, 2004) and diversity of bacterial community (e.g. Crump *et al.*, 1999; Troussellier *et al.*, 2002).

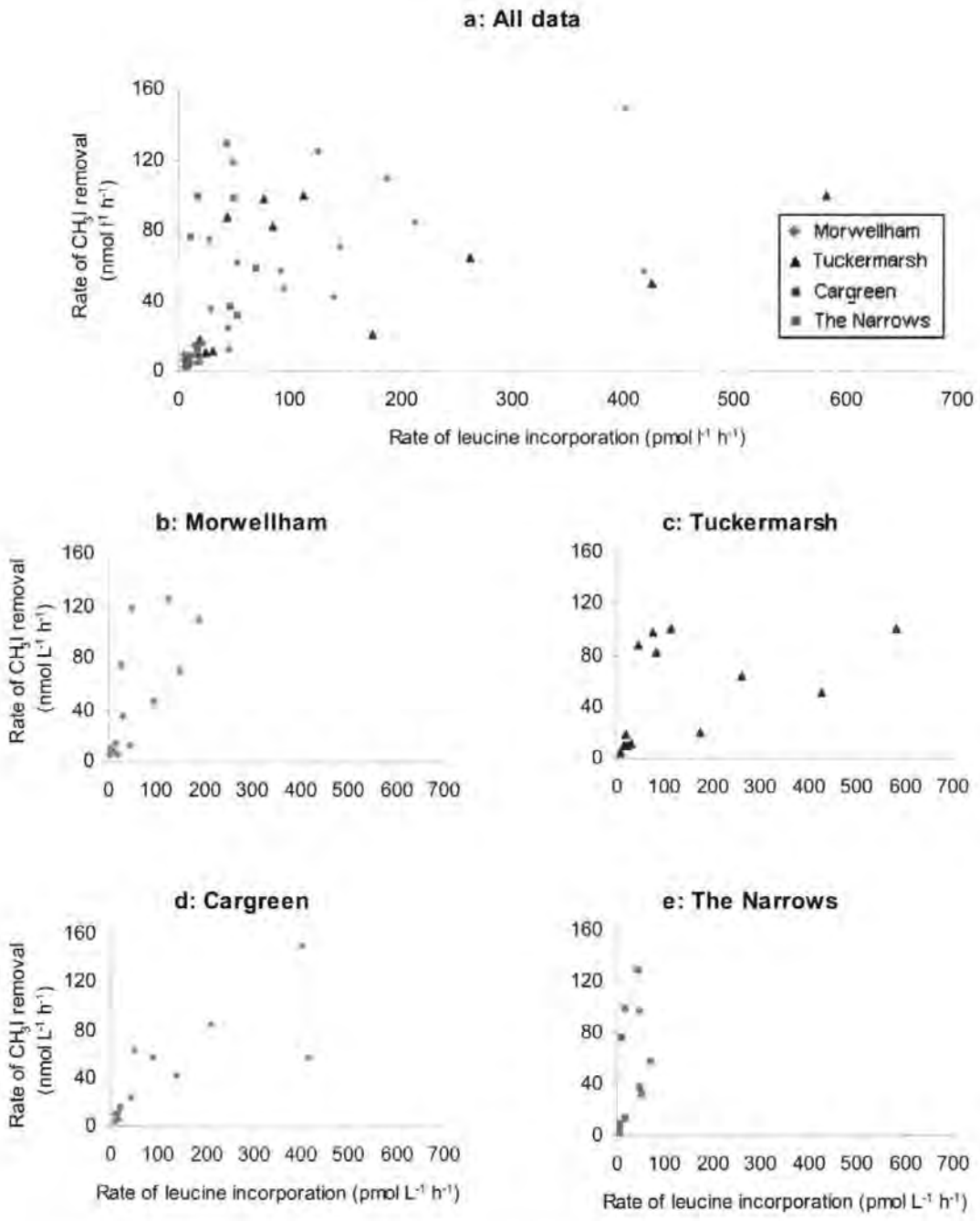

Figure 5.20a-e: Scatter plots of the relationship between the rate of bacterial CH_3I removal (nmol $L^{-1}h^{-1}$) and leucine incorporation (pmol L^{-1} h^{-1}) along the Tamar Estuary. **(a)** All data (with a linear curve), **(b)** Morwellham, **(c)** Tuckermarsh, **(d)** Cargreen, **(e)** The Narrows.

5.5.4.4. Rate of methyl iodide removal vs. methyl iodide concentration

In this section, the activity of methylotrophs in this study in the Tamar estuary was plotted against CH_3I concentration to investigate the relationship between the two variables.

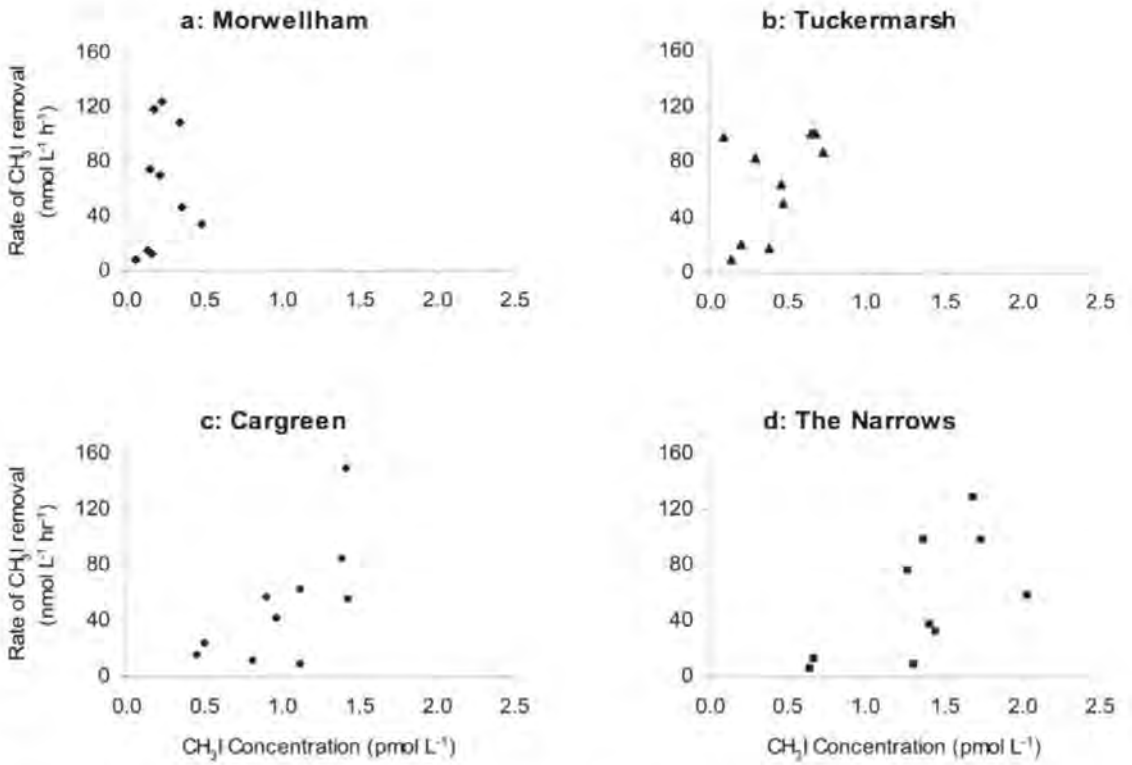

Figure 5.21a-d: Scatter plots of the relationship between relationship between the rate of bacterial CH_3I removal (nmol $L^{-1}h^{-1}$) and CH_3I concentration (pmol L^{-1}) along the Tamar Estuary. **(a)** Morwellham, **(b)** Tuckermarsh, **(c)** Cargreen, **(d)** The Narrows.

From section 5.5.4.3, methylotrophic CH_3I removal in the Tamar estuary is enhanced by the rate of bacterial production. Since the rate of bacterial CH_3I production investigated in Chapter Three is also linked to the rate of bacterial production, it implies that both microbially-mediated CH_3I production and removal increase with increasing microbial activity and eventually impact on CH_3I concentration in the estuary. However, from the results (shown by the scatter plots in Figure 5.21a-d), the rate of bacterial CH_3I removal in the estuary increased with increasing CH_3I concentration. This could suggest a net increase in CH_3I production as microbial activity in the estuary increases.

However, based on the extent of the scatter (Figure 5.21a-d), the relationship between methylotrophic CH_3I removal and CH_3I concentration at all four sampling stations do not appear to have well-defined correlations. This may be due to the complex interplay of biological and physico-chemical activity (i.e. continuous spatial and temporal variations and interrelations of physical and chemical conditions such as salinity, temperature, SPM on biological activities such as microbial production and removal of CH_3I) which together determines the net surface water concentration of CH_3I in the Tamar estuary (especially within the low salinity region). In addition, nucleophilic substitution reactions involving the transformation of CH_3I to yield more stable forms of CH_3Cl and CH_3Br (Lovelock *et al.*, 1973; Zafiriou, 1975) may compete for CH_3I uptake. This together with chemical (Vogel *et al.*, 1987) and photochemical (Moore & Zafiriou, 1994) production that may occur in surface water could have impacted on CH_3I concentration in the estuary. These therefore might have lead to the poor correlations between the rate of bacterial removal and CH_3I concentration in the Tamar estuary.

5.5.4.5. Bacterial abundance vs. temperature

In the estuarine environment, bacterial abundance (as well as other bacterial activities) is very temperature dependent (e.g. White *et al.*, 1991; Shiah & Ducklow, 1995). Generally, since nutrient is a non-limiting factor for bacterial growth in estuarine systems (e.g. Kromkamp, *et al.*, 1995), variations in environmental temperature serve as the major regulating factor for variations in bacterial abundance (e.g. Goosen *et al.*, 1997). During the course of the study, abundance of bacterial cells in the Tamar estuary was found to increase with increasing temperature (see Figure 5.22a-d) and hence follow the seasonal cycle of temperature. This was true for all the selected sampling locations although the response by bacteria to temperature increase was more pronounced in the mid-estuary (Tuckermarsh and Cargreen). This may be as results of higher nutrients (POC and DOC) availability in the mid-estuary (Goosen *et al.*, 1997).

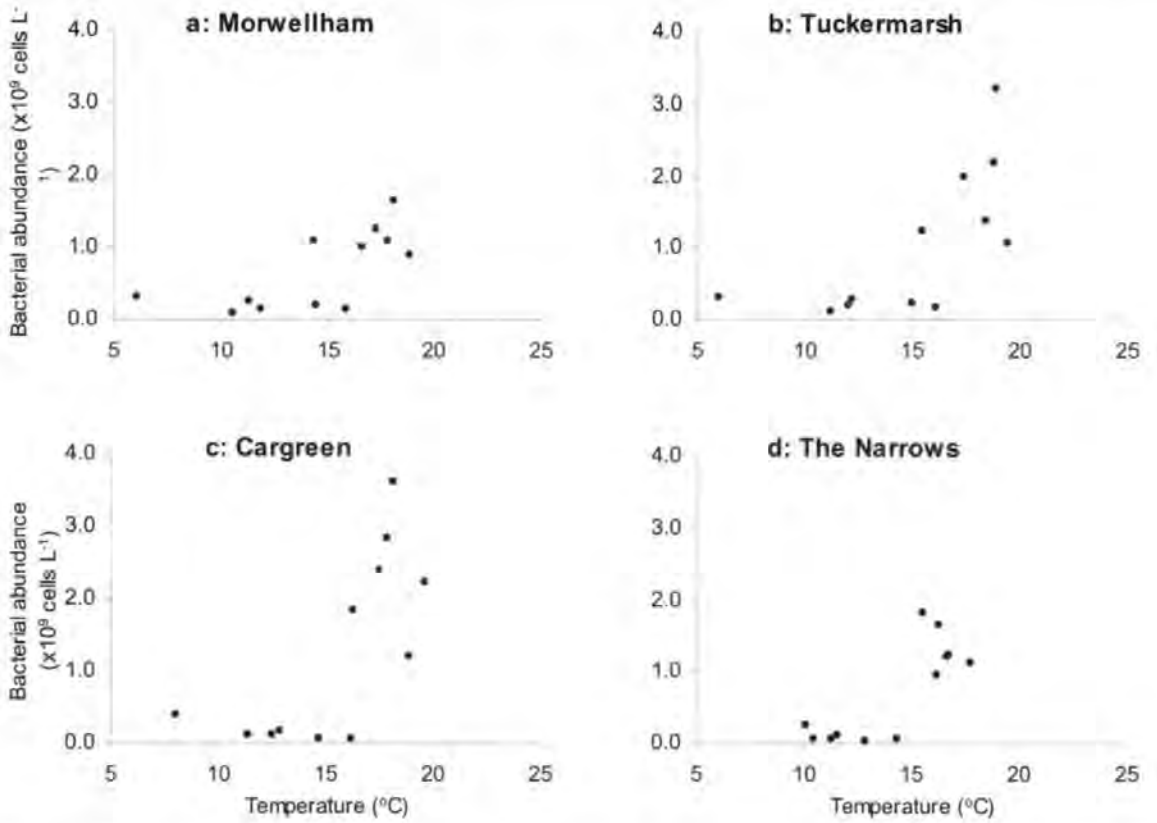

Figure 5.22a-d: Scatter plots of the relationship between bacterial abundance (cells L^{-1}) and temperature (°C) along the Tamar Estuary. (a) All data, (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

5.5.4.6. Methyl iodide concentration vs. bacterial abundance

Bacterial production of CH_3I in the Tamar estuary is significant (see Chapter Three). It could therefore be expected that a relationship between bacterial abundance and CH_3I concentration in any natural system may occur. In the Tamar estuary, this relationship was poorly correlated. As presented by the scatter plots of Figure 5.23a-d, the observed relationship between the two variables at all sampling locations is such that an increase in bacterial abundance corresponds to an increase in CH_3I concentration. The correlation between the two factors was however statistically weak for all selected sampling stations. Since the bacterial community consists of both CH_3I producers and consumers, it is likely a correlation between CH_3I concentration and total bacterial abundance in the Tamar estuary will be poor due to possible temporal differences in microbially-mediated CH_3I production and removal.

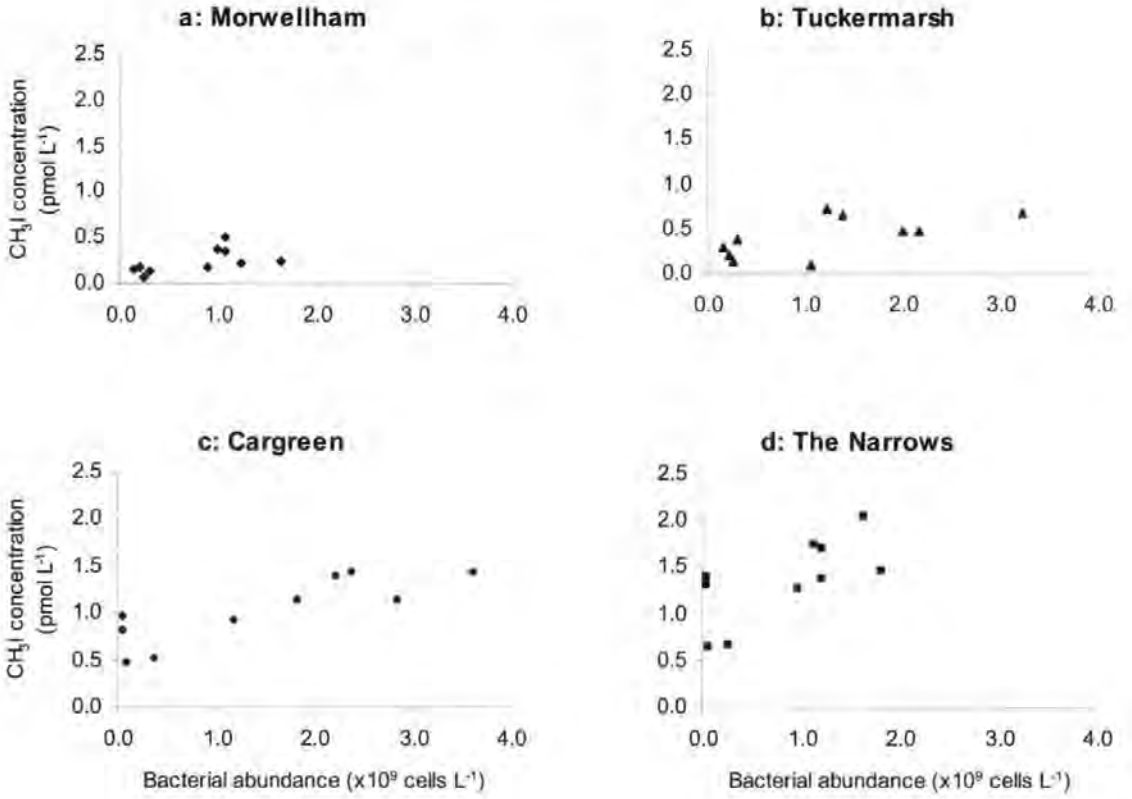

Figure 5.23a-d: Scatter plots of the relationship between bacterial abundance (cells L^{-1}) and CH_3I concentration (pmol L^{-1}) along the Tamar Estuary. (a) All data, (a) Morwellham, (b) Tuckermarsh, (c) Cargreen, (d) The Narrows.

5.5.4.7. Methyl iodide concentration vs. Salinity

Recorded CH_3I concentration along the Tamar estuary during this study was found to be correlated to salinity changes in the estuary (Figure 5.24a-d). In this sense, spatially, CH_3I concentration increases from the freshwater towards the seawater region (from Morwellham through to The Narrows). However, the rate of CH_3I concentration increase in the high salinity region was about two times higher than that of the low salinity region.

