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PREFACE

This ship sourced pollution study originated from a research program conducted by the South East Queensland Regional Water Quality Management Strategy (SEQRWQMS), a multi-agency community/industry/government partnership group. The first phase of the study produced a final report on ship-sourced pollution in the Brisbane River and Moreton Bay. The Moreton Bay Waterways and Catchment Partnership (or commonly referred to Healthy Waterways Partnership), as the successor organisation to the SEQRWQMS and BRMG, continued the association with the Sustainable Tourism CRC and funded further research into ship sourced pollution.

This report summarises work for the first collaborative project jointly funded by the two bodies involved in previous work. Imminent changes to sewage discharge regulations for recreational vessels operating in Queensland’s coastal waters and concerns about nutrients derived from sewage emissions fuelled demand for a study aimed at identifying sewage signatures from recreational and tourist vessels. Popular anchor sites in Moreton Bay were considered as the locations where discharges from on-board toilets were most likely to occur. The principal aim of this study was to use sterol fingerprinting of sediments as an indicator of ongoing inputs of sewage together with traditional bacterial indicator methods.

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SUMMARY

Objectives of Study
The principal objective of this research was to investigate whether (mostly untreated) sewage released from recreational vessels can be detected at popular anchor sites in southern Moreton Bay and the Gold Coast Broadwater. More specifically, the main objectives of this study were to:

- gain a better understanding of the use of selected popular anchor sites by members of the recreational boating community in southern Moreton Bay and the Gold Coast Broadwater;
- determine changes in short- and long-term sewage-related signatures associated with recreational vessels moored at these anchorages using bacteriological indicators and sterol fingerprints; and
- estimate the amount of nutrient input and health risks associated with recreational vessels at popular anchor sites.

Methodology
After initial site inspections and consultation with relevant authorities (Maritime Services Queensland and Queensland Parks and Wildlife Service (Moreton Bay Marine Park)), five anchor sites (Myora, One Mile, Horseshoe Bay, Tipplers and the Marine Stadium) and three reference sites [Banana Banks, Jumpinpin Bar (Squire Island) and Wavebreak Island] were monitored repeatedly over a year for microbial sewage indicators (faecal coliforms and \textit{E. coli}) in the water column and sterol fingerprints in sediments.

Key Findings
Results from this monitoring exercise indicated that:

- the number of recreational vessels varied considerably (up to an order of magnitude) between weekdays and long weekends or holidays, and between times of sunny and calm weather and stormy or rainy conditions;
- levels of faecal coliforms and \textit{E. coli} were generally low at anchor and reference sites and in accordance with other studies in Moreton Bay and the Broadwater;
- in regard to ratios of faecal sterol indicators, almost all sterol fingerprints obtained for sediments at anchor sites were dominated by natural assimilation processes and not by inputs of human-derived sewage;
- although both microbial sewage indicators and sterol biomarkers indicate a rapid dispersion of sewage inputs, recreational vessels contributed significantly to overall levels of microbial sewage indicators;
- based on existing national and international water quality guidelines, surface waters at popular anchorages were—for most of the year—safe for recreational activities that require primary contact with the water column (swimming and diving); and
- based on vessel counts, conservative estimates of sewage-related inputs of nitrogen and phosphorous were low for most of the year (e.g. ~ 1.2 kg d-1 N for the entire area between Tangalooma and Tipplers) but could peak to ~ 3 kg d-1 N for a site like Tipplers holding up to 180 vessels during peak holiday times.