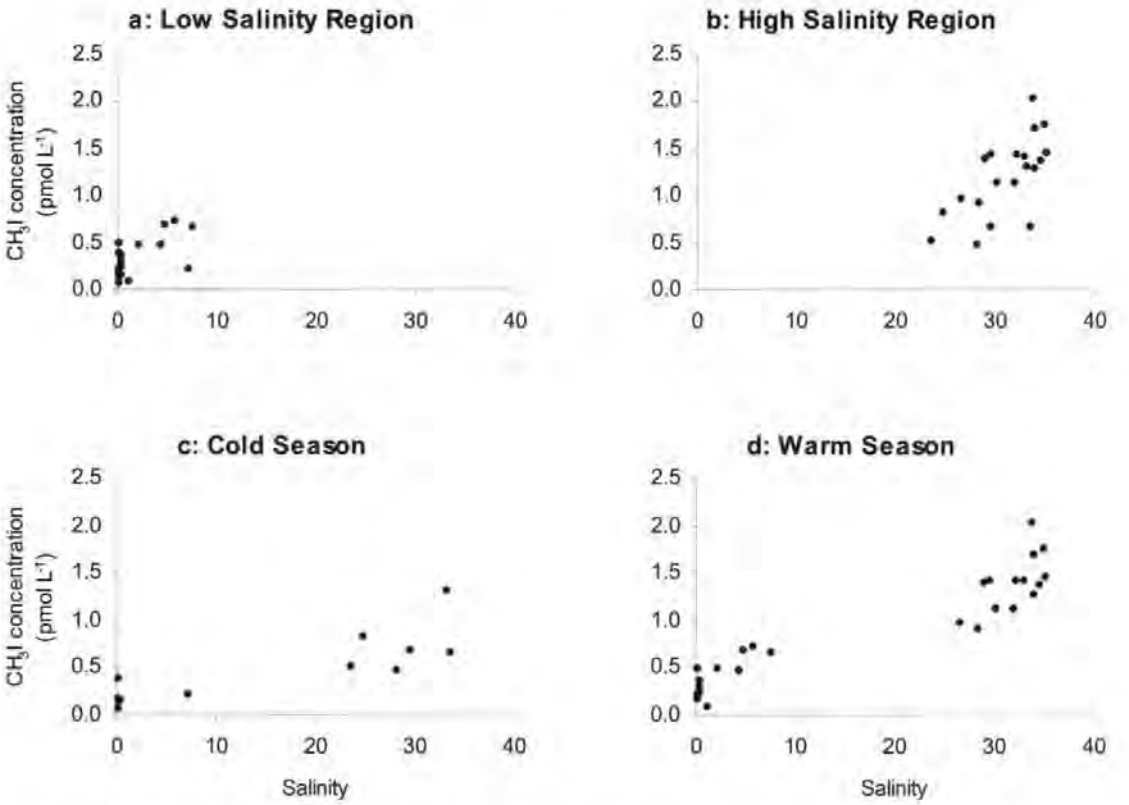

Figure 5.24a-d: Scatter plots of the relationship between CH_3I concentration (pmol L^{-1}) and salinity gradient along the Tamar Estuary. **(a)** low salinity region (both season), **(b)** high salinity region (both season), **(c)** cold season (all sites), **(d)** warm season (all sites).

Both seasonal and temporal variations of CH_3I concentration were also correlated to salinity changes during the two seasons (cold and warm seasons) and within the two specified geographical regions (low and high salinity regions) of the estuary. The rate of CH_3I concentration increase during the warm season was higher than during the cold season (refer to Figure 5.19c-d). The spatial and temporal changes in CH_3I concentration could be attributed to the conservative mixing of seawater and freshwater but based on the findings of this study, it is more likely to be as a result of a net spatial increase in biologically-mediated production such as that of macroalgae (Nightingale *et al.*, 1995) with increasing salinity and a net increase in microbially-mediated removal with decreasing salinity.

5.5.5. Modelling the measured biological, physical and chemical variables

In order to confidently establish the extent of spatial and seasonal variations with respect to the relationships existing between the measured bacterial activity and the physico-chemical variables surveyed in the Tamar estuary, regression modelling was carried out (see Appendix 24-28) with bacterial CH_3I removal as the response factor. A summary of significant relationships existing between bacterial activity and the measured physical and chemical variables in the Tamar estuary has also been presented in Figure 5.25.

The type and degree of physico-chemical variables that were found to have significant relationship with the rate of methylotrophic bacterial CH_3I removal in the Tamar estuary varied both spatially and seasonally. Generally, temperature was found to be the single most influential variable explaining approximately 48% of the variations in the rate of bacterial CH_3I removal in the Tamar estuary (see Table 5.12). In this respect, temperature plays an important role in determining both spatial and seasonal variations in bacterial activity in the estuary (McManus *et al.*, 2004). However, spatial variation during the cold season with respect to bacterial CH_3I removal does not share any significant relationship with temperature.

Table 5.12: Bacterial CH_3I removal in the Tamar estuary: variations in the influence of spatial and seasonal predictors (see Appendix 24 – 28 for details). The predictor variables and their percentage influence on the spatial and temporal variations of bacterial-mediated CH_3I degradation in the Tamar estuary were obtained through regression analysis using Minitab statistical package. The best fit regression model is only meant to look at how best the measured variables are related to the rate of methylotrophic bacterial removal of CH_3I in each data set.

Data Set	**Best single Predictor variable**	**Influence (%)**	**Best double Predictor variables**	**Influence (%)**	**Best fit regression model** (based on 5% level of significance)
Low salinity region (both seasons)	Temperature	56.5	Temperature & SPM	65.6	Ln ($R.^{14}C$) = 0.29 Temp. + 0.31 Ln(SPM) + 0.54
High salinity region (both seasons)	CH_3I concentration	25.7	CH_3I concentration & Temperature	28.2	Ln ($R.^{14}C$) = 1.3 [CH_3I] + 2.2
Warm season (all sites)	Temperature	10.7	Temperature & Bacterial abundance	11.8	$R.^{14}C$ = 8.7 Temp. - 73
Cold season (all sites)	Bacterial abundance	41.8	Bacterial abundance & DO concentration	61.3	Ln ($R.^{14}C$) = 3.4 B.Nos. - 0.24 [DO] + 3.3
General relationship (all data)	Temperature	48.3	Temperature & Bacteria leucine incorporation.	52.9	Ln ($R.^{14}C$) = 0.31 Ln ($R.^{3}H$) + 0.14 Temp. + 0.17

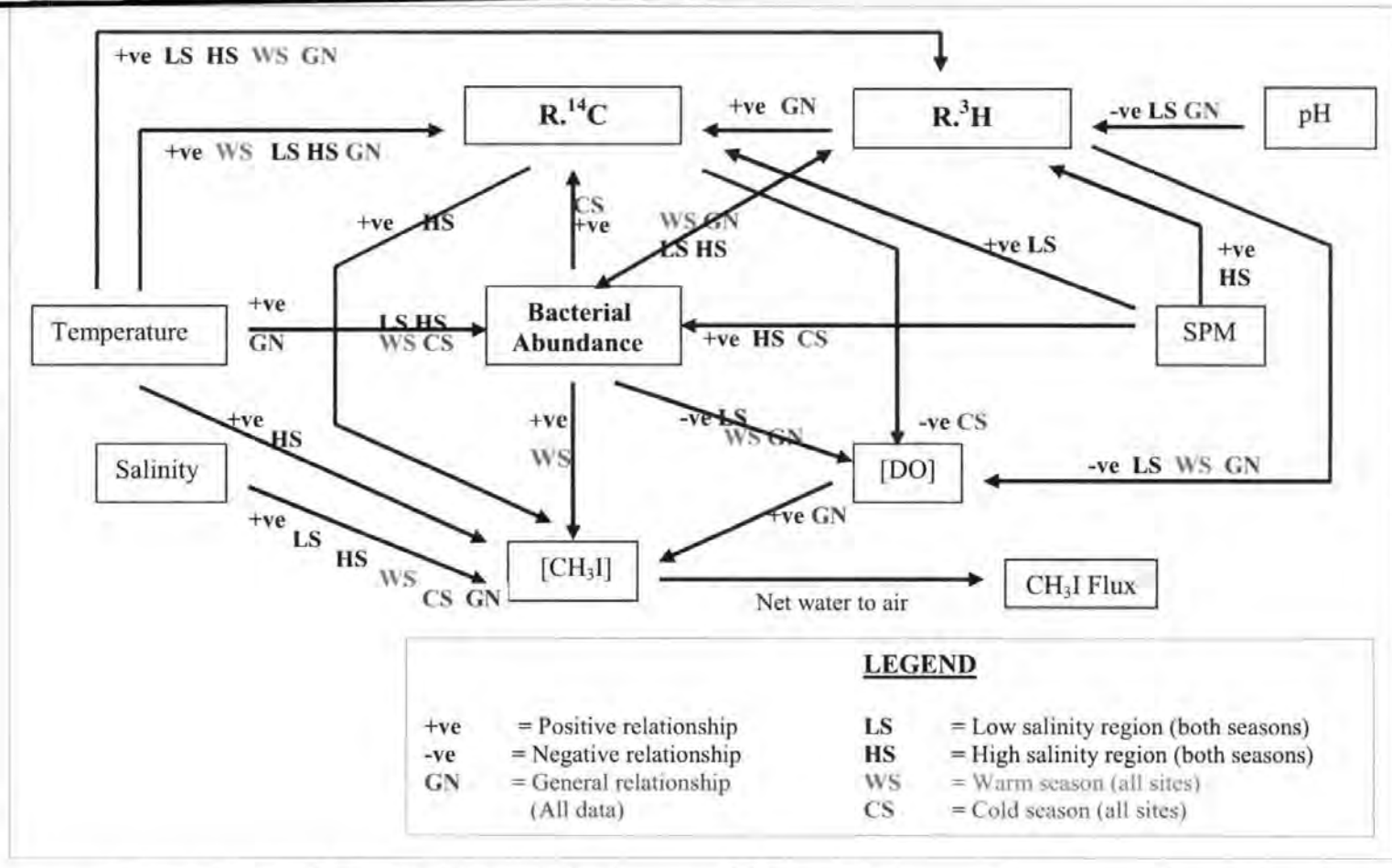

Figure 5.25: A summary of statistically significant (at 5%) spatial and seasonal relationships between the measured bacterial activity and physico-chemical variables in the Tamar estuary (with respect to seasonal relationship at low/high salinity regions and spatial relationship during cold/warm seasons). R.^{14}C = rate of bacterial CH_3I removal, R.^{3}H = rate of bacterial leucine incorporation, [CH_3I] = CH_3I concentration in surface water, [DO] = dissolved oxygen saturation, SPM = suspended particulate matter load.

The influence of temperature was also found to significantly affect seasonal variation in CH_3I concentration within the high salinity region of the estuary. This influence could however be indirect since if an adequate supply of allochthonous nutrients (Huller *et al.*, 1996; Goosen *et al.*, 1997) reach the seaward end of the estuary (high salinity region), an increase in temperature may also result in increases in biological activities and hence in addition to microbial production (Manley & Dastoor, 1988), a possible increase in algal production (Manley & Dastoor, 1988; Ekdahl *et al.*, 1998) of CH_3I at the seaward end of the estuary. Since CH_3I serves as a substrate for the oxidative processes of methylotrophic bacteria (e.g. King & Saltzman, 1997; Goodwin *et al.*, 1998), an increase in temperature within the high salinity region may result in an increase in CH_3I production which in turn may fuel methylotrophic bacterial activity within the region.

Suspended particles are known to influence the activity (e.g. Goosen *et al.*, 1999; Herman & Heip, 1999) and abundance of particle-attached bacteria (e.g. Joint & Pomroy, 1982; Plummer *et al.*, 1987). Based on this, the seasonal variation in SPM was found to reflect the seasonal variation in bacterial activity within the low salinity region of the estuary where the highest SPM concentration was recorded. SPM was found to positively influence the rate of bacterial CH_3I removal although this relationship may be indirect.

Statistically, DO concentration was identified to be significantly influenced by spatial variation in the rate of bacterial CH_3I removal during the cold season in the Tamar estuary. This relationship was however negative which implies that an increase in bacterial CH_3I removal result in a decrease in DO concentration. This supports the suggestion that the bacterial consortia found within the water column of the estuary are generally aerobic bacteria (Goosen *et al.*, 1997).

Among the physico-chemical variables measured in the Tamar estuary, the influences of salinity and pH on methylotrophic bacterial removal of CH_3I were statistically insignificant. However salinity was the only variable that significantly correlates to both spatial and seasonal variations in CH_3I concentrations in the Tamar estuary. Salinity may not necessarily have a direct influence on the rate of bacterial activity but it may play a role in the selection of different consortia of bacteria at different locations of the estuary (Schultz Jr. *et al.*, 2003; McManus *et al.*, 2004) which may in turn reflect in variations in bacterial activity along the estuary. Although this may be unlikely, it is possible that salinity may (to a minor degree) have an indirect influence on spatial variations in bacterial activity in the Tamar estuary. With respect to pH, there was no evidence of its influence (no matter how remote) on bacterial activity or abundance in the Tamar estuary.

CH_3I concentration in an estuarine system will be dependent on a complex interplay of biological, physical and chemical processes that result in the production, removal and transfer of CH_3I. The present study only focused on the interaction of physico-chemical variables with microbial activity in relation to the removal of CH_3I by methylotrophic bacteria and considered CH_3I transfer between water and air.

Through mixing which is facilitated by tidal movements, the concentration of CH_3I in the Tamar estuary was dependent on salinity changes in both salinity regions, during both seasons and with all data sets put together. An increase in salinity corresponds to an increase in CH_3I concentration and vice versa. With air concentration of CH_3I over the Tamar estuary assumed to be the same during the entire study period (1 ppt(v)), the rate of transfer between water and air was therefore dependent on the recorded surface water concentration of CH_3I along the Tamar estuary over the study period and the estimated transfer velocity.

In an estuarine system, a general hypothesis could be made that, (i) an increase in environment temperature facilitates bacterial productivity which in turn influences bacterial abundance (including methylotrophic bacteria) and vice versa and in effect influence the rate of CH_3I removal, (ii) the rate of methylotrophic bacterial CH_3I removal (in relation to bacterial abundance) influence CH_3I concentration. Both parts of the hypothesis are summarized in Figure 5.26.

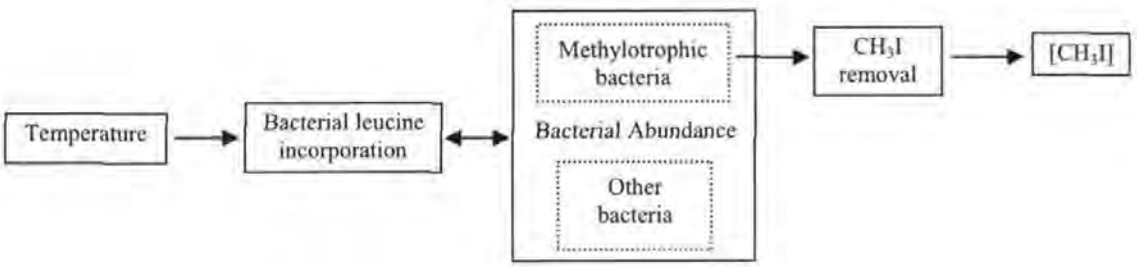

Figure 5.26: A summary of general hypothesis involving the relationship between microbial activity and physicochemical variables in an estuarine system.

This general hypothesis could be tested for the Tamar estuary under different environmental conditions (i.e. all data sets, low and high salinity regions, cold and warm seasons).

For the combined data sets (All data), it was observed that in the Tamar estuary, an increase in temperature promotes bacterial productivity, which results in a relationship with bacterial abundance whilst influencing methylotrophic bacterial removal of CH_3I. However, the observed increases in bacterial abundance and CH_3I removal by methylotrophic bacteria did not translate into a statistically significance relationship with CH_3I concentration in the estuary (see Figure 5.27a).

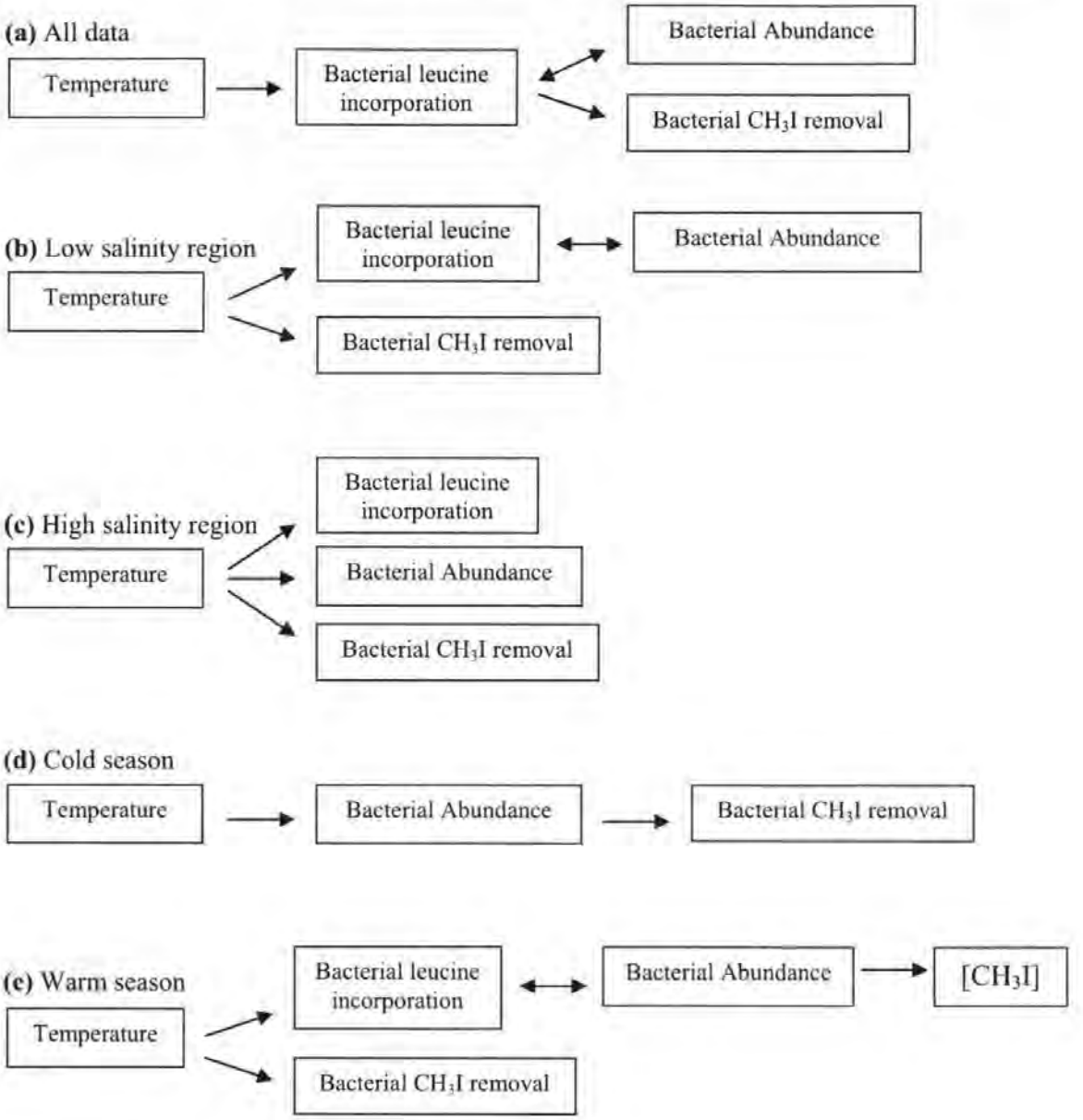

Figure 5.27a-e: A summary of the observed scenarios in relation to the presented general hypothesis for the various data sets.