Future Action
More research is needed to determine whether boat wake-induced sediment re-suspension in intertidal habitats can contribute to levels of microbial sewage indicators and nutrients in the water column.
Chapter 1

INTRODUCTION

Study Background

Research conducted during Stage 2 of the Moreton Bay Study (later SEQRWQMS, now the Moreton Bay Waterways and Catchments Partnership) indicated that nutrients released from sewage treatment plants (STPs) and other non-point sources were a major factor contributing to the deterioration of ecological health in (mostly western) parts of Moreton Bay (Moreton Bay Waterways and Catchment Partnership 2001). The possibility of enumerable, yet small amounts of sewage being released into Moreton Bay, associated with approximately 85,000 recreational vessels registered in south east Queensland (Murphy 2003, pers. comm.), raised concerns about additional loads associated with the use of these vessels. Per annum, the amount of nutrients released from recreational vessels likely to be used in Moreton Bay waters was estimated to be in the range of 5–15 tons of nitrogen (Warnken & Byrnes 2000). In addition, sewage from boats is considered a potential risk to human health: few vessels were, at the time of this study, equipped with on-board treatment facilities or holding tanks and most sewage generated on such vessels was likely to be released directly into surrounding waters. Where swimmers or divers use the same areas as recreational vessels and, therefore, come in contact with raw sewage released from these vessels, digestion of human pathogens and, ultimately, infection could result.

As a precautionary measure, the Queensland Government introduced amendments to its Transport Operations (Marine Pollution) Act 1995 (TOMPA) in 1998, which required all vessels with more than 10 m in overall length to be equipped with sewage holding tanks by the year 2000. A number of practical constraints and considerable opposition amongst members of the boating community prevented these provisions from being implemented. Further consultation with relevant stakeholders, government authorities and the general community prompted a second review of sewage-related provisions under TOMPA. One of the key principles for managing sewage from recreational vessels under these new provisions related to developing more streamlined criteria for discharges into coastal waters. This included definitions for nil-discharge areas and nil-discharge zones depending on their sensitivity to sewage inputs and the number of people on-board a vessel, or in other terms the likelihood of creating an impact.

In practice, the highest densities of recreational vessels with on-board toilet facilities (vessels per volume of water) can be found in purpose-built marinas. A body of research has shown that increased levels of pollutants can be detected in sediments and waters of marinas (Sawyer & Golding 1990; Gaines & Solow 1990; Fisher et al. 1987; Milliken & Lee 1990; Augier, Aubert & Guillemeau 1984, 1985). Most members of the general public are probably aware of these problems and therefore rarely use these areas for other recreational activities – particularly any activity that will lead to primary contact with the water column.

Theoretically, a much greater risk for user conflicts exists at popular anchor sites. On weekends and during holidays, these sites can attract a large number of vessels. Particularly during longer stays at an anchorage, people will use on-board facilities, including toilets. On the other hand, it is quite common for other users to jump overboard for a swim, snorkel or dive. These types of activities result in primary contact with water that could be contaminated with raw sewage released from nearby vessels (e.g. Faust 1982).

At the time of this study, no epidemiological data were available that indicated a dose/response relationship between boat numbers and higher infection risks amongst other recreational users of the same waterways in the Moreton Bay area. Similarly, little or no quantitative data was available in regard to the number of vessels or frequency of recreational uses at such anchor sites. As a result, actual sewage-related impacts associated with recreational vessels using popular anchorages were largely unknown. Initial recommendations for nil-discharge zones for the second review of TOMPA amendments were therefore based on hydrological modelling data of discharge plumes from ships (Lawson & Treloar 2000). In a second phase, results from this modelling exercise were to be complemented by a field study determining sewage-related signatures in a number of characteristic anchor and reference sites.

Traditional indicators of sewage inputs are elevated levels of standard bacterial water quality parameters. Unfortunately, though, concentrations of these indicators can be influenced by other, i.e. general environmental, factors, and are not source-specific (see discussion next section), nor are they unique to the human digestive system. Nevertheless, major national and international public health organisations have developed guidelines and threshold concentrations based on these indicators. Over time, a large body of data has been collected about these indicator organisms and, gradually, water quality guidelines have been streamlined to recommend various standardised sampling strategies for most freshwater and marine systems (e.g. APHA 1998; ANZECC & ARMCANZ 2000; NZ Ministry for the Environment 2003; WHO 2003).

A better understanding about the likely source of faecal contamination can be gained by analysing specific biomarkers found in sediments and in the water column, e.g. concentrations of specific steroid compounds, fatty acids or bile acids (Bull et al. 2002). Determining sterol fingerprints of sediments has been used with great
success to trace faecal contamination from sewage treatment works up to 20 km off the coast near Sydney (Nichols et al. 1993). Recently, a similar methodology was applied for determining the source of pollution in the water of several recreational beaches in Moreton Bay (Webb 2003). Similarly, small traces of faecal contamination have been detected along beaches in Antarctica about 150 m away from a treatment plant outlet that served a research station occupied by around 30 persons for most of the year (Green et al. 1992).

**Rationale and Study Overview**

The magnitude of impacts commonly associated with small to medium recreational vessels depends on the number of incidents and the concentration of pollutants emitted per area or volume of water, the resilience and health of the ecosystem or the flushing rate of the water body affected, and, in the case of pollutants, the concentration of neutralising agents (e.g. particulate organic matter) present (see equ. 1 and 2). In the marine environment, all these parameters constantly change depending on monthly variations in tidal prisms, wind conditions and general seasonal changes (e.g. wet vs. dry season). In other terms, long-term effects depend on the magnitude of disturbances or pollution and the time interval between these events and the recovery period between two consecutive events.

**Equation 1**

\[ \text{Magnitude of impact (disturbances)} = F(\text{incidents}, 1/\text{area}, 1/\text{resilience (or health) of ecosystem (or species)} \text{affected}, t) \]

**Equation 2**

\[ \text{Magnitude of impact (pollutants)} = F(\text{[pollutant emitted]}, 1/\text{volume}, 1/\text{flushing rate}, 1/\text{[neutralising agents]}, t) \]

In equations 1 and 2, the terms describing the number of disturbance incidents or the concentration of pollutants largely depend on the number and size of vessels using a particular area and, in regard to sewage, the number of people on-board and whether they use on-board facilities.

Based on these equations, the largest amounts of boating-related sewage emissions likely to occur outside purpose-built marinas were considered to be popular anchorages, because:

- These sites are limited, i.e. there are only a small number of locations in the Moreton Bay area that are popular amongst members of the boating community. As a result, vessels congregate at these sites from late afternoon to early morning;
- They usually provide sheltered calm waters, which encourages passengers and crew to use on-board facilities, i.e. toilets and galleys; and
- They are often protected from wind and currents and therefore likely to have lower volume exchange rates than open waters, which can lead to accumulation rather than dispersion of introduced materials.

In Moreton Bay popular anchor sites vary considerably in regard to their general environmental settings. Some are more open providing shelter against winds from a narrow range of directions. Others are more enclosed and rarely exposed to strong winds. Furthermore, some anchorages are attractive to members of the boating community for their extended sandy beaches, while other anchor sites are favoured for their surrounding mangrove habitats and their fishing opportunities. It was therefore considered necessary to investigate a range of sites ranging from high-use, open channel-like systems to semi-enclosed, U-shaped areas with a more limited exchange rate.

Secondly, larger amounts of sewage inputs from recreational vessels are emitted discontinuously, i.e. usually at times of high boating activities (e.g. holidays and weekends). In between these periods, or during periods of rough to very rough weather, most recreational vessels remain safely moored in marinas or at designated swing moorings. During these periods of unfavourable boating weather, the use of popular anchor sites, and therefore the input of boating related sewage, is likely to be minor.

Prior to this study, little was known about the number of recreational vessels using popular anchorages in Moreton Bay and the Broadwater at a given time and fluctuations between holidays, weekends and normal weekdays. This prompted a three-tiered sampling approach based on five popular anchorages and three reference sites. Each anchor site was selected to represent different types of environmental settings and different boat use patterns. Reference sites were selected to provide sediment characteristics similar to nearby anchor sites and water quality parameters typical for the area. Each site was sampled repeatedly over a whole year for:

- the number of vessels moored at these sites (by aerial survey and/or survey from a research vessel)
- short-term signals (pulses) of faecal pollution from boats using bacterial water quality indicators; and
- for longer-term signals of boating-related sewage pollution using sterol fingerprints in bottom sediments.