Within the low salinity region of the Tamar estuary, the influence of temperature on both bacterial productivity and CH_3I removal was apparent. Bacterial productivity in turn is related to bacterial abundance. This implies that an increase in temperature results in an increase in the rate of bacterial leucine incorporation, abundance and CH_3I removal but however does not lead to significant impact on CH_3I concentration (see Figure 5.27b).

In the high salinity region, there were very low statistically significant relationships with respect to the presented general hypothesis. The only statistically significant observation

made within the high salinity region of the estuary was the influence of temperature on bacterial productivity, abundance and CH_3I removal (see Figure 5.27c). An increase in temperature resulted in a corresponding increase in the rate of bacterial leucine incorporation, bacterial abundance and bacterial CH_3I removal in the estuary but these do not lead to any significant relationship with CH_3I concentration.

During the cold season, bacterial abundance was found to be significantly dependent on temperature variation which in effect influences the rate of methylotrophic bacterial removal of CH_3I. An increase in temperature resulted in an increase in bacterial abundance and eventually bacterial removal of CH_3I. These again however had no significant relationship with CH_3I concentration during the period (see Figure 5.27d).

The closest scenario supporting the hypotheses occurred during the warm season. Temperature was found to influence bacterial productivity and bacterial CH_3I removal. The influence on bacterial productivity resulted in a significant relationship with bacterial abundance which in turn was related to CH_3I concentration. In this sense, an increase in temperature resulted in an increase in the rate of bacterial leucine incorporation and an increase in bacterial abundance eventually influencing the concentration of CH_3I. However, an increase in the rate of methylotrophic bacterial CH_3I removal was not statistically significant to variations in CH_3I concentration in the estuary during this period (see Figure 5.27e). This may be due to the presence of CH_3I production through microbial activity (based on the findings of this study) as well as from other biotic sources such as macroalgae and phytoplankton.

5.6. Conclusions

From Chapter four, it was apparent that bacterial activity in aquatic systems can result in the consumption of CH_3I. The work presented in this chapter was conducted to investigate

spatial and seasonal trends in bacterial activity and physico-chemical variables in estuarine systems and in particular, the effect of biological and physico-chemical variables on spatial and seasonal variations in CH_3I concentration. Microbially-mediated production and removal of CH_3I in the estuary may be tightly coupled. Although high potential removal rate of CH_3I was observed in the estuary, the net effect of the production/removal coupling was in favour of CH_3I production (i.e. microbial production was larger than removal). From the various statistical analyses conducted from biological, physical and chemical variables recorded in the Tamar estuary during this study as well as the interpretations obtained, the following conclusions could be drawn;

1. CH_3I concentration in the Tamar estuary varied spatially with changing salinity. The concentration increased with increasing salinity.

2. There occurred spatial and seasonal variations in bacterial productivity and bacterial CH_3I removal in the Tamar estuary.

3. Methylotrophic bacterial removal of CH_3I was highest during the warm season of the year and least during the cold season of the year.

4. Higher rates of methylotrophic bacterial CH_3I removal were generally observed within the low salinity region of the estuary where the TMZ is localized especially during the warm season.

5. Spatial variation in bacterial activity was influenced by temperature and SPM whilst seasonal variation was influenced by temperature and bacterial abundance. Therefore temperature could be considered the single physical variable that influences both spatial and seasonal trend in bacterial productivity and

methylotrophic bacterial CH_3I removal in the Tamar estuary.

CHAPTER SIX

Conclusions and Future Work

6. Conclusions and Future Work

6.1. Conclusions

The present study has highlighted the contribution of microbial communities to the production and removal of CH_3I in an estuarine system. A summary of the investigations carried out in this thesis has been diagrammatically presented in Figure 6.1. From the investigations, bacterial association with POM was found to serve as a source of CH_3I whilst the rate of formation of the aggregates influences the rate at which CH_3I is produced. Methylotrophic bacterial activity was also found to be capable of removing CH_3I after developing and rigorously testing the analytical technique in the laboratory to carryout the investigation in the Tamar estuary. Spatial and seasonal trends in bacterial productivity and methylotrophic bacterial CH_3I removal in the Tamar estuary was primarily influenced by variations in temperature.

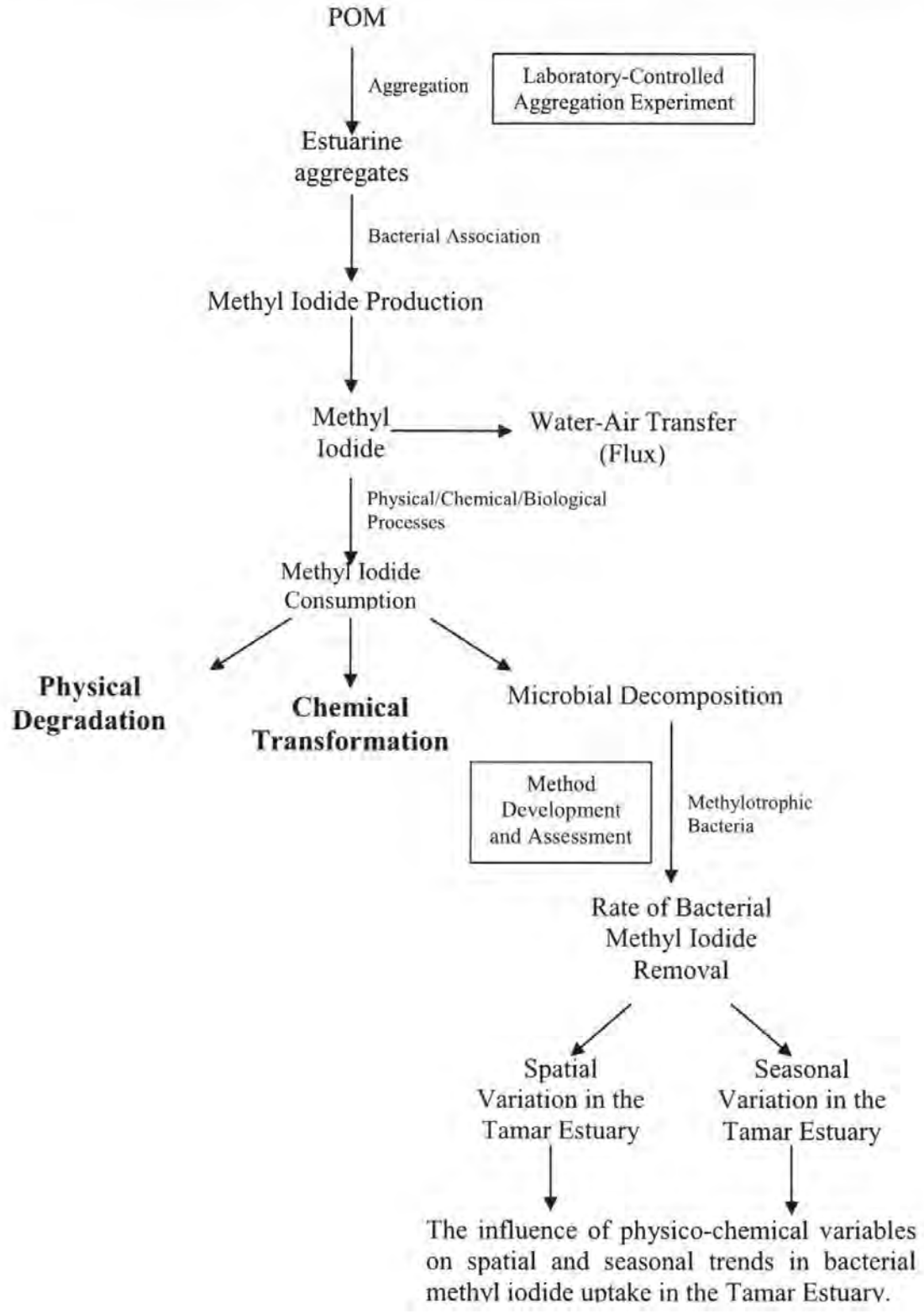

Figure 6.1: A diagram showing the progression of the work carried out during the study presented in this thesis (with exception of those in bold).

6.1.1. Bacterially-mediated methyl iodide production

During the initial part of the study (Chapter Three), bacterial communities that were associated with macroaggregates were responsible (through the degradation of organic matter present in the aggregates) for the release of CH_3I as by-products of presumably POM breakdown. Bacterial activity accounted for the production of 19% of the total observed CH_3I concentration during the aggregation experiment (average from both Rolled and Unrolled samples). Aggregate-attached bacteria, which represented about 17% of the total bacterial population, could have been responsible for most of this production since their activity is often higher than the free-living forms (Alldredge & Youngbluth, 1985; Turley & Stutt, 2000). With the significant elevation in CH_3I concentration, this work has provided strong evidence that the relationship between bacteria and aggregates in the aquatic environment results in the production of CH_3I during the process of organic matter degradation. With an estimated net global microbial CH_3I production of $1.7 \pm 0.5 \times 10^{12}$ g y^{-1}, findings of this study support the presence of other sources of CH_3I in the aquatic environment as proposed by Carpenter *et al.* (2000). Since this estimated value is a net value, it may represent the fraction of the total global microbially-mediated CH_3I production which can potentially transfer to the atmosphere through water to air flux. In the global cycling of CH_3I, this however is only up to 1.4% of the reported total global CH_3I flux of 128 – 355 Gg y^{-1} (Moore & Groszko, 1999).

Heterotrophic production of CH_3I by bacteria will depend on the level of distribution of CH_3I precursors (I^-) and substrate (POM). In the Tamar estuary, the horizontal distribution of CH_3I concentration was found to decrease from the seawater towards the freshwater region. This pattern of distribution conforms to the reported pattern of distribution of iodine species in inshore waters as a result of conservative mixing (Upstill-Goddard & Elderfield, 1988; Truesdale & Upstill-Goddard, 2003).

6.1.2. Bacterial methyl iodide removal

Based on this study, it was apparent that microbial mediation serves as both a source (production) and sink (removal) of CH_3I in the estuarine environment. Amongst the known mechanisms of CH_3I removal (e.g. photochemical transformation, chemical substitution) is the biologically-mediated transformation. From this study, bacterial activity in estuarine systems has been identified to contribute significantly to CH_3I removal in the aquatic environment. However, this removal was also influenced by several other environmental physical and chemical variables that were also investigated concurrently with bacterial activity. Measured physico-chemical variables (salinity, temperature, pH, SPM, DO and CH_3I concentration) in the Tamar estuary were found to exert varying influences on the spatial and seasonal trends of bacterial CH_3I removal. In general, however not surprisingly, temperature was identified to be the variable that has the most influence on bacterial activity in the Tamar estuary.

6.1.3. Water to Air exchange (flux) of methyl iodide in estuarine systems

Besides the production and removal of CH_3I in estuarine systems, exchange between media also play an important role in establishing the net concentration of CH_3I in surface waters of estuarine systems. The exchange could be either horizontal or vertical. Horizontal diffusivity of CH_3I which occurs through the process of mixing mainly due to tidal effects between freshwater and seawater resulting in dilution was not investigated in this study. Vertical transfer which occurs through exchange with the atmosphere (water to air flux) has however been estimated, and from this, the contribution of estuarine systems to the total global water to air CH_3I flux also estimated from this study as 3.9×10^7 g y^{-1} (from section 5.5.2). This represents only a maximum of 0.03% of the total global flux of CH_3I. This percentage is estimated as the fraction between the global estuarine water to air CH_3I flux and the minimum total global water to air CH_3I flux (estimate obtained from Moore & Groszko, 1999) presented as $(3.9 \times 10^7 / 128 \times 10^9$ g $y^{-1}) \times 100$. With the total

surface area of global estuarine systems (1.4×10^6 km^2) representing about 0.4% of the total surface area of the world's oceans and seas (361.1×10^6 km^2; Moiseev, 1971), the estimated maximum contribution of global estuarine systems (0.03%) to the total global flux of CH_3I is negligible.

From all the estimates of this study, estuarine systems may possible not serve as a net source of CH_3I due to their negligible water to air flux rate of CH_3I. However, through the high bacterially-mediated production and the potentially high bacterially-mediated removal of CH_3I in estuarine systems, they may be important to the global cycling of CH_3I in the aquatic environment.

With analytical findings and experimental observations from the various investigations carried out in the Tamar estuary (in addition to information from literature), a conceptual model could be generated. This conceptual model highlighted in Figure 6.2 represents the contributions of investigated microbial activity to the production and removal of CH_3I in an estuarine system.

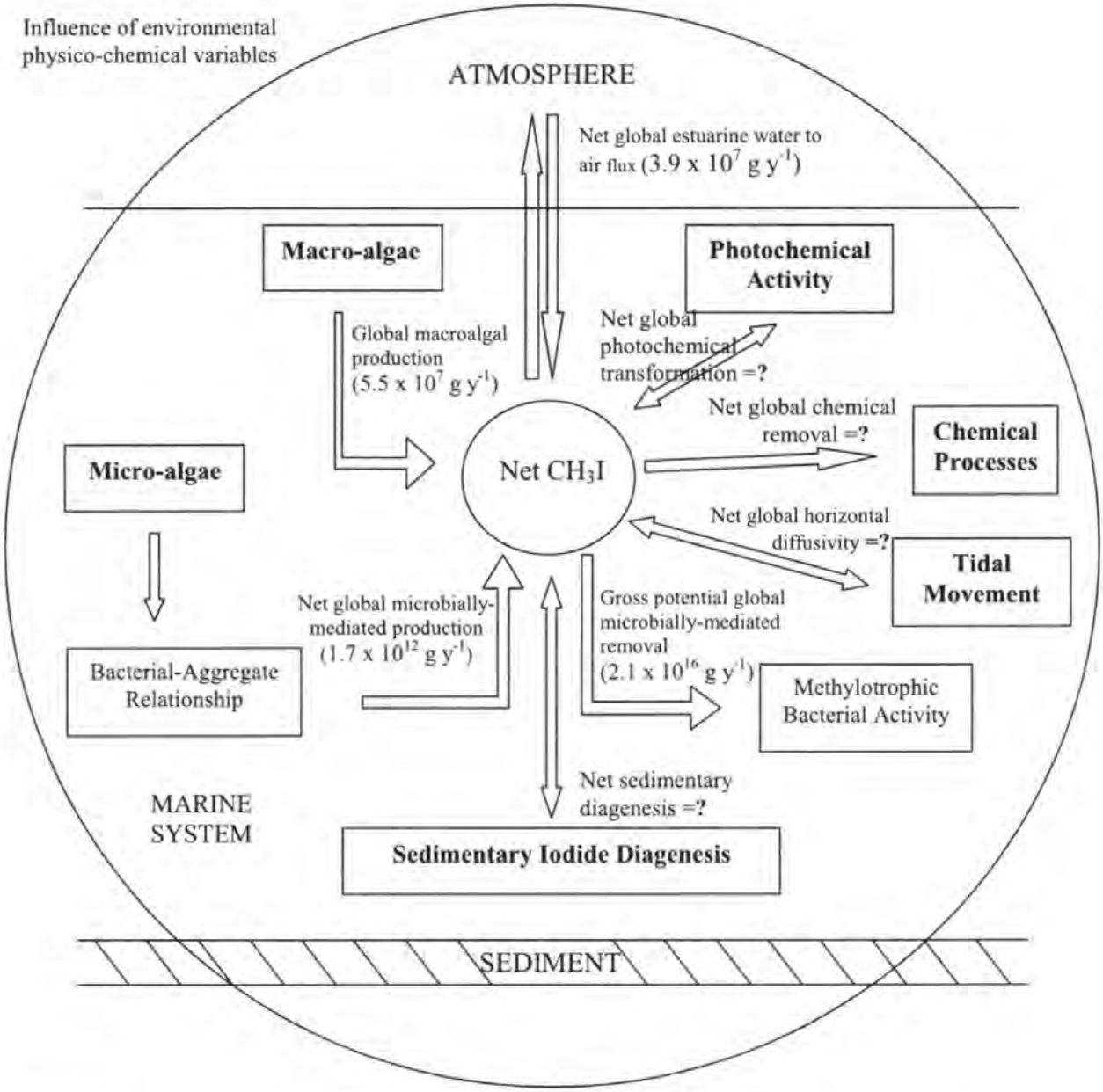

Figure 6.2: A conceptual model of biological, physical and chemical contribution to the net CH_3I concentration in the aquatic environment through production and removal (based on investigation in an estuarine system except those in bold) with global estimates. Arrows directed toward "Net CH_3I" represent production and arrows directed away from "Net CH_3I" represent removal of CH_3I. The large circle represents the influence of environmental physical and chemical variables (e.g. temperatures, DO, Salinity, pH, wind speed) on the various biological, physical and chemical processes that result in the production and removal of CH_3I in estuarine systems. Global macroalgal production value was obtained from Nightingale *et al.* (1995). The question mark (?) represents unknown global estimates.

6.1.4. Limitations of the study

During the aggregation experiment in this study, time did not allow the investigation of the effect of variations of other important physical parameters such as temperature on the rate of production of CH_3I. This prevented the appropriate conclusion regarding the role of other physical factors in regulating CH_3I production from bacterial-aggregate relationship. In addition, since the removal of CH_3I was identified during the aggregation experiment and investigated in the Tamar estuary, it would have been important to carryout controlled laboratory experiments that would have involved the investigation of both bacterially-mediated CH_3I production and removal in the same experimental medium in order to estimate the gross CH_3I production as a result of bacterial-aggregate associations. In addition, tidal effects and salinity stratification that may be present in the Tamar estuary could not be investigated due to the inaccessibility of the research vessel to the upper reaches of the estuary during high tide and time constraints. Information from these studies would have allowed a better understanding of the distribution of CH_3I concentration in the estuary and assisted in estimating the horizontal diffusivity of CH_3I in the Tamar estuary. Although wind speed data required for water to air CH_3I flux calculation during each sampling date was obtained from the local weather records at Plymouth Marine Laboratory, this information was not obtained directly from the Tamar estuary. Therefore, a direct measurement of wind speed data at each sampling location during each sampling period would have allowed a better estimate of water to air flux of CH_3I over the Tamar estuary.

6.2. Future work

In section 6.1.4, a number of limitations were encountered during the course of the present study. Bacterial activity in the estuarine environment (whether in the production or removal of CH_3I) as shown in this study is highly dependant on environmental physical

variables. Therefore, further laboratory and field investigations need to be carried out to better define the effects of physico-chemical variables on bacterial CH_3I production and removal in the estuarine environment.

6.2.1. Laboratory Experiments

Laboratory-controlled experiments are required to (i) Obtain quantitative information on the enhancement of CH_3I concentrations as a result of bacteria-aggregate relationship as well as bacterial CH_3I removal. Experimental techniques that will simultaneously measure both production and removal of CH_3I by bacteria in the same experimental medium need to be developed. (ii) To investigate the effect of temperature on bacteria-aggregation activity and the impact on the net CH_3I concentration. The development of this technique will involve adaptation and modification of the methods used in the investigation of bacterial CH_3I production and removal in the previous studies into a single reliable, applicable and reproducible technique.

6.2.2. Field Investigations

The Tamar estuary fieldwork conducted in this study should be continued and attempt to assess the effects of (i) tidal movement and (ii) salinity stratification (halocline) on methylotrophic bacterial CH_3I removal and CH_3I concentrations. Future field studies would include the measurement of (1) chlorophyll *a*, (2) wind speed and atmospheric CH_3I concentration measurements, (3) sedimentary diagenesis of CH_3I, (4) chemical and photochemical production/removal of CH_3I, and (5) methylotrophic bacterial CH_3I removal mechanisms. In addition, there would be the investigation into (6) bacterial CH_3I production and removal in the aquatic environment.

1. Chlorophyll a measurement

As already known, the most dominant identified source of CH_3I in the aquatic environment is through primary production. But it is also known that primary production in estuarine systems is highly limited by lack of light penetration as a result of high load of suspended particulate matter (especially in the turbidity maximum region of the estuary). Therefore the measurement of chlorophyll *a* as an indicator of autotrophic activity would aid in understanding spatial and seasonal trends in CH_3I distribution in the Tamar estuary (through autotrophic CH_3I production). Although there are a number of methods that could be applied in estimating chlorophyll *a* level in the estuary, this would be achieved through *in situ* chlorophyll fluorescence measurement.

2. Atmospheric methyl iodide concentration and wind speed measurement

During water to air exchange of gases, the transfer velocity of any gas of interest is affected by wind speed among other environmental factors. More often, the estimation of water to air flux of gases is achieved by making several assumptions regarding these environmental factors that are known to affect the transfer velocity. Therefore in order to minimize the error associated with these assumptions, estimation of water to air flux of CH_3I over the Tamar estuary in future studies would include the direct measurement of wind speed over the estuary. In addition, atmospheric concentration of CH_3I over the Tamar estuary is required to accurately estimate the concentration gradient for water to air flux calculation of CH_3I over the Tamar estuary and therefore will also be measured in future studies.

3. Investigating sedimentary diagenesis of methyl iodide

Microbial activity in estuarine sediments, just like in the overlying water body could lead to the production and removal of biogenic gases through the process of diagenesis. Based on a study conducted in the Tamar estuary by Upstill-Goddard & Elderfield (1988), it is

likely that diagenetic production of CH_3I may occur through I^- scavenging by CH_3 radicals either close to the sediment-water interface or on particles in the overlying water. On the other hand, the diagenetic removal of CH_3I may occur through the oxidative breakdown of the bond between iodide and carbon (Malcolm & Price, 1984) of the CH_3I chemical structure. Sedimentary diagenesis of CH_3I is therefore expected to affect the concentration of CH_3I in the overlying water through transfer across the sediment-water interface (diagenetic flux). Through mixing, this may eventually affect surface water concentration of CH_3I of the estuary. It will therefore be very important to assess the contributions and role of sedimentary diagenesis in CH_3I regulation in the Tamar estuary in establishing sources and sinks of CH_3I.

4. Chemical and photochemical activities in the production/removal of methyl iodide

From literature, it has already been established that both chemical and photochemical processes in the aquatic environment result in either the production or removal of CH_3I. The influence of chemical processes on CH_3I concentration is through iodide substitution reactions as well as reaction of CH_3I with other radicals (e.g. OH^-) that results in the transformation of CH_3I into other compounds (based on the stability of the compounds). Since CH_3I with the highest molecular weight is the least stable amongst the monohalomethanes (CH_3X), chemical processes involving iodide species in an estuary may result in the removal of CH_3I through substitution. Photochemical activity (especially in surface water) could lead to both production and removal of CH_3I. The type of photochemical reaction that may result in the production of CH_3I probably utilizes the UV portion of the light spectrum (Manley & de la Cuesta, 1997). According to Moore & Zafiriou (1994), the photochemical production of CH_3I will also depend on the source of I atoms and CH_3 radicals in the aquatic system. Photochemical removal of CH_3I may occur through UV photolysis in surface waters (Zika *et al.*, 1984), which may result in the splitting of CH_3I into I atoms and CH_3 radicals. In this sense, investigating the role of

chemical and photochemical activities in the production and removal of CH_3I in an estuary in future studies will be paramount for CH_3I budgeting in estuarine systems.

5. Methylotrophic bacterial methyl iodide removal mechanisms

Methylotrophic bacterial removal of CH_3I in natural water samples was identified and investigated in the previous studies. A portion of the removed CH_3I was oxidized whilst the remaining portion was simply recorded in the bacterial cells. However the process through which the removal was carried out by the methylotrophic bacteria is not known. Future work would therefore include an investigation into the mechanisms of CH_3I removal by the methylotrophic bacterial community in aquatic systems.

6. Microbial methyl iodide production and removal in the marine environment

From the previous investigations, it was apparent that concentration level and water to air flux of CH_3I, one of the focal points of the investigation was very low in the freshwater reaches compared to the seawater reaches of the estuarine system. This increase in CH_3I concentration and water to air flux rate with increasing salinity suggests a possible higher marine origin of CH_3I. It is therefore important to conduct similar investigations to the previous one with regard to microbial CH_3I production and removal through their association with POM in the marine environment in future studies.

All information that would be derived from these future investigations could be used (in addition to existing information from the previous study) to highlight on the cycling of CH_3I in estuarine systems as well as reflect in the role estuaries play in the global cycling of iodide in general.

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Appendices

Appendix 1: List of chemicals used in field and laboratory analyses

1a. List of Chemicals

i. Antimycotic cocktail
ii. Hydrochloric Acid (HCl)
iii. L-[4, 5-^{3}H] leucine
iv. Magnesium Perchlorate ($Mg(ClO_4)_2$)
v. Methanol (CH_3OH)
vi. Methyl Iodide Standard (CH_3I)
vii. [^{14}C] Methyl Iodide ($^{14}CH_3I$)
viii. OptiPhase 'HiSafe'3
ix. Paraformaldehyde
x. Phosphate Buffered Saline (PBS)
xi. Sodium Carbonate (Na_2CO_3)
xii. Sodium Hydroxide (NaOH)
xiii. Strontium Chloride Hexahydrate ($SrCl_2.6H_2O$)
xiv. SYBR Green I
xv. Trichloroacetic Acid (TCA)
xvi. Tri-Potassium Citrate

1b. List of chemicals and their intended purpose within the experimental procedures.

CHEMICAL	PURPOSE
Antimycotic cocktail	For terminating bacterial growth and activity in water samples.
Hydrochloric Acid (HCl)	Used for the preparation of Phosphate Buffered Saline.
L-[4, 5-^{3}H] leucine	Used as substrate for bacterial protein synthesis.
Magnesium Perchlorate ($Mg(ClO_4)_2$)	As drying agent in the purge and trap system of a GC system.
Methanol (CH_3OH)	For preparing methyl iodide standards.
Methyl Iodide Standard	Used for system calibration in the analysis of the concentrations of methyl iodide in samples.
[^{14}C] Methyl Iodide ($^{14}CH_3I$)	Used as substrate for bacterial uptake of methyl iodide in experimental samples.
OptiPhase 'HiSafe'3	Used as cocktail for scintillation counting.
Paraformaldehyde	For fixing bacteria in water sample before storage/counting.
Phosphate Buffered Saline (PBS)	Used for the preparation of tri-potassium citrate solution.
Sodium Carbonate (Na_2CO_3)	Used for enhancing precipitation of CO_2 from bacterial oxidation.
Sodium Hydroxide (NaOH)	For converting volatile CO_2 in samples into non-volatile CO_3^- before

	precipitation.
Strontium Chloride Hexahydrate ($SrCl_2.6H_2O$)	For precipitating resulting CO_2 in the form of Sr_2CO_3 in the sample medium.
SYBR Green I	For staining bacteria before counting.
Trichloroacetic Acid (TCA)	For denaturing bacterial protein in control sample replicates.
Tri-Potassium Citrate	For maintaining bacterial cell integrity during the staining process before counting.

Appendix 2: Preparation of Chemicals

2a. Methyl Iodide Standard

In order to accurately estimate how much of each compound is present in a given sample there is the need to analyze the instrumental response with a known concentration of the compounds of interest.

The calibration standard for methyl iodide (CH_3I) detection was prepared using a stock iodomethane 99% pure by weight (Aldrich) and methanol, HPLC grade (Fisher) as the solvent in the following manner.

i. A 15ml amber glass vial was weighed on an electronic balance (Metler AE240) together with its aluminium septum cap.

ii. It was then filled with methanol leaving only a small headspace and capped tightly with the aid of a hand crimper.

iii. This was weighed again to obtain the weight of the methanol in the vial. (The actual volume of methanol was subsequently estimated with the density of methanol at room temperature).

iv. A small amount of CH_3I standard was injected into the methanol through the septum with the aid of a microsyringe and weighed.

v. The weight of the injected standard was determined and its mass concentration calculated.

vi. Serial dilution from the prepared standard of known concentration was conducted by taking a small volume of known CH_3I into another vial with known volume of methanol until the desired range of concentration was achieved.

2b. [^{14}C] Methyl Iodide (stock)

Radiochemical Information: Pack Size = 1mCi (under vacuum)
Specific Activity = 2.04GBq (55mCi/mmol)
Molecular Weight = 142 (144 at Sp. Activity)
Chemical Purity = 99.9%
Manufacturer = Amersham Ltd

i. The vial containing the radiochemical was clamped with a vice in a fume cupboard.

ii. 3 ml of milli Q as solvent was pipetted down the vial to cover the hook seal in the upper chamber.

iii. The hook was then broken using a glass rod allowing solvent to drain into the lower chemical chamber as a result of the negative pressure in the lower chamber.

iv. The vial with its content was allowed to homogenize for at least 30 minutes.

v. The upper chamber was then emptied of any residual solvent into a new vial.

vi. The edge of the lower chamber was scratched with the aid of a metal file.

vii. A hot burner was applied to the scratched area to cause a crack.

viii. The upper chamber was then broken off at the crack giving access to the content of the lower chamber.

ix. The content of the lower chamber was then emptied into the new vial containing the residual solvent, 2 ml of milli Q then added and homogenized.

x. Since methyl iodide is highly volatile, the resulting stock solution was divided into 5 aliquots of 1 ml each to prevent over exposure of the whole content during usage which result some loss of concentration.

xi. All aliquots were tightly sealed, labelled and stored at -20°C in a freezer as recommended by manufacturer.

2c. 20% Paraformaldehyde solution

i. 10g of paraformaldehyde GPR (VWR 29447 4L) was weighed in a clean beaker.

ii. 50ml of phosphate buffered saline (PBS) solution was measured using a 50ml volumetric flask and added to the paraformaldehyde.

iii. The mixture was then heated to approximately 80°C in a hot water bath while swirling the beaker to mix.

iv. A drop of 0.5M NaOH was added to the solution and continuously warmed while swirling until the paraformaldehyde powder has completely dissolved.

v. The pH was then corrected to neutral by the addition of a few drops of 0.5M HCl and the solution filtered into a clean bottle and labelled.

vi. The solution was kept in a refrigerator until use.

2d. 10% Tri-Potassium citrate solution

i. 2.5g of tri-potassium citrate GPR (VWR 29599 4L) was weighed in a clean beaker.

ii. 25ml of de-ionized water (Milli-Q) was measured in a volumetric flask and transferred into the beaker.

iii. The content of the beaker was thoroughly mixed by swirling until the tri-potassium citrate has completely dissolved.

iv. This was then transferred into a clean bottle and labelled accordingly and stored in the refrigerator until used.

2e. 1% SYBR green I

i. For each set of samples to be analysed, 495µl of Milli-Q water was added to a microcentrifuge tube containing 5µl of Sybr Green removed from a freezer where stored.

ii. This was vortex-mixed and kept in the refrigerator a moment before used.

2f. Trichloroacetic acid

100% Trichloroacetic acid (TCA)

i. Approximately 250g of stock TCA (BDH 10286) was weighed in a clean beaker.

ii. Milli-Q water was measured in a clean 250ml capacity volumetric flask (Pyrex).

iii. This was then added to the weighed TCA in small volumes while mixing by swirling the beaker until the TCA has completely dissolved.

iv. The rest of the milli-Q was added to the solution and homogenized to obtain the 100% concentration.

5% TCA

i. The preparation follows the same trend as above but 25g of stock crystal TCA was used in a volume of 500ml milli-Q instead.

2g. (1M) Strontium Chloride Hexahydrate solution

To prepare a strontium chloride hexahydrate solution, the following information was initially established.

Molecular mass = 267g

Required molarity = 1

Volume of solution to be made = 100ml

Molarity = No. of moles/ 1L

Hence No. of moles = Molarity x 1L

= 1 x 1L = 1 mole

Also No. of moles = Mass (g)/ Molecular mass

Hence Mass (g) = No. of moles x Molecular mass
= 1 mole x 267 = 267g (for 1L volume)
For 100ml, Mass = (267g x100ml)/ 1000ml = 26.7g

i. A clean 50ml beaker (Pyrex) was pre-weighed with an electronic balance in a fume cupboard.

ii. The balance was then zeroed before the actual weighing.

iii. 26.7g of strontium chloride hexahydrate salt was weighed with the aid of a spatula.

iv. About 30ml of milli-Q water was added to the content of the beaker to pre dissolve the salt by stirring with the spatula.

v. This was then transferred into a 100ml volumetric flask (Pyrex) and the beaker rinsed with milli-Q into the volumetric flask.

vi. The content of the flask was top up to the mark with more milli-Q and homogenized.

vii. This was then stored in the refrigerator until use.

2h. (1M) Sodium Carbonate solution

Molecular mass = 106g
Required molarity = 1
Volume of solution to be made = 50ml
Mass (g) = 1 mole x 106g = 267g (for 1L volume)
For 50ml, Mass = (106g x 50ml)/ 1000 ml = 5.3g

i. A clean 50 ml beaker (Pyrex) was pre-weighed with an electronic balance in a fume cupboard.

ii. The balance was then zeroed for the actual weighing.

iii. 5.3g of sodium carbonate salt was fetched into the beaker with a spatula and weighed.

iv. About 20 ml of milli-Q was added to the content of the beaker to pre dissolve the salt by stirring with the spatula.

v. This was then transferred into a 50ml volumetric flask (Pyrex) and the beaker rinsed with milli-Q into the volumetric flask.

vi. The content of the flask was top up to the mark with more milli-Q and homogenized.

vii. This was then stored in the refrigerator until use.

viii.

Appendix 3: RISK ASSESSMENT (Use of radiochemical [^{14}C] Methyl Iodide)

This risk assessment is for the use of radiolabelled methyl iodide with carbon 14 as a substrate for bacterial incubation experiment in the radiochemical laboratory.

Location of activity: PML laboratories 317 and 318
Person responsible: Noble Asare, Anyone who may use this radiochemical
Supervising PML staff: Fred Staff

Who may be at risk?

Scientific users of the laboratories
Non-scientific staff (e.g. Cleaners)

Procedures involved

Bacterial incubation
Centrifugation
Aspiration

Equipments involved

Incubator
Centrifuge
Vortex mixer
Vacuum pump

Main Hazards

The following hazards are likely to take place if working precautions and good working practices are not employed.

1. Contamination through spillages during pipetting of radiochemical or samples containing the radiochemical may occur.
2. Exposure to harmful beta radiation if spillage occurs.
3. Very toxic when the radiochemical is inhaled or swallowed.
4. Injury may occur when the radiochemical is in contact with the skin or eyes.

RISK = PROBABILITY x SEVERITY

PROBABILITY OF HAZARD OCCURRING:		
Certain	High	3
Reasonably likely	Medium	2
Unlikely	Low	1

SEVERITY OF HAZARD:		
Death	Medium	2
Injury/Illness/Disease	Medium	2
Minor injury	Low	1

Hazards Identified	Likelihood	Hazard Rating	Risk
Spillage	Medium	Medium	4 -High
Contamination	High	Low	3 -Medium
Radiation exposure	High	Low	3 -Medium
Inhalation	High	Medium	6 -Very high

Reduction of Risk

Spillage Stock radiochemical vial and all other sample vials in use should be handled and opened with extreme care. A piece of tissue paper dipped in milli Q should be used with the aid of forceps to swipe the neck of the stock vial and checked for spillage with a low energy emitting detector before opening. During pipetting of stock or samples from one vial into another, care should be taking to reduce the risk of spillage of droplets.