Using the latter two methodologies was based on the following assumptions: most small to medium recreational vessels that anchor in these areas for a lunch break, afternoon tea stopover or overnight stay have on-board toilet facilities (and are therefore likely to carry people that have to use these facilities). Most marine toilets use pumps (electric or manual) to pump out faecal material from on-board toilet facilities. During this pumping process, most faecal material will be macerated into small particles and flushed out into marine waters.
As a result of this flushing process, a large number of bacteria will be dissolved in the flushing water or remain attached to particulate matter and, after their release, remain suspended in the water column for long enough to eventually die off, depending on temperature, UV light intensity and salinity (Allen, Pasly & Pierce 1952; Faust, Aotaky & Hargadon 1975; Curtis, Mara & Sila 1992; Mezrioui et al. 1995; Burkhardt et al. 2000). Intense natural sunlight, saltwater or environments with brackish water limit the survival of most indicator organisms (including FCF, *E.coli*) to several hours or a few days (El-Sharkawi et al. 1989; Mezrioui et al. 1995; Davies et al. 1995). As such, elevated concentrations of bacteriological indicator organisms were used to indicate short-term effects associated with recreational boating activities. Unfortunately, sewage indicator bacteria can also be emitted from other warm blooded animals and, more importantly, through surface water runoff after rain or sediment re-suspension during rough weather. On the other hand, tidal flushing can increase substantially during spring tides in a shallow estuarine system. In order to account for these effects, concentrations of faecal indicator bacteria were measured against a range of other parameters including rainfall up to 72 hours prior to sampling, tide difference, water temperature, salinity, wind speed and direction, etc. The effects of these parameters together with vessel numbers counted at the time of sampling were to be determined by multiple regression analyses or multifactorial ANOVAs.

Sterols, on the other hand, are hydrophobic and therefore remain attached to faecal material or other particulate matter. More than 60% of the sterols contained in human faeces is coprostanol (5β(H)-cholestan-3β-ol) (Leeming et al. 1994) which is only present in trace amounts in faecal material from most other species (Leeming et al. 1997). Once macerated, most of the material from marine toilets will settle on the bottom sediment and, once deposited there, gradually degrade over time (depending on oxygen availability, temperature, bioturbation, composition of microbiological communities, etc.). Naturally occurring sterols, i.e. sterols in plant material (algae, mangroves, seagrasses) and in bird or animal faeces, can also be deposited, and then degraded, in marine sediments. During this degradation process, a small proportion of naturally occurring sterols will be converted to coprostanol (for discussion see Bull et al. 2002). In other terms, elevated coprostanol levels *per se* are not an indication of human sewage input, particularly in cases where inputs of other sterols are high. Grimalt et al. (1990) and Leeming and Ashbolt (1998) therefore suggest to use ratios of (coprostanol (and other sterols)): (5α-stanol (and thermodynamically stable isomers) to discriminate between different sources of sewage input. For the purpose of this study, elevated coprostanol to 5α-stanol ratios of 0.5 or higher in lower, and therefore mostly anoxic, sections of sediments were considered a strong indication of persistent inputs of sewage from human origin.

**Objectives**

The main objectives of this study were to:

- gain a better understanding of the use of selected popular anchor sites by members of the recreational boating community in Southern Moreton Bay and the Gold Coast Broadwater;
- determine changes in short-term and long-term sewage related signatures associated with recreational vessels moored at these anchorages using bacteriological indicators and sterol fingerprints; and
- estimate the amount of nutrient input and health risks associated with recreational vessels at popular anchor sites.
Chapter 2