Contamination Work involving the use of this radiochemical should always be carried out in trays with the internal surface lined with absorbent paper in to serve as containment for all spillages. The working bench should likewise be covered with absorbent paper to reduce spread of contamination in case spillage occurs. Working gloves should be changed frequently especially when moving from one working area to another. Working area should always be monitored for contaminations with a detector or tissue swipes and the area thoroughly cleaned before the next user.

Radiation exposure All the necessary working gear as stated in the SAFE WORKING PRACTICES. This includes wearing a lab coat, safety specs and gloves at all times. Personal radiation exposure monitor should be worn at all times to estimate the rate of exposure.

Inhalation Since this radiochemical is highly volatile, work involving its use should always be carried out in fume cupboard to avoid inhalation or reduce the rate of inhalation.

Assessor Name	Signature	Date
Noble K. Asare		

Appendix 4: Safe Working Practices (Use of [^{14}C] CH_3I)

This safe working practices sheet is for the transferring, diluting and use of the radiolabelled methyl iodide as a biological tracer in the radiochemical labs.

Person Responsible: Noble K. Asare

Safe Practices

1. This procedure must always be carried out in fume cupboards.
2. Wear safety specs, lab coat and gloves at all times during work.
3. Monitor for contamination with low energy emitting detector periodically.
4. Change gloves each time contamination is detected on them or when moving to another working area.
5. In case of minor spillage, clean area of contamination thoroughly with wet tissue and monitor until detection is just background noise.
6. In the case of major spillage, call the radiochemical officer immediately who will take the proper action.
7. Conduct swipe tests of the working area periodically which is more effective in detecting low levels of contaminations.
8. Remove and dispose properly all potentially contaminated equipments prior to leaving the area where the radiochemical was used.
9. Handle all radioactive sample/solutions in trays large enough to contain the material in the event of spillage.
10. Never eat, drink, smoke, handle contact lenses, apply cosmetics or take/apply any medication in the radiochemical laboratories.
11. Clearly label each radioactive material container with the radioactive tape and in the case of samples/stock solutions, the isotope, activity and concentration.
12. Clearly outline radioactive material working areas with radioactive tape.
13. Follow the general laboratory policy for security of radioactive materials.
14. Plan experiments to minimize external exposures by reducing exposure times.
15. Reduce internal and external radiation dose by monitoring yourself and the working area after each use of radioactive methyl iodide.
16. Provide for safe disposal of all radioactive waste by following the laboratory policy on radioactive waste handling and disposal.

Appendix 5: Statistical test (student's t-test) between methyl iodide concentrations from different treatments but the same periods (Study 1) at 95% confidence level of significance.

Day	Comparison Between	s	t-values	*P*-values	Significantly Diffeent
2	Live-Rolled & Live-Unrolled	0.027	3.313	<0.05	Yes
4	Live-Rolled & Live-Unrolled	0.020	4.654	<0.01	Yes
6	Live-Rolled & Live-Unrolled	0.223	0.423	>0.10	No
8	Live-Rolled & Live-Unrolled	0.029	6.212	<0.01	Yes
10	Live-Rolled & Live-Unrolled	0.044	0.836	>0.10	No
12	Live-Rolled & Live-Unrolled			>0.10	No
14	Live-Rolled & Live-Unrolled			>0.10	No

Appendix 6: Statistical test (student's t-test) between methyl iodide concentrations from different treatments but the same periods (Study 2) at 95% confidence level.

SIGNIFICANT TEST BETWEEN (L+L and D+L) & (L+D and D+D)						
Sample	Mean	SD (+/-)	s	t-values	P-values	Significantly Different
Pre-00-L+L	0.373	0.022	0.016	4.622	<0.01	Yes
Pre-00-D+L	0.312	0.005				
00-L+L	0.341	0.003	0.005	1.067	>0.10	No
00-D+L	0.336	0.007				
00-L+D	0.341	0.003	0.005	1.067	>0.10	No
00-D+D	0.336	0.007				
01-L+L	0.328	0.009	0.007	4.111	<0.01	Yes
01-D+L	0.303	0.005				
01-L+D	0.354	0.002	0.018	1.710	>0.10	No
01-D+D	0.329	0.025				
02-L+L	0.379	0.016	0.012	3.564	<0.02	Yes
02-D+L	0.344	0.005				
02-L+D	0.366	0.003	0.003	8.141	<0.001	Yes
02-D+D	0.343	0.004				
04-L+L	0.570	0.034	0.024	5.186	<0.01	Yes
04-D+L	0.467	0.003				
04-L+D	0.561	0.062	0.044	3.620	<0.02	Yes
04-D+D	0.431	0.008				
06-L+L	0.747	0.037	0.040	7.092	<0.001	Yes
06-D+L	0.513	0.043				
06-L+D	0.606	0.042	0.034	3.644	<0.02	Yes
06-D+D	0.505	0.022				
08-L+L	0.559	0.030	0.022	3.690	<0.02	Yes
08-D+L	0.494	0.008				
08-L+D	0.605	0.024	0.018	5.101	<0.01	Yes
08-D+D	0.528	0.011				

L+L = Live in Light Samples
D+L = Dead in Light Samples
L+D = Live in Dark Samples
D+D = Dead in Dark Samples

Appendix 7: A table of significant test for bacterial uptake of 14-C labelled methyl iodide (Experiment 1.1).

Significant test between same targets of different concentrations (nmol/l)								
Sample Target	**Conc.**	**SD (+/-)**	**$\sum$(x-mean)^2**	**s**	***t*-values**	**Comparison**	***P*-values**	**Significantly Different?**
^{14}C (3h-C1)	0.7	0.3	1.1	0.7068	0.117	**^{14}C-3h(C1 vs C2)**	>0.10	**No**
^{14}C (3h-C2)	0.7	0.1	2.9	0.8985	0.167	**^{14}C-3h(C2 vs C3)**	>0.10	**No**
^{14}C (3h-C3)	0.8	0.5	3.5	0.7609	0.088	**^{14}C-3h(C3 vs C1)**	>0.10	**No**
$^{14}CO_2$ (3h-C1)	0.2	0.1	0.0	0.0684	1.405	**$^{14}CO_2$.3h (C1 vs C2)**	>0.10	**No**
$^{14}CO_2$ (3h-C2)	0.2	0.1	0.0	0.0759	0.886	**$^{14}CO_2$.3h (C2 vs C3)**	>0.10	**No**
$^{14}CO_2$ (3h-C3)	0.2	0.1	0.0	0.0979	0.295	**$^{14}CO_2$.3h (C3 vs C1)**	>0.10	**No**
$^{14}CH_3I$b (3h-C1)	1.9	0.2	0.2	0.2403	18.197	**$^{14}CH_3I$b-3h (C1 vs C2)**	<0.001	**Yes**
$^{14}CH_3I$b (3h-C2)	4.6	0.5	0.3	0.6056	7.150	**$^{14}CH_3I$b-3h (C2 vs C3)**	<0.001	**Yes**
$^{14}CH_3I$b (3h-C3)	7.4	0.6	2.7	0.5993	14.521	**$^{14}CH_3I$b-3h (C3 vs C1)**	<0.001	**Yes**
^{14}C (6h-C1)	0.8	0.3	0.9	0.6298	0.356	**^{14}C-6h(C1 vs C2)**	>0.10	**No**
^{14}C (6h-C2)	0.9	0.4	2.2	1.2966	0.075	**^{14}C-6h(C2 vs C3)**	>0.10	**No**
^{14}C (6h-C3)	1.0	0.5	11.2	1.2320	0.261	**^{14}C-6h(C3 vs C1)**	>0.10	**No**
$^{14}CO_2$ (6h-C1)	0.2	0.1	0.0	0.0638	0.643	**$^{14}CO_2$.6h (C1 vs C2)**	>0.10	**No**
$^{14}CO_2$ (6h-C2)	0.2	0.1	0.0	0.1279	1.023	**$^{14}CO_2$.6h (C2 vs C3)**	>0.10	**No**
$^{14}CO_2$ (6h-C3)	0.3	0.1	0.1	0.1130	1.520	**$^{14}CO_2$.6h (C3 vs C1)**	>0.10	**No**
$^{14}CH_3I$b (6h-C1)	2.3	0.2	0.3	0.3891	14.258	**$^{14}CH_3I$b-6h (C1 vs C2)**	<0.001	**Yes**
$^{14}CH_3I$b (6h-C2)	5.8	0.3	0.9	0.5336	10.620	**$^{14}CH_3I$b-6h (C2 vs C3)**	<0.001	**Yes**
$^{14}CH_3I$b (6h-C3)	9.4	0.7	1.3	0.4497	24.940	**$^{14}CH_3I$b-6h (C3 vs C1)**	<0.001	**Yes**

Where **h** = number of hours and **C** = concentration level

Appendix 8: A table of significant test for bacterial uptake of 14-C labelled methyl iodide (Experiment 1.2).

Significant test between same targets of different concentrations (nmol/l)								
Sample Target	Conc.	SD (+/-)	$\sum$(x-mean)^2	s	*t*-values	Comparison	*P*-values	Significantly Different?
^{14}C (3h-C1)	56.1	2.4	22.8	3.7800	26.290	^{14}C-3h(C1 vs C2)	<0.001	Yes
^{14}C (3h-C2)	118.9	4.8	91.5	27.0522	7.245	^{14}C-3h(C2 vs C3)	<0.001	Yes
^{14}C (3h-C3)	242.9	37.6	5763.1	26.8932	10.983	^{14}C-3h(C3 vs C1)	<0.001	Yes
$^{14}CO_2$ (3h-C1)	29.6	0.8	0.6	1.6631	27.190	$^{14}CO_2$.3h (C1 vs C2)	<0.001	Yes
$^{14}CO_2$ (3h-C2)	58.2	4.6	21.5	4.7183	19.520	$^{14}CO_2$.3h (C2 vs C3)	<0.001	Yes
$^{14}CO_2$ (3h-C3)	116.5	12.5	156.6	4.4334	30.974	$^{14}CO_2$.3h (C3 vs C1)	<0.001	Yes
$^{14}CH_3I$b (3h-C1)	329.6	31.1	6098.7	33.8744	11.639	$^{14}CH_3I$b-3h (C1 vs C2)	<0.001	Yes
$^{14}CH_3I$b (3h-C2)	578.6	59.2	3081.1	48.3559	15.693	$^{14}CH_3I$b-3h (C2 vs C3)	<0.001	Yes
$^{14}CH_3I$b (3h-C3)	1058.9	52.0	15625.2	52.1104	22.128	$^{14}CH_3I$b-3h (C3 vs C1)	<0.001	Yes
^{14}C (6h-C1)	58.6	1.3	6.3	5.1407	19.577	^{14}C-6h(C1 vs C2)	<0.001	Yes
^{14}C (6h-C2)	122.2	6.2	205.1	5.8707	39.120	^{14}C-6h(C2 vs C3)	<0.001	Yes
^{14}C (6h-C3)	267.5	3.7	70.6	3.1010	106.514	^{14}C-6h(C3 vs C1)	<0.001	Yes
$^{14}CO_2$ (6h-C1)	29.6	0.8	0.6	1.9627	25.013	$^{14}CO_2$.6h (C1 vs C2)	<0.001	Yes
$^{14}CO_2$ (6h-C2)	60.7	5.9	30.2	3.2461	28.909	$^{14}CO_2$.6h (C2 vs C3)	<0.001	Yes
$^{14}CO_2$ (6h-C3)	120.0	7.6	54.1	2.6161	54.637	$^{14}CO_2$.6h (C3 vs C1)	<0.001	Yes
$^{14}CH_3I$b (6h-C1)	316.4	59.1	1726.0	33.2824	11.843	$^{14}CH_3I$b-6h (C1 vs C2)	<0.001	Yes
$^{14}CH_3I$b (6h-C2)	565.7	40.5	7135.8	39.8641	19.038	$^{14}CH_3I$b-6h (C2 vs C3)	<0.001	Yes
$^{14}CH_3I$b (6h-C3)	1045.7	176.1	5577.4	30.2146	38.164	$^{14}CH_3I$b-6h (C3 vs C1)	<0.001	Yes
^{14}C (72h-C1)	56.0	1.8	12.6	3.0772	55.031	^{14}C-72h(C1 vs C2)	<0.001	Yes
^{14}C (72h-C2)	163.1	3.5	63.2	10.6967	41.714	^{14}C-72h(C2 vs C3)	<0.001	Yes
^{14}C (72h-C3)	445.3	14.4	852.2	10.3966	59.206	^{14}C-72h(C3 vs C1)	<0.001	Yes
$^{14}CO_2$ (72h-C1)	18.8	1.2	1.9	0.5271	72.597	$^{14}CO_2$.72h (C1 vs C2)	<0.001	Yes
$^{14}CO_2$ (72h-C2)	43	0.6	0.3	1.9245	49.377	$^{14}CO_2$.72h (C2 vs C3)	<0.001	Yes
$^{14}CO_2$ (72h-C3)	103.1	5.0	29.3	1.9777	67.398	$^{14}CO_2$.72h (C3 vs C1)	<0.001	Yes
$^{14}CH_3I$b (72h-C1)	193.3	8.2	143.8	6.9711	56.341	$^{14}CH_3I$b-72h (C1 vs C2)	<0.001	Yes
$^{14}CH_3I$b (72h-C2)	441.7	12.3	245.0	19.5935	47.361	$^{14}CH_3I$b-72h (C2 vs C3)	<0.001	Yes
$^{14}CH_3I$b (72h-C3)	1028.6	41.1	2826.3	19.2681	68.545	$^{14}CH_3I$b-72h (C3 vs C1)	<0.001	Yes

Where **h** = number of hours and **C** = concentration level

Appendix 9: One-way analysis of variance (ANOVA) for bacterial methyl iodide uptake rate between cold and warm seasons at the selected sampling locations along the Tamar estuary.

9a. Morwellham

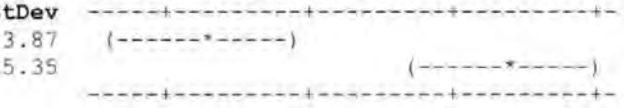
Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	17283	17283	25.11	0.000
Error	11	7571	688		
Total	12	24854			

Season	N	Mean	StDev
Cold	6	9.52	3.87
Warm	7	82.66	35.35

Pooled StDev = 26.23

Individual 95% CIs For Mean Based on Pooled StDev



9b. Tuckermarsh

Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	16083	16083	73.81	0.000
Error	11	2397	218		
Total	12	18479			

Season	N	Mean	StDev
Cold	6	12.52	6.06
Warm	7	83.07	19.21

Pooled StDev = 14.76

Individual 95% CIs For Mean Based on Pooled StDev

9c. Cargreen

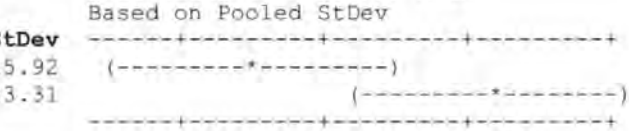
Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	8230	8230	7.23	0.021
Error	11	12520	1138		
Total	12	20750			

Season	N	Mean	StDev
Cold	6	14.80	15.92
Warm	7	65.27	43.31

Pooled StDev = 33.74

Individual 95% CIs For Mean Based on Pooled StDev



9d. The Narrows

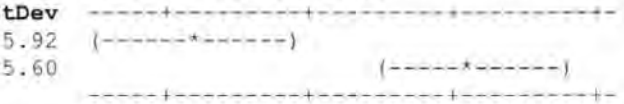
Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	14760	14760	20.87	0.001
Error	11	7779	707		
Total	12	22538			

Season	N	Mean	StDev
Cold	6	7.57	5.92
Warm	7	75.16	35.60

Pooled StDev = 26.59

Individual 95% CIs For Mean Based on Pooled StDev

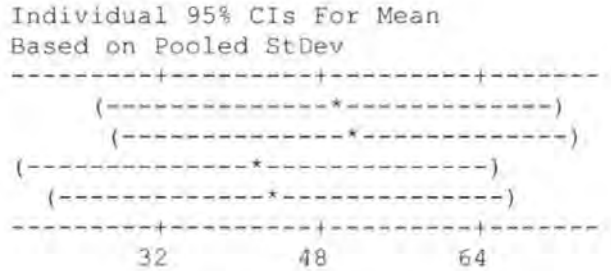


Appendix 10: One-way analysis of variance (ANOVA) in spatial variations for variables measured along the Tamar estuary (with sampling location as a factor).

10a. Rate of bacterial methyl iodide uptake

Source	DF	SS	MS	F	P
Location	3	632	211	0.12	0.950
Error	48	86622	1805		
Total	51	87254			

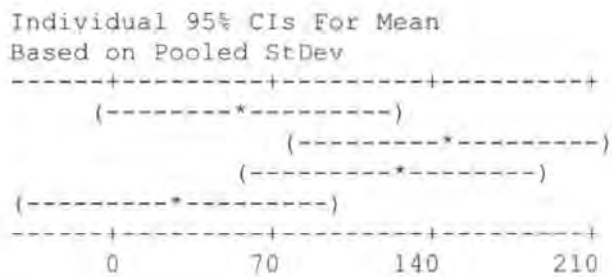
Location	N	Mean	StDev
Morwellham	13	48.90	45.51
Tuckermarsh	13	50.51	39.24
Cargreen	13	41.98	41.58
The Narrows	13	43.96	43.34
Pooled StDev =		42.48	



10b. Rate of bacterial leucine incorporation

Source	DF	SS	MS	F	P
Location	3	115594	38531	2.70	0.056
Error	48	685127	14273		
Total	51	800721			

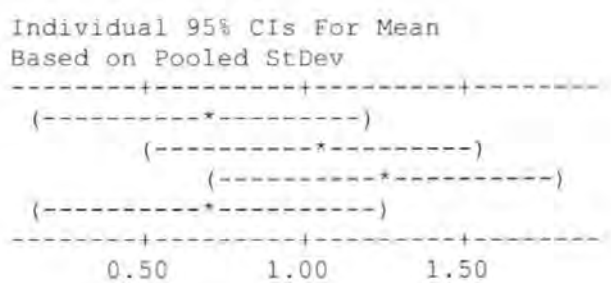
Location	N	Mean	StDev
Morwellham	13	58.4	60.7
Tuckermarsh	13	143.0	179.0
Cargreen	13	123.7	144.4
The Narrows	13	27.1	22.6
Pooled StDev =		119.5	



10c. Bacterial Numbers

Source	DF	SS	MS	F	P
Location	3	2.702	0.901	1.05	0.378
Error	44	37.618	0.855		
Total	47	40.321			

Location	N	Mean	StDev
Morwellham	12	0.6751	0.5380
Tuckermarsh	12	1.0284	1.0094
Cargreen	12	1.2498	1.2809
The Narrows	12	0.7083	0.6861
Pooled StDev =		0.9246	

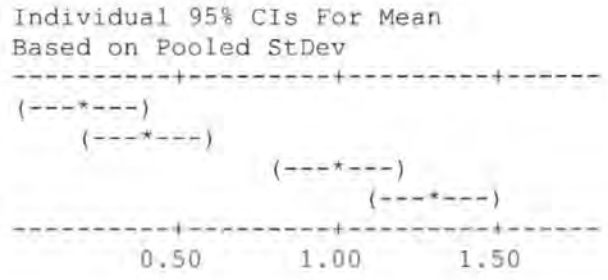


10d. Methyl iodide concentration

Source	DF	SS	MS	F	P
Location	3	7.9384	2.6461	28.51	0.000
Error	36	3.3409	0.0928		
Total	39	11.2794			

Location	N	Mean	StDev
Morwellham	10	0.2190	0.1300
Tuckermarsh	10	0.3930	0.2204
Cargreen	10	1.0010	0.3635
The Narrows	10	1.3180	0.4166

Pooled StDev = 0.3046

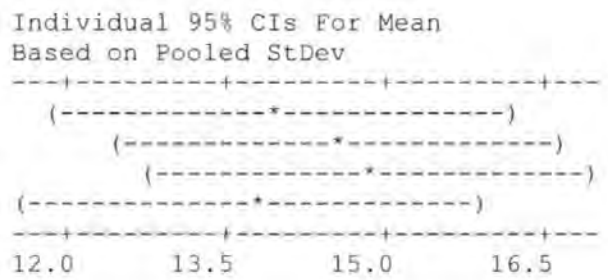


10e. Temperature

Source	DF	SS	MS	F	P
Location	3	9.6	3.2	0.22	0.883
Error	48	704.7	14.7		
Total	51	714.4			

Location	N	Mean	StDev
Morwellham	13	14.015	3.970
Tuckermarsh	13	14.538	4.351
Cargreen	13	14.831	3.812
The Narrows	13	13.731	3.082

Pooled StDev = 3.832

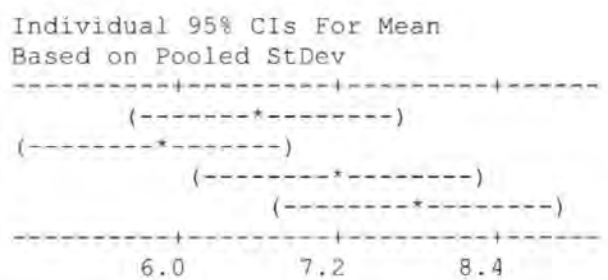


10f. Dissolved oxygen concentration

Source	DF	SS	MS	F	P
Location	3	25.91	8.64	2.53	0.068
Error	48	163.84	3.41		
Total	51	189.75			

Location	N	Mean	StDev
Morwellham	13	6.638	2.186
Tuckermarsh	13	5.854	1.810
Cargreen	13	7.162	1.956
The Narrows	13	7.777	1.331

Pooled StDev = 1.848

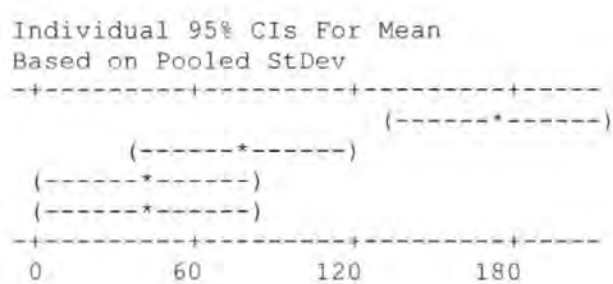


10g. Suspended particulate matter (SPM) concentration

Source	DF	SS	MS	F	P
Location	3	153539	51180	9.14	0.000
Error	48	268904	5602		
Total	51	422442			

Location	N	Mean	StDev
Morwellham	13	174.60	133.12
Tuckermarsh	13	78.36	58.02
Cargreen	13	41.02	21.78
The Narrows	13	42.49	29.11

Pooled StDev = 74.85

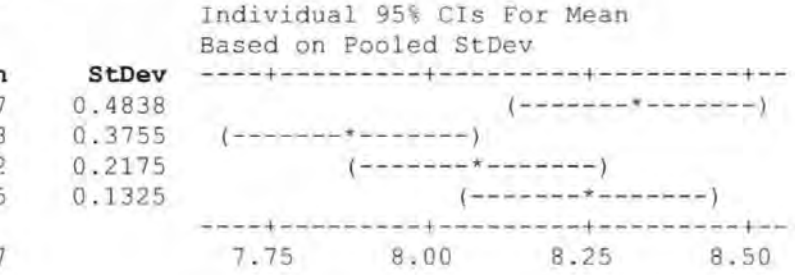


10h. pH

Source	DF	SS	MS	F	P
Location	3	1.594	0.531	4.83	0.005
Error	48	5.280	0.110		
Total	51	6.874			

Location	N	Mean	StDev
Morwellham	13	8.3077	0.4838
Tuckermarsh	13	7.8538	0.3755
Cargreen	13	8.0692	0.2175
The Narrows	13	8.2385	0.1325

Pooled StDev = 0.3317

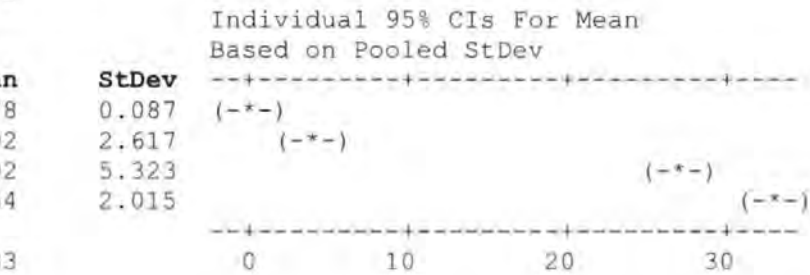


10i. Salinity

Source	DF	SS	MS	F	P
Location	3	10475.02	3491.67	355.79	0.000
Error	48	471.06	9.81		
Total	51	10946.08			

Location	N	Mean	StDev
Morwellham	13	0.178	0.087
Tuckermarsh	13	3.392	2.617
Cargreen	13	26.292	5.323
The Narrows	13	33.054	2.015

Pooled StDev = 3.133



Appendix 11: One-way ANOVA for spatial variations in the measured variables between Low/High Salinity Regions of the Tamar Estuary.

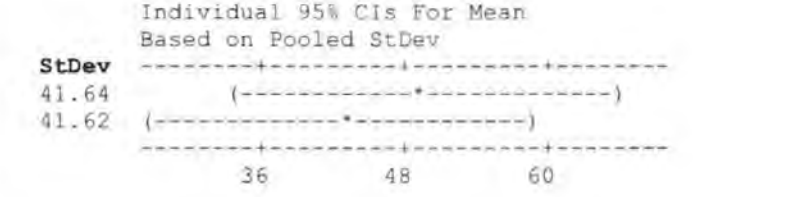
11a. Rate of bacterial methyl iodide uptake (R.^{14}C)

Analysis of Variance for R.^{14}C

Source	DF	SS	MS	F	P
Location	1	590	590	0.34	0.562
Error	50	86664	1733		
Total	51	87254			

Location	N	Mean	StDev
Low Sal	26	49.70	41.64
High Sal	26	42.97	41.62

Pooled StDev = 41.63



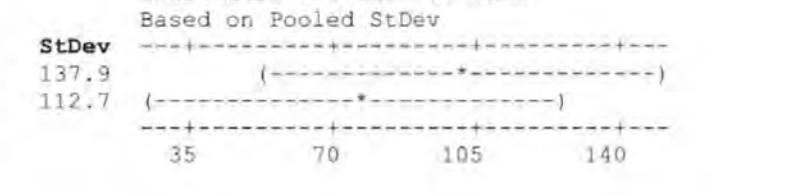
11b. Rate of bacterial leucine incorporation (R.^{3}H)

Analysis of Variance for R.^{3}H

Source	DF	SS	MS	F	P
Location	1	8301	8301	0.52	0.473
Error	50	792420	15848		
Total	51	800721			

Location	N	Mean	StDev
Low Sal	26	100.7	137.9
High Sal	26	75.4	112.7

Pooled StDev = 125.9



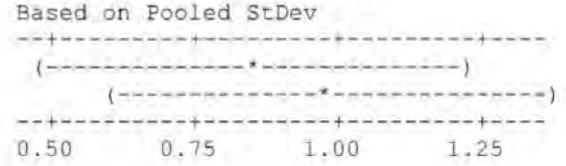
11c. Bacterial abundance (B.Nos)

Analysis of Variance for B.Nos

Source	DF	SS	MS	F	P
Location	1	0.194	0.194	0.22	0.639
Error	46	40.126	0.872		
Total	47	40.321			

Location	N	Mean	StDev
Low Sal	24	0.8518	0.8113
High Sal	24	0.9790	1.0423
Pooled StDev =		0.9340	

Individual 95% CIs For Mean Based on Pooled StDev



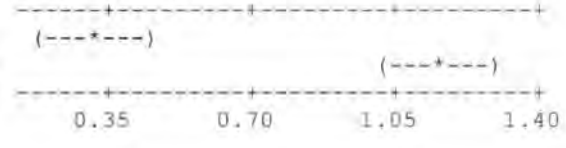
11d. Methyl iodide concentration [CH_3I]

Analysis of Variance for [CH_3I]

Source	DF	SS	MS	F	P
Location	1	7.285	7.285	69.29	0.000
Error	38	3.995	0.105		
Total	39	11.279			

Location	N	Mean	StDev
Low Sal	20	0.3060	0.1974
High Sal	20	1.1595	0.4138
Pooled StDev =		0.3242	

Individual 95% CIs For Mean Based on Pooled StDev



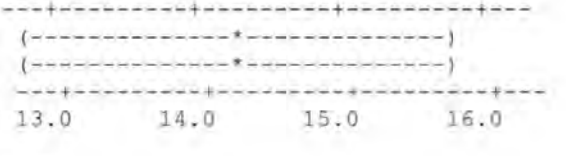
11e. Temperature

Analysis of Variance for Temp.

Source	DF	SS	MS	F	P
Location	1	0.0	0.0	0.00	0.997
Error	50	714.4	14.3		
Total	51	714.4			

Location	N	Mean	StDev
Low Sal	26	14.277	4.089
High Sal	26	14.281	3.443
Pooled StDev =		3.780	

Individual 95% CIs For Mean Based on Pooled StDev



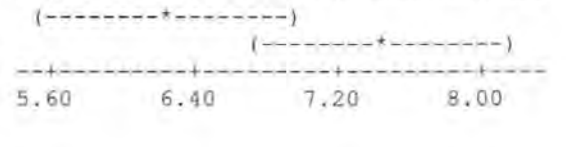
11f. Dissolved oxygen concentration [DO]

Analysis of Variance for [DO]

Source	DF	SS	MS	F	P
Location	1	19.45	19.45	5.71	0.021
Error	50	170.30	3.41		
Total	51	189.75			

Location	N	Mean	StDev
Low Sal	26	6.246	2.006
High Sal	26	7.469	1.669
Pooled StDev =		1.846	

Individual 95% CIs For Mean Based on Pooled StDev



11g. Suspended particulate matter (SPM)

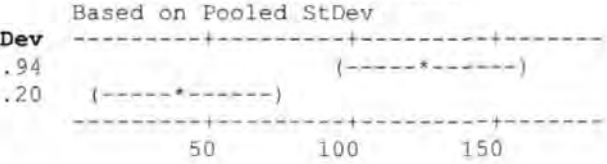
Analysis of Variance for SPM

Source	DF	SS	MS	F	P
Location	1	93322	93322	14.18	0.000
Error	50	329120	6582		
Total	51	422442			

Location	N	Mean	StDev
Low Sal	26	126.48	111.94
High Sal	26	41.75	25.20

Pooled StDev = 81.13

Individual 95% CIs For Mean Based on Pooled StDev



11h. pH

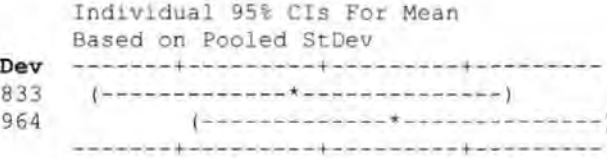
Analysis of Variance for pH

Source	DF	SS	MS	F	P
Location	1	0.069	0.069	0.51	0.478
Error	50	6.805	0.136		
Total	51	6.874			

Location	N	Mean	StDev
Low Sal	26	8.0808	0.4833
High Sal	26	8.1538	0.1964

Pooled StDev = 0.3689

Individual 95% CIs For Mean Based on Pooled StDev



11i. Salinity

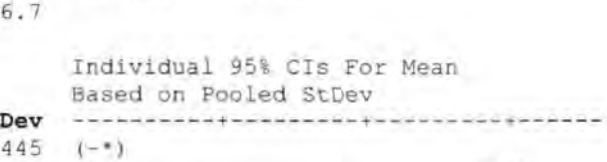
Analysis of Variance for Salinity

Source	DF	SS	MS	F	P
Location	1	10110.7	10110.7	605.14	0.000
Error	50	835.4	16.7		
Total	51	10946.1			

Location	N	Mean	StDev
Low Sal	26	1.785	2.445
High Sal	26	29.673	5.238

Pooled StDev = 4.088

Individual 95% CIs For Mean Based on Pooled StDev



Appendix 12: One-way ANOVA for bacterial methyl iodide uptake rate at the selected sampling locations (Morwellham, Tuckermarsh, Cargreen, The Narrows).

12a. Cold Season

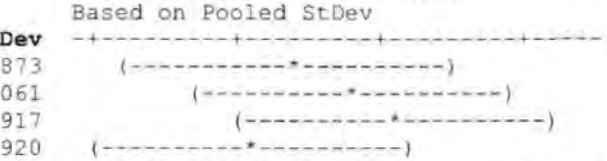
Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	184.1	61.4	0.72	0.551
Error	20	1700.7	85.0		
Total	23	1884.8			

Level	N	Mean	StDev
Morwellham	6	9.517	3.873
Tuckermarsh	6	12.517	6.061
Cargreen	6	14.800	15.917
The Narrows	6	7.567	5.920

Pooled StDev = 9.221

Individual 95% CIs For Mean Based on Pooled StDev



50

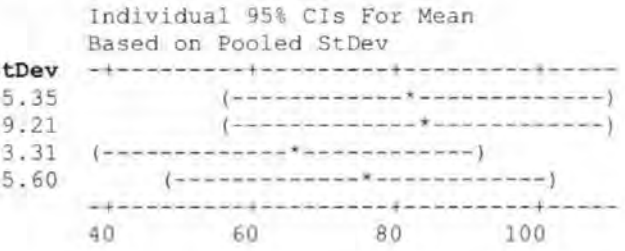
12b. Warm Season

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	1463	488	0.41	0.747
Error	24	28566	1190		
Total	27	30029			

Level	N	Mean	StDev
Morwellham	7	82.66	35.35
Tuckermarsh	7	83.07	19.21
Cargreen	7	65.27	43.31
The Narrows	7	75.16	35.60

Pooled StDev = 34.50



Appendix 13: One-way ANOVA for bacterial leucine incorporation rate at the selected sampling locations (Morwellham, Tuckermarsh, Cargreen, The Narrows).

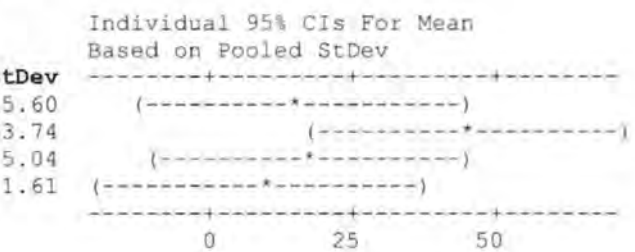
13a. Cold Season

Analysis of Variance

Source	DF	SS	MS	F	P
Location	3	4413	1471	1.36	0.284
Error	20	21674	1084		
Total	23	26087			

Location	N	Mean	StDev
Morwellham	6	16.15	15.60
Tuckermarsh	6	44.80	63.74
Cargreen	6	17.70	5.04
The Narrows	6	9.23	1.61

Pooled StDev = 32.92



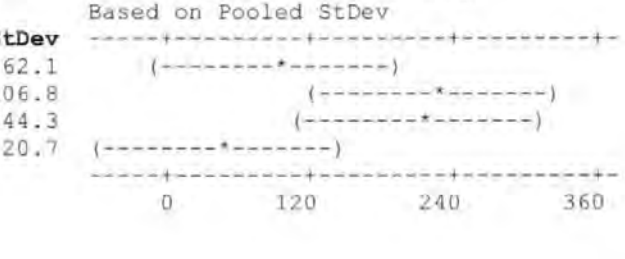
13b. Warm Season

Analysis of Variance

Source	DF	SS	MS	F	P
Location	3	172748	57583	3.39	0.034
Error	24	407357	16973		
Total	27	580106			

Location	N	Mean	StDev
Morwellham	7	94.6	62.1
Tuckermarsh	7	227.1	206.8
Cargreen	7	214.6	144.3
The Narrows	7	42.3	20.7

Pooled StDev = 130.3



Appendix 14: One-way ANOVA for bacterial numbers at the selected sampling locations (Morwellham, Tuckermarsh, Cargreen, The Narrows).