METHODOLOGY

Sampling Strategy for Determining Bacteriological Water Quality Indicators

Indicator Organisms

In order to facilitate comparison with previous studies (Moss & Cox 1999) and a survey conducted simultaneously (Webb 2003), this study used the same microbial indicators of marine recreational water quality, i.e. faecal coliforms (FC) and E.coli, and not faecal enterococci as recommended in several recently published guidelines (APHA 1998; ANZECC & ARMCANZ 2000; NZ Ministry for the Environment 2003; WHO 2003). Another reason for choosing FC and E.coli was the fact that most of the sites under investigation were part of a large estuarine system and therefore subject to (occasionally major) freshwater inputs. Under such conditions, most guidelines recommend to use both FC or E.coli and faecal streptococci. For logistical reasons, i.e. exceeding a time of 6-8 hrs (APHA 1998) between sampling and sample processing, only one set of bacterial indicator could be analysed.

Furthermore, more uncertainty appears to exist about streptococci compared to E.coli in regard to their survival in sediments (Davies et al. 1995) and inputs of streptococci from estuarine communities such as mangroves, algae and seagrass (Anderson et al. 1997).

Sampling strategy: general overview

As mentioned previously, information about spatial and temporal variations of bacterial water quality indicators at popular anchor sites was limited. Under these circumstances, various guidelines (ANZECC & ARMCANZ 2000; NZ Ministry for the Environment 2003; WHO 2003) recommend to conduct prior testing of the sampling methodology selected for the main study. Accordingly, an initial pilot study was conducted at the Marine Stadium to gauge sampling volumes for different conditions, and to determine the accuracy of the methodology for detecting indicator bacteria.

The main study included sampling of four principal parameters:
1. Vessel numbers;
2. Concentrations of E.coli and FC in the water column;
3. Sterol fingerprints in sediments; and
4. Grain size and total organic matter to characterise bottom sediment conditions.

Each of these parameters was determined for the same locations but for different temporal scales. Vessel numbers and concentrations of bacterial indicator organisms were counted approximately every four to five weeks over 10 months. Sediments for analysis of sterol fingerprints were collected on four to five occasions over the same 10 months. Sediment samples for grain size analysis were collected only once.

Another additional experiment was conducted on two different occasions to determine concentrations of E.coli and FC in intertidal sediments at two anchor sites.

Sampling strategy for pilot study: transect sampling at the Marine Stadium, Gold Coast

Samples were collected over several months along a transect of five stations across the entrance of the Marine Stadium (see Figure 1), the southern-most anchorage in the Moreton Bay/Broadwater system and close to Australia’s biggest purpose-built tourist destination, the Gold Coast. Sampling times included winter and summer aspects and periods of high and low boating activities. Apart from one occasion, sampling times were separated by 5-6 days and, therefore, considered independent. During each sampling time surface water temperature, tide level, salinity and boat numbers were recorded. At each station along the transect, three replicate water samples were collected in sterile 250 ml glass bottles about -20 cm to -30 cm from the surface. For the three stations in the middle, three additional replicates were collected at a depth of about -1 m from the surface. Furthermore, water samples for nutrient analyses were collected in three acid washed plastic bottles across the same transect. All samples were put on ice immediately after collection.
Sampling strategy for main study: broad scale sampling of anchor and reference sites in Southern Moreton Bay and the Broadwater

Sampling for the main study focused on demonstrating differences in bacteriological water quality and other parameters at a larger scale, i.e. between five popular boat anchorages and three reference sites with little boating activity in the Moreton Bay/Broadwater system (see Figure 2). Sampling was restricted to times of favourable
boating conditions and included periods of peak and off-peak use, i.e. holiday weekends and weekdays after normal weekends.

At each site, 250ml water samples were collected at three randomly spread locations in the middle of each anchor or reference site every 4-5 weeks over several months. Each time, water temperatures were measured and noted in increments of 0.5°C. Wind velocity, precipitation 72 hours prior to sampling and the average differences between high and low tides for 24 hours prior to sampling were obtained from the Australian Bureau of Meteorology and the Australian Tide Tables for the nearest reference station, the Gold Coast Seaway and the Brisbane River Bar. Tide differences were considered important as they can vary between 0.1 m and > 2 m for neap and spring tides, respectively. With a depth of only 3-4 m at most anchor sites such variation in differences between tide and low tide was regarded to have a considerable effect on flushing rates, and therefore dilution of bacteria concentrations.