14a. Cold Season

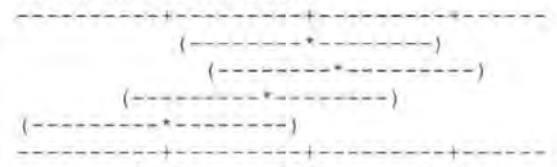
Analysis of Variance

Source	DF	SS	MS	F	P
Location	3	0.04649	0.01550	1.68	0.212
Error	16	0.14796	0.00925		
Total	19	0.19446			

Location	N	Mean	StDev
Morwellham	5	0.20000	0.08544
Tuckermarsh	5	0.22400	0.06877
Cargreen	5	0.16600	0.12582
The Narrows	5	0.09600	0.09555

Pooled StDev = 0.09616

Individual 95% CIs For Mean Based on Pooled StDev



14b. Warm Season

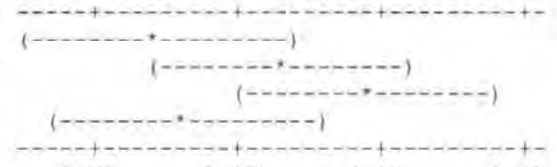
Analysis of Variance

Source	DF	SS	MS	F	P
Location	3	4.455	1.485	2.13	0.122
Error	24	16.699	0.696		
Total	27	21.154			

Location	N	Mean	StDev
Morwellham	7	1.0143	0.4502
Tuckermarsh	7	1.6029	0.9697
Cargreen	7	2.0243	1.1473
The Narrows	7	1.1443	0.5691

Pooled StDev = 0.8341

Individual 95% CIs For Mean Based on Pooled StDev



Appendix 15: One-way analysis of variance (ANOVA) for bacterial methyl iodide uptake rate between cold and warm seasons at the selected sampling locations along the Tamar estuary.

15a. Morwellham

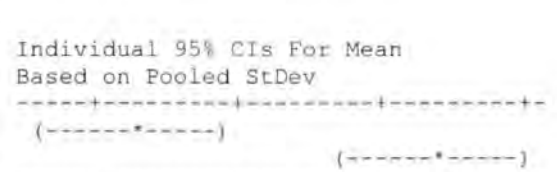
Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	17283	17283	25.11	0.000
Error	11	7571	688		
Total	12	24854			

Season	N	Mean	StDev
Cold	6	9.52	3.87
Warm	7	82.66	35.35

Pooled StDev = 26.23

Individual 95% CIs For Mean Based on Pooled StDev



15b. Tuckermarsh

Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	16083	16083	73.81	0.000
Error	11	2397	218		
Total	12	18479			

Season	N	Mean	StDev
Cold	6	12.52	6.06
Warm	7	83.07	19.21

Pooled StDev = 14.76

Individual 95% CIs For Mean Based on Pooled StDev

15c. Cargreen

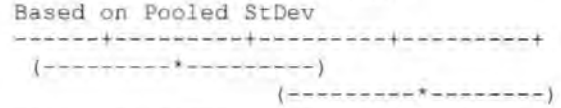
Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	8230	8230	7.23	0.021
Error	11	12520	1138		
Total	12	20750			

Season	N	Mean	StDev
Cold	6	14.80	15.92
Warm	7	65.27	43.31

Pooled StDev = 33.74

Individual 95% CIs For Mean Based on Pooled StDev



15d. The Narrows

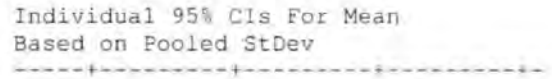
Analysis of Variance

Source	DF	SS	MS	F	P
Season	1	14760	14760	20.87	0.001
Error	11	7779	707		
Total	12	22538			

Season	N	Mean	StDev
Cold	6	7.57	5.92
Warm	7	75.16	35.60

Pooled StDev = 26.59

Individual 95% CIs For Mean Based on Pooled StDev



Appendix 16: One-way analysis of variance (ANOVA) for bacterial leucine incorporation rate between cold and warm seasons at the selected sampling locations along the Tamar estuary.

16a. Morwellham

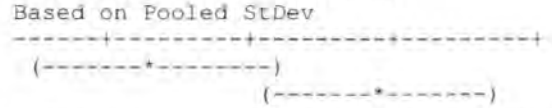
Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	19862	19862	8.96	0.012
Error	11	24375	2216		
Total	12	44237			

Level	N	Mean	StDev
Cold Sea	6	16.15	15.60
Warm Sea	7	94.56	62.13

Pooled StDev = 47.07

Individual 95% CIs For Mean Based on Pooled StDev



16b. Tuckermarsh

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	107386	107386	4.26	0.063
Error	11	277023	25184		
Total	12	384409			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Cold Sea	6	44.8	63.7	(---------*--------)
Warm Sea	7	227.1	206.8	(--------*--------)
Pooled StDev =		158.7		0 150 300

16c. Cargreen

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	125310	125310	11.02	0.007
Error	11	125060	11369		
Total	12	250371			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Cold Sea	6	17.7	5.0	(-------*-------)
Warm Sea	7	214.6	144.3	(-------*------)
Pooled StDev =		106.6		0 120 240

16d. The Narrows

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	3539	3539	15.13	0.003
Error	11	2572	234		
Total	12	6111			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Cold Sea	6	9.23	1.61	(------*-----)
Warm Sea	7	42.33	20.65	(-----*------)
Pooled StDev =		15.29		0 20 40 60

Appendix 17: One-way analysis of variance (ANOVA) for bacterial abundance between cold and warm seasons at the selected sampling locations along the Tamar estuary.

17a. Morwellham

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	1.934	1.934	15.53	0.003
Error	10	1.245	0.125		
Total	11	3.179			

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Cold Sea	5	0.2000	0.0854	(--------*--------)
Warm Sea	7	1.0143	0.4502	(------*-------)
Pooled StDev =		0.3529		0.00 0.40 0.80 1.20

17b. Tuckermarsh

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	5.545	5.545	9.80	0.011
Error	10	5.661	0.566		
Total	11	11.206			

Level	N	Mean	StDev
Cold Sea	5	0.2240	0.0688
Warm Sea	7	1.6029	0.9697

Pooled StDev = 0.7524

Individual 95% CIs For Mean Based on Pooled StDev

```
-------+---------+---------+---------
(---------*---------)
                    (-------*-------)
-------+---------+---------+---------
     0.00      0.80      1.60
```

17c. Cargreen

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	10.072	10.072	12.65	0.005
Error	10	7.961	0.796		
Total	11	18.033			

Level	N	Mean	StDev
Cold Sea	5	0.1660	0.1258
Warm Sea	7	2.0243	1.1473

Pooled StDev = 0.8922

Individual 95% CIs For Mean Based on Pooled StDev

```
--------+---------+---------+---------
 (---------*---------)
                     (------*-------)
--------+---------+---------+---------
      0.0       1.0       2.0
```

17d. The Narrows

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	1	3.205	3.205	16.19	0.002
Error	10	1.980	0.198		
Total	11	5.185			

Level	N	Mean	StDev
Cold Sea	5	0.0960	0.0956
Warm Sea	7	1.1443	0.5691

Pooled StDev = 0.4449

Individual 95% CIs For Mean Based on Pooled StDev

```
------+---------+---------+---------+
(-------*------)
                      (-----*-----)
------+---------+---------+---------+
    0.00      0.60      1.20      1.80
```

Appendix 18: One-way ANOVA: Overall seasonal variations of the measured variables between Warm/Cold Seasons in the Tamar Estuary.

18a. Rate of bacterial methyl iodide uptake (R.^{14}C) versus Season

Analysis of Variance for R.^{14}C

Source	DF	SS	MS	F	P
Season	1	55340	55340	86.70	0.000
Error	50	31913	638		
Total	51	87254			

Season	N	Mean	StDev
Warm	28	76.54	33.35
Cold	24	11.10	9.05

Pooled StDev = 25.26

Individual 95% CIs For Mean Based on Pooled StDev

```
----------+---------+---------+------
                              (---*--)
(---*----)
----------+---------+---------+------
          25        50        75
```

18b. Rate of bacterial leucine incorporation (R.^{3}H) versus Season

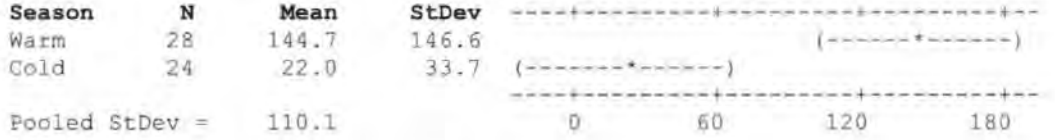
Analysis of Variance for R.^{3}H

Source	DF	SS	MS	F	P
Season	1	194529	194529	16.05	0.000
Error	50	606192	12124		
Total	51	800721			

Season	N	Mean	StDev
Warm	28	144.7	146.6
Cold	24	22.0	33.7

Pooled StDev = 110.1

Individual 95% CIs For Mean Based on Pooled StDev



18c. Bacterial Numbers (B.Nos) versus Season

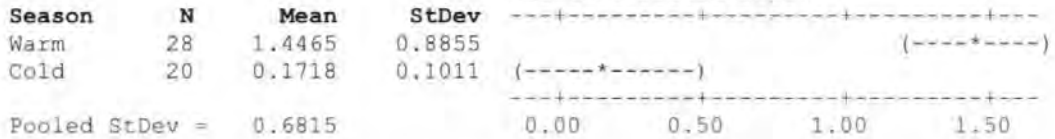
Analysis of Variance for B.Nos

Source	DF	SS	MS	F	P
Season	1	18.958	18.958	40.82	0.000
Error	46	21.363	0.464		
Total	47	40.321			

Season	N	Mean	StDev
Warm	28	1.4465	0.8855
Cold	20	0.1718	0.1011

Pooled StDev = 0.6815

Individual 95% CIs For Mean Based on Pooled StDev



18d. Methyl iodide concentration [CH_3I] versus Season

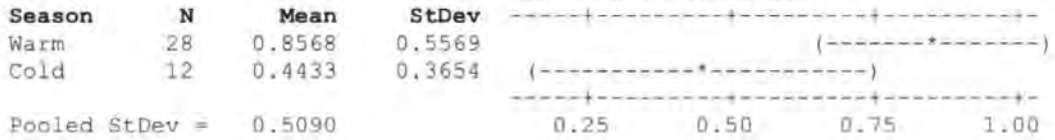
Analysis of Variance for [CH_3I]

Source	DF	SS	MS	F	P
Season	1	1.436	1.436	5.54	0.024
Error	38	9.843	0.259		
Total	39	11.279			

Season	N	Mean	StDev
Warm	28	0.8568	0.5569
Cold	12	0.4433	0.3654

Pooled StDev = 0.5090

Individual 95% CIs For Mean Based on Pooled StDev



18e. Temperature (Temp.) versus Season

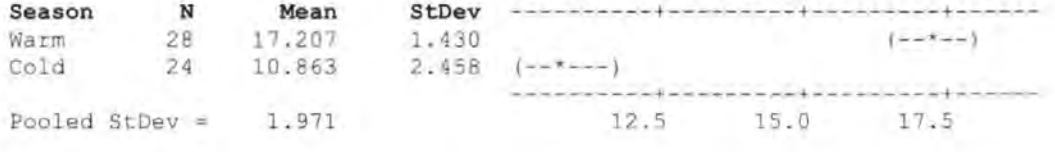
Analysis of Variance for Temp.

Source	DF	SS	MS	F	P
Season	1	520.21	520.21	133.97	0.000
Error	50	194.15	3.88		
Total	51	714.37			

Season	N	Mean	StDev
Warm	28	17.207	1.430
Cold	24	10.863	2.458

Pooled StDev = 1.971

Individual 95% CIs For Mean Based on Pooled StDev



18f. Dissolved oxygen concentration [DO] versus Season

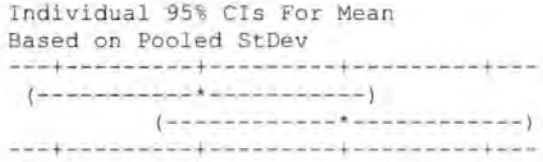
Analysis of Variance for [DO]

Source	DF	SS	MS	F	P
Season	1	4.61	4.61	1.24	0.270
Error	50	185.14	3.70		
Total	51	189.75			

Season	N	Mean	StDev
Warm	28	6.582	2.195
Cold	24	7.179	1.546

Pooled StDev = 1.924

Individual 95% CIs For Mean Based on Pooled StDev



18g. Suspended particulate matter (SPM) versus Season

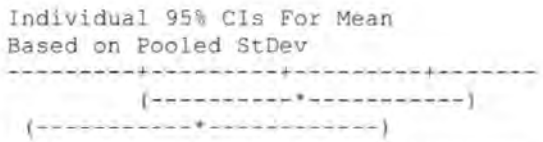
Analysis of Variance for SPM

Source	DF	SS	MS	F	P
Season	1	6242	6242	0.75	0.391
Error	50	416200	8324		
Total	51	422442			

Season	N	Mean	StDev
Warm	28	94.26	90.72
Cold	24	72.28	91.84

Pooled StDev = 91.24

Individual 95% CIs For Mean Based on Pooled StDev



18h. pH versus Season

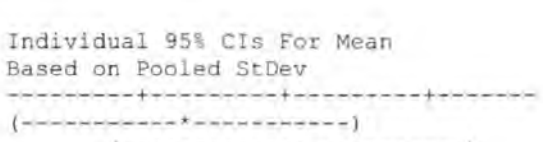
Analysis of Variance for pH

Source	DF	SS	MS	F	P
Season	1	0.109	0.109	0.80	0.375
Error	50	6.766	0.135		
Total	51	6.874			

Season	N	Mean	StDev
Warm	28	8.0750	0.3417
Cold	24	8.1667	0.3964

Pooled StDev = 0.3679

Individual 95% CIs For Mean Based on Pooled StDev



18i. Salinity versus Season

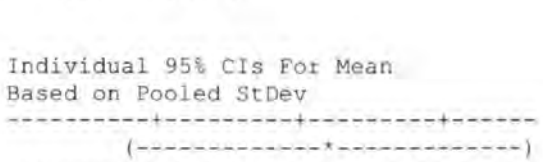
Analysis of Variance for Salinity

Source	DF	SS	MS	F	P
Season	1	94	94	0.43	0.513
Error	50	10852	217		
Total	51	10946			

Season	N	Mean	StDev
Warm	28	16.97	15.48
Cold	24	14.28	13.81

Pooled StDev = 14.73

Individual 95% CIs For Mean Based on Pooled StDev



Appendix 19: Correlation matrix of all variables for all sampling locations put together (Tamar estuary).

	Ln($R.^{14}C$)	Ln($R.^{3}H$)	B.Nos	Ln[CH_3I]	Temp.	[DO]	%DO	SPM	pH	Salinity
Ln($R.^{3}H$)	0.693									
	0.000									
B.Nos	0.564	0.769								
	0.000	**0.000**								
Ln[CH_3I]	0.216	0.202	0.380							
	0.181	**0.210**	**0.016**							
Temp.	0.749	0.743	0.670	0.254						
	0.000	**0.000**	**0.000**	**0.114**						
[DO]	-0.329	-0.363	-0.257	0.255	-0.217					
	0.017	**0.008**	**0.078**	**0.112**	**0.122**					
%DO	-0.178	-0.258	-0.127	0.303	0.013	0.932				
	0.208	**0.064**	**0.391**	**0.057**	**0.929**	**0.000**				
Ln(SPM)	0.281	0.357	0.259	-0.169	0.461	-0.166	-0.078			
	0.043	**0.009**	**0.076**	**0.298**	**0.001**	**0.239**	**0.583**			
pH	-0.099	-0.309	-0.276	-0.124	-0.268	0.289	0.222	-0.227		
	0.485	**0.026**	**0.058**	**0.446**	**0.055**	**0.038**	**0.113**	**0.105**		
Salinity	-0.031	-0.057	0.157	0.807	0.072	0.305	0.401	-0.275	0.088	
	0.828	**0.686**	**0.288**	**0.000**	**0.612**	**0.028**	**0.003**	**0.049**	**0.535**	
Wnd Speed	0.077	-0.008	-0.012	0.037	-0.243	-0.241	-0.363	-0.118	0.214	0.021
	0.586	**0.955**	**0.937**	**0.821**	**0.082**	**0.086**	**0.008**	**0.404**	**0.129**	**0.883**

Cell Contents: Pearson correlation

P-Value, P-Values at 5% significance level

Appendix 20: Correlation matrix of all variables at Morwellham (Tamar estuary).

	Ln(R.^{14}C)	Ln(R.^{3}H)	B.Nos	[CH_3I]	Temp.	[DO]	%DO	SPM	pH	Salinity
Ln(R.^{3}H)	0.812									
	0.001									
B.Nos	0.770	0.809								
	0.003	**0.001**								
[CH_3I]	0.399	0.508	0.569							
	0.254	**0.134**	**0.086**							
Temp.	0.843	0.755	0.684	0.383						
	0.000	**0.003**	**0.014**	**0.275**						
[DO]	-0.651	-0.646	-0.709	-0.489	-0.543					
	0.016	**0.017**	**0.010**	**0.151**	**0.055**					
%DO	-0.460	-0.501	-0.554	-0.400	-0.307	0.964				
	0.114	**0.081**	**0.062**	**0.253**	**0.307**	**0.000**				
SPM	0.214	0.553	0.472	0.373	0.443	-0.586	-0.553			
	0.483	**0.050**	**0.121**	**0.288**	**0.129**	**0.035**	**0.050**			
pH	-0.465	-0.421	-0.657	-0.423	-0.532	0.229	0.099	-0.265		
	0.109	**0.152**	**0.020**	**0.223**	**0.062**	**0.452**	**0.748**	**0.382**		
Salinity	0.614	0.816	0.732	0.538	0.666	-0.858	-0.789	0.728	-0.285	
	0.026	**0.001**	**0.007**	**0.109**	**0.013**	**0.000**	**0.001**	**0.005**	**0.345**	
WndSpeed	-0.104	-0.044	-0.099	0.342	-0.274	-0.285	-0.417	0.279	0.313	0.107
	0.734	**0.887**	**0.761**	**0.333**	**0.365**	**0.346**	**0.156**	**0.357**	**0.298**	**0.727**

Cell Contents: Pearson correlation

P-Value, P-Values at 5% significance level

Appendix 21: Correlation matrix of all variables at Tuckermarsh (Tamar estuary).

	Ln(R.^{14}C)	Ln(R.^{3}H)	B.Nos	[CH_3I]	Temp.	[DO]	%DO	SPM	pH	Salinity
Ln(R.^{3}H)	0.743									
	0.004									
B.Nos	0.755	0.756								
	0.005	**0.004**								
[CH_3I]	0.514	0.369	0.647							
	0.128	**0.293**	**0.043**							
Temp.	0.773	0.762	0.700	0.214						
	0.002	**0.002**	**0.011**	**0.553**						
[DO]	-0.600	-0.719	-0.794	-0.387	-0.615					
	0.030	**0.006**	**0.002**	**0.270**	**0.025**					
%DO	-0.380	-0.587	-0.641	-0.410	-0.338	0.940				
	0.201	**0.035**	**0.025**	**0.239**	**0.258**	**0.000**				
SPM	-0.205	0.112	-0.215	-0.648	0.056	0.157	0.211			
	0.502	**0.716**	**0.503**	**0.043**	**0.857**	**0.608**	**0.489**			
pH	-0.291	-0.519	-0.509	-0.384	-0.593	0.265	0.152	0.014		
	0.336	**0.069**	**0.091**	**0.274**	**0.033**	**0.381**	**0.621**	**0.964**		
Salinity	0.157	0.287	0.276	0.561	0.300	-0.169	-0.142	-0.006	-0.603	
	0.609	**0.343**	**0.385**	**0.092**	**0.320**	**0.582**	**0.644**	**0.984**	**0.029**	
WndSpeed	-0.018	-0.058	0.029	0.030	-0.271	-0.202	-0.342	-0.067	0.306	-0.048
	0.953	**0.852**	**0.929**	**0.935**	**0.370**	**0.509**	**0.253**	**0.828**	**0.309**	**0.877**

Cell Contents: Pearson correlation

P-Value, P-Values at 5% significance level

Appendix 22: Correlation matrix of all variables at Cargreen (Tamar estuary).

	Ln(R.^{14}C)	Ln(R.^{3}H)	B.Nos	[CH_3I]	Temp.	[DO]	%DO	SPM	pH	Salinity
Ln(R.^{3}H)	0.729 **0.005**									
B.Nos	0.581 **0.048**	0.747 **0.005**								
[CH_3I]	0.684 **0.029**	0.898 **0.000**	0.711 **0.021**							
Temp.	0.582 **0.037**	0.801 **0.001**	0.623 **0.030**	0.805 **0.005**						
[DO]	-0.074 **0.811**	0.162 **0.596**	-0.101 **0.756**	0.321 **0.366**	0.354 **0.236**					
%DO	0.028 **0.927**	0.296 **0.327**	0.066 **0.838**	0.368 **0.296**	0.570 **0.042**	0.897 **0.000**				
SPM	0.430 **0.142**	0.626 **0.022**	0.780 **0.003**	0.418 **0.230**	0.580 **0.038**	-0.186 **0.543**	0.016 **0.959**			
pH	0.543 **0.055**	0.601 **0.030**	0.247 **0.440**	0.406 **0.244**	0.556 **0.048**	0.220 **0.470**	0.160 **0.601**	0.276 **0.361**		
Salinity	0.635 **0.020**	0.769 **0.002**	0.657 **0.020**	0.692 **0.026**	0.614 **0.025**	-0.030 **0.921**	0.169 **0.581**	0.660 **0.014**	0.386 **0.192**	
WndSpeed	0.237 **0.435**	0.076 **0.804**	0.013 **0.968**	-0.015 **0.967**	-0.284 **0.347**	-0.347 **0.245**	-0.513 **0.073**	-0.329 **0.272**	0.144 **0.639**	0.216 **0.478**

Cell Contents: Pearson correlation

P-Value, P-Values at 5% significance level

Appendix 23: Correlation matrix of all variables at The Narrows (Tamar estuary).

	Ln(R.^{14}C)	Ln(R.^{3}H)	B.Nos	[CH_3I]	Temp.	[DO]	%DO	SPM	pH	Salinity
Ln(R.^{3}H)	0.712 **0.006**									
B.Nos	0.727 **0.007**	0.726 **0.007**								
[CH_3I]	0.649 **0.042**	0.796 **0.006**	0.645 **0.044**							
Temp.	0.897 **0.000**	0.743 **0.004**	0.797 **0.002**	0.830 **0.003**						
[DO]	0.141 **0.645**	0.347 **0.246**	0.220 **0.492**	0.513 **0.129**	0.206 **0.499**					
%DO	0.391 **0.187**	0.522 **0.067**	0.417 **0.178**	0.609 **0.061**	0.582 **0.037**	0.824 **0.001**				
SPM	0.583 **0.036**	0.560 **0.047**	0.628 **0.029**	0.489 **0.151**	0.676 **0.011**	0.208 **0.496**	0.443 **0.129**			
pH	0.666 **0.013**	0.347 **0.246**	0.172 **0.592**	0.082 **0.821**	0.472 **0.103**	0.048 **0.876**	0.191 **0.532**	0.303 **0.314**		
Salinity	0.539 **0.057**	0.476 **0.100**	0.632 **0.027**	0.585 **0.075**	0.591 **0.033**	0.034 **0.913**	0.363 **0.223**	0.517 **0.071**	0.500 **0.082**	
WndSpeed	0.171 **0.576**	-0.012 **0.970**	-0.024 **0.940**	-0.159 **0.661**	-0.132 **0.667**	-0.186 **0.543**	-0.341 **0.254**	-0.412 **0.162**	0.192 **0.529**	0.115 **0.707**

Cell Contents: Pearson correlation

P-Value, P-Values at 5% significance level

Appendix 24: Seasonal relationship of bacterial methyl iodide uptake in the low salinity (<8) region of the Tamar estuary.

24a. Best Subsets Regression: R.^{14}C versus all other variables

Response variable is Ln(R.^{14}C)

20 cases used 6 cases contain missing values.

Vars	R-Sq	R-Sq(adj)	C-p	S	Ln(3H).	Ln(Bact.)	[Mem.]	Temp	[DO]	SPM	Salinity
1	58.8	56.5	3.6	0.58958				X			
1	39.5	36.2	12.8	0.71410	X						
2	69.3	65.6	0.6	0.52391				X		X	
2	63.7	59.5	3.2	0.56893			X	X			
3	71.3	66.0	1.6	0.52150		X		X		X	
3	71.1	65.7	1.7	0.52358				X	X	X	
4	76.0	69.6	1.4	0.49296			X	X		X	X
4	74.0	67.1	2.4	0.51305				X	X	X	X
5	76.8	68.5	3.0	0.50129			X	X	X	X	X
5	76.1	67.6	3.3	0.50862	X		X	X		X	X
6	76.8	66.2	5.0	0.51999		X	X	X	X	X	X
6	76.8	66.1	5.0	0.52009	X		X	X	X	X	X
7	76.9	63.4	7.0	0.54109	X	X	X	X	X	X	X
7	76.9	63.3	7.0	0.54111		X	X	X	X	X	X
8	76.9	60.0	9.0	0.56498	X	X	X	X	X	X	X

24b. Stepwise Regression: R.^{14}C versus all other variables

Forward selection. Alpha-to-Enter: 0.05

Response is Ln(R.^{14}C) on 7 predictors, with N = 24

Step	1	2
Constant	0.2351	0.5415
Temp.	0.222	0.290
T-Value	5.86	7.11
P-Value	0.000	0.000
Ln(SPM)		0.31
T-Value		2.83
P-Value		0.010
S	0.702	0.611
R-Sq	60.93	71.69
R-Sq(adj)	59.15	69.00
C-p	13.2	6.1

24c. Regression Analysis: R.^{14}C versus Temperature & SPM

The regression equation is

Ln(R.^{14}C) = 0.542 + 0.290 Temp. + 0.308 Ln(SPM)

Predictor	Coef	SE Coef	T	P
Constant	0.5415	0.5156	1.05	0.306
Temp.	0.28982	0.04079	7.11	0.000
Ln(SPM)	0.3081	0.1090	2.83	0.010

S = 0.6112 R-Sq = 71.7% R-Sq(adj) = 69.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	19.8731	9.9366	26.60	0.000
Residual Error	21	7.8460	0.3736		
Total	23	27.7192			

Appendix 25: Seasonal relationship of bacterial methyl iodide uptake in the high salinity (>8) region of the Tamar estuary.

25a. Best Subsets Regression: R.^{14}C versus all other variables

Response is Ln(R.^{14}C)

20 cases used 6 cases contain missing values.

Vars	R-Sq	R-Sq(adj)	C-p	S	Ln(Bact.)	[Me]I	Temp.	[DO]I	Ln(SPM)	pH	Salinity
1	29.6	25.7	-0.9	0.84056		X					
1	26.7	22.7	-0.3	0.85766			X				
2	35.8	28.2	-0.2	0.82637		X					X
2	34.5	26.8	0.1	0.83471		X	X				
3	41.5	30.6	0.6	0.81276	X	X					X
3	38.6	27.1	1.2	0.83268		X	X				X
4	43.7	28.7	2.1	0.82354	X	X	X				X
4	42.7	27.4	2.3	0.83091	X	X		X			X
5	44.1	24.1	4.0	0.84950	X	X	X			X	X
5	43.9	23.9	4.0	0.85068	X	X	X	X			X
6	44.1	18.3	6.0	0.88140	X	X	X		X	X	X
6	44.1	18.3	6.0	0.88156	X	X	X	X		X	X
7	44.1	11.5	8.0	0.91738	X	X	X	X	X	X	X

25b. Stepwise Regression: R.^{14}C versus all other variables

Forward selection. Alpha-to-Enter: 0.05

Response is Ln(R.^{14}C) on 8 predictors, with N = 20
N(cases with missing observations) = 4 N(all cases) = 24

Step	1
Constant	2.145
[CH_3I]	1.28
T-Value	2.75
P-Value	0.013
S	0.841
R-Sq	29.63
R-Sq(adj)	25.73
C-p	-2.1

25c. Regression Analysis: R.^{14}C versus [CH_3I]

The regression equation is

Ln(R.^{14}C) = 2.15 + 1.28 [CH_3I]

Predictor	Coef	SE Coef	T	P
Constant	2.1451	0.5720	3.75	0.001
[CH_3I]	1.2829	0.4660	2.75	0.013

S = 0.8406 R-Sq = 29.6% R-Sq(adj) = 25.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	5.3561	5.3561	7.58	0.013
Residual Error	18	12.7177	0.7065		
Total	19	18.0738			

Appendix 26: Spatial relationship of bacterial methyl iodide uptake in the Tamar estuary during the warm (>15°C) season.

26a. Best Subsets Regression: R.^{14}C versus all other variables

Response variable is R.^{14}C

Vars	R-Sq	R-Sq(adj)	C-p	S	Ln(3H)	Bact.Nos	[Me]I	Temp.	[DOM]	Ln(SPM)	pH	Salinity
1	14.0	10.7	2.6	31.515				X				
1	6.0	2.4	5.1	32.945					X			
2	18.3	11.8	3.3	31.322		X		X				
2	18.1	11.6	3.4	31.362	X			X				
3	22.6	12.9	4.0	31.129	X			X	X			
3	21.6	11.8	4.3	31.318		X		X	X			
4	30.2	18.1	3.6	30.178	X		X	X				X
4	29.0	16.7	4.0	30.446		X	X	X				X
5	32.6	17.3	4.9	30.332	X	X	X	X				X
5	32.4	17.0	4.9	30.387		X	X	X	X			X
6	35.9	17.6	5.8	30.273	X	X	X	X	X			X
6	34.4	15.6	6.3	30.636	X		X	X	X	X		X
7	38.6	17.2	7.0	30.353	X	X	X	X	X	X		X
7	36.1	13.7	7.8	30.980	X	X	X	X	X		X	X
8	38.6	12.8	9.0	31.141	X	X	X	X	X	X	X	X

26b. Stepwise Regression: R.^{14}C versus all other variables

Forward selection. Alpha-to-Enter: 0.05

Response is R.^{14}C on 8 predictors, with N = 28

Step	1
Constant	-73.67
Temp.	8.7
T-Value	2.06
P-Value	0.050
S	31.5
R-Sq	14.01
R-Sq(adj)	10.70
C-p	2.6

26c. Regression Analysis: R.^{14}C versus Temperature

The regression equation is

R.^{14}C = - 73.7 + 8.73 Temp.

Predictor	Coef	SE Coef	T	P
Constant	-73.67	73.23	-1.01	0.324
Temp.	8.729	4.242	2.06	0.050

S = 31.51 R-Sq = 14.0% R-Sq(adj) = 10.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	4206.1	4206.1	4.24	0.050
Residual Error	26	25822.5	993.2		
Total	27	30028.6			

Appendix 27: Spatial relationship of bacterial methyl iodide uptake in the Tamar estuary during the cold (<15°C) season.

27a. Best Subsets Regression: R.^{14}C versus all other variables

Response is Ln(R.^{14}C)

Vars	R-Sq	R-Sq(adj)	C-p	S	Ln(3H)	Bact. Nos.	Temp.	Ln([DOM])	Ln(SPM)	pH	Salinity
1	44.8	41.8	7.0	0.50554		X					
1	41.9	38.7	8.2	0.51858			X				
2	65.4	61.3	0.4	0.41186		X		X			
2	55.6	50.4	4.5	0.46670	X	X					
3	69.2	63.5	0.8	0.40044	X	X		X			
3	68.0	62.0	1.3	0.40828		X	X	X			
4	69.9	61.9	2.5	0.40906	X	X		X	X		
4	69.5	61.4	2.7	0.41145	X	X		X			X
5	70.3	59.7	4.4	0.42036	X	X	X	X	X		
5	70.3	59.7	4.4	0.42046	X	X		X	X		X
6	71.0	57.6	6.1	0.43113	X	X	X	X	X		X
6	70.4	56.7	6.3	0.43576	X	X		X	X	X	X
7	71.2	54.4	8.0	0.44756	X	X	X	X	X	X	X

27b. Stepwise Regression: R.^{14}C versus all other variables

Forward selection. Alpha-to-Enter: 0.05

Response is Ln(R.^{14}C) on 7 predictors, with N = 20

Step	1	2
Constant	1.533	3.326
B.Nos	4.39	3.36
T-Value	3.82	3.40
P-Value	0.001	0.003
[DO]		-0.239
T-Value		-3.18
P-Value		0.005
S	0.506	0.412
R-Sq	44.82	65.41
R-Sq(adj)	41.76	61.34
C-p	7.0	0.4

27c. Regression Analysis: R.^{14}C versus B.Nos & [DO]

The regression equation is

Ln(R.^{14}C) = 3.33 + 3.36 B.Nos. - 0.239 [DO]

Predictor	Coef	SE Coef	T	P
Constant	3.3261	0.5934	5.60	0.000
B.Nos	3.3594	0.9891	3.40	0.003
[DO]	-0.23867	0.07503	-3.18	0.005

S = 0.4119 R-Sq = 65.4% R-Sq(adj) = 61.3%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	5.4535	2.7267	16.07	0.000
Residual Error	17	2.8837	0.1696		
Total	19	8.3372			

Appendix 28: General relationship of bacterial methyl iodide uptake in the Tamar estuary throughout the whole sampling period.

28a. Best Subsets Regression: Rate(14C) versus all other variables

Response is Ln(R.^{14}C)

Vars	R-Sq	R-Sq(adj)	C-p	S	Ln(3H)	Bact. Nos	Temp	Ln([DOM])	Ln(SPM)	pH	Salinity
1	49.4	48.3	6.2	0.81656			X				
1	47.1	46.0	8.4	0.83470	X						
2	54.9	52.9	2.7	0.77915	X		X				
2	52.3	50.2	5.3	0.80157			X	X			
3	56.9	54.0	2.7	0.77019	X		X			X	
3	55.7	52.7	3.9	0.78093			X	X		X	
4	58.6	54.8	3.0	0.76347	X		X	X		X	
4	57.3	53.3	4.3	0.77551			X	X	X	X	
5	59.5	54.7	4.1	0.76426	X		X	X	X	X	
5	58.6	53.7	5.0	0.77237	X	X	X	X		X	
6	59.6	53.7	6.0	0.77272	X	X	X	X	X	X	
6	59.6	53.7	6.1	0.77284	X		X	X	X	X	X
7	59.6	52.6	8.0	0.78195	X	X	X	X	X	X	X

28b. Stepwise Regression: R.^{14}C versus all other variables

Forward selection. Alpha-to-Enter: 0.05

Response is Ln(R.^{14}C) on 7 predictors, with N = 48

Step	1	2
Constant	0.01843	0.17191
Temp.	0.230	0.140
T-Value	6.70	2.79
P-Value	0.000	0.008
Ln(R^{3}H)		0.31
T-Value		2.35
P-Value		0.023
S	0.817	0.779
R-Sq	49.38	54.91
R-Sq(adj)	48.28	52.91
C-p	6.2	2.7

28c. Regression Analysis: R.^{14}C vs. R.^{3}H & temperature

The regression equation is

Ln(R.^{14}C) = 0.172 + 0.313 Ln(R.^{3}H) + 0.140 Temp.

Predictor	Coef	SE Coef	T	P
Constant	0.1719	0.5004	0.34	0.733
Ln(R.^{3}H)	0.3126	0.1330	2.35	0.023
Temp.	0.14021	0.05023	2.79	0.008

S = 0.7792 R-Sq = 54.9% R-Sq(adj) = 52.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	33.271	16.636	27.40	0.000
Residual Error	45	27.319	0.607		
Total	47	60.590			