Sediment samples for determining sterol fingerprints were collected on four or five occasions when sampling bacterial water quality. Large sediment cores for grain size analysis and sediments from the intertidal area were collected during an extra field trip.

Sample Sites

Sample sites for the main study were selected based on three criteria:

1. being located distant to, or isolated from, other known or potential point sources of raw or treated sewage (as much as this was possible given the spatial constraints particularly in the Broadwater);
2. being a site popular with recreational users (based on information obtained with relevant staff from Queensland Maritime Safety and boating enthusiasts); and
3. having different site and use characteristics.

Reference sites were chosen to reflect ambient water quality in the study area, i.e. with practically no use as overnight anchor sites.

Accordingly, the study sites chosen included Myora Anchorage and 1 Mile Anchorage (or Little Ships Club Anchorage) on the western shores of North Stradbroke Island, Horseshoe Bay on Peel Island, Tipplers Passage near Tipplers Resort on South Stradbroke Island, and the Marine Stadium north of Sea World Nara Resort on the Gold Coast Spit (see Figure 2). Reference sites included the northern tip of Banana Banks in southern Moreton Bay, the northern channel west of Squire Island near the southern entrance of Canaipa Passage (near Jumpinpin Bar), and the lower slopes of a sand bank near the southern passage around Wavebreak Island on the Gold Coast.

More detailed information about the environmental settings of each sample site is provided in Chapter 3.
Enumeration of Bacteria in Water and Resuspended Sediment Samples

Depending on boat numbers at each sampling site, a range of sub-sample aliquots from each of triplicate sample bottle from each site was tested for accurate dilution factors. This became easier to predict over time with the exception of stormy weather periods.

Typical volumes used for filtration at each site were as follows:
Stage 2 sewage pollution

- Tipplers, Marine Stadium and One Mile Beach
  - High boating periods- triplicates of 50, 75, and 100 ml
  - Low boating periods- triplicates of 100 and 150 ml
- Reference sites, Squire Island (near Jumpinpin Bar) and Wavebreak Island-
  - High boating periods- triplicates of 100 and 150 ml
  - Low boating periods- triplicates of 250 ml

Autoclavable plastic filtration units were used to extract bacteria from water samples onto sterile nitrocellulose membrane filters (Millipore S-pak type HA, pre size of 0.45 µm, 45 mm in diameter). Previously prepared agar (10% w/v) with membrane lauryl sulphate broth (MLSB, Oxoid) and 100mg L-1 MUG (4-methylumbelliferyl-ß-D-glucuronide) supplement (Oxoid BR071E) was poured into 55 mm gamma irradiated Sarstard petri dishes. These were kept at 4°C in a fridge until use.

Sample collection, preservation and membrane filtration followed standard procedures (9060, 9222) as recommended by the American Public Health Association (APHA 1998). Sample bottles were vigorously shaken before a required volume of sample water was measured and poured into the filtration unit. After filtration, filters were carefully removed and placed on selective growth media. All plates were pre-incubated at 28°C ± 1°C for 4 hours, then placed at 44 ± 1°C for a further 14 hours. Yellow colony forming units (CFUs) were reported as presumptive faecal coliforms in accordance with Apte, Davies and Peterson (1995). Red CFUs were considered as non- *E.coli* non-FC thermotolerant bacteria. Both yellow and red colonies were initially counted to calculate relevant concentrations and to allow comparison to ANZECC/ARMCANZ and New Zealand water quality guidelines for marine and freshwater.

Presumptive *E.coli* colonies were detected based on their fluorescence under long wave UV light. After removing agar plates from the incubator, plates were placed at 4 oC for 1 to 1.5 hours. Such treatment intensifies fluorescence of colonies with β-galactosidase activity. Plates were then examined under a handheld UV light and target colonies exhibiting blue-white fluorescence or a fluorescent halo were counted as presumptive *E.coli* colonies. The proportion of false MUG-positive and false MUG-negative colonies for this fluorogenic essay were identified previously (Pratt 2000) and found to be 2% and 1%, respectively.

Sediment Analysis for Faecal Coliforms

Small amounts of sediment (50-100 g) were scooped up at low tide in duplicates using sterilised 250 ml glass bottles along 4 stations of a transect line running perpendicular to the high water mark. This procedure was repeated for 2 sites (Myora and the Marine Stadium) along 4 randomly located transects for each site. Approximately 20 g wet weight were used from each glass bottle and resuspended in 100 ml sterile salt solution (3.7g NaCl/100ml sterile milliQ water) to avoid osmotic stress. The sediment-water suspension was mildly sonicated for five minutes, then allowed to settle for a few minutes (varied, depending on site) to remove excessive sediment materials from the supernatant. Finally, 10 ml and 20 ml of supernatant were diluted with sterile salt water to a final volume of 50 ml and then processed as for water samples described above. Results are presented as number of CFU per gram wet weight of sediment.

Sediment Samples for Sterol Fingerprints

Sediment cores were collected in triplicates by diver-operated, modified 60 ml plastic syringes in order to avoid disturbance of the surface sediment. Each corer was immediately sealed from the bottom using an extra plunger (see Figure 3). Samples were taken from sediments near vessels at anchor or close to depth markers at reference sites. Corers were placed away from animal burrows or very dense patches of seagrass or algae. Each syringe was immediately sealed and returned to the surface. Once on-board, triplicate samples were sloughed into plastic vials for three depth segments [0 cm to -3 cm (top), -3 cm to -6 cm (middle), and -6 cm to -9 cm (bottom)], pooled and immediately stored on ice. All samples were kept frozen until further analysis.
Lipid extraction, saponification and derivitisation of neutral lipids

Sediments were extracted quantitatively by a modified version of the Bligh and Dyer one-phase CHCL₃/MeOH/H₂O (1:2:0.8 v/v/v) method (Bligh & Dyer 1959). Approximately 30 g of sediment was scooped into a 500 ml glass separatory funnel (with a teflon stopcock) and the solvents added, 30 ml water, 75 ml methanol, 37.5 ml chloroform. In addition, each funnel was spiked with 10 µg of internal standard, 5α-cholestane. The recoveries of internal standard were calculated with each analysis to assess extraction efficiency and to track losses. A complete procedural blank was run with every batch of sediment extracts.

After phase separation, the lower solvent lipid layer was removed and transferred into glass screw cap tubes for saponification. After the sample was reduced to dryness by nitrogen, 2 ml of a 10% NaOH mixture in methanol: water (80:20) was added and the samples heated for two hours at 80°C.

Then added was 3 ml Milli-Q water and 2 ml 4:1 Hexane: chloroform, followed by physical shaking and centrifugation. The products were thus extracted into the upper solvent layer. This was repeated three times to ensure complete extraction efficiency.

The samples were then transferred to vials, reduced to dryness by N₂ and derivitised with bis(trimethylsilyl) trifluoroacetamide (BSTFA, 100ul, 60°C, 24hr). The samples were then made up to 300-500 µl for gas chromatography (GC) analysis.

Dry weight determination

Sub-samples of each sediment were used for dry weight determination. The weights of the wet sediment and aluminium pan were recorded and these were placed into an oven at 105°C and dried overnight. The sample was allowed to cool the following day and the weight of the sample was recorded. The percent solids for each sediment sample was then calculated.

Total organic matter

Sediments were sub-sampled for total organic matter. Sediments were dried and powdered using a mortar and pestle, and carbonates were removed by acid treatment. Samples were then placed into a 5000°C muffle furnace for 24 hours after which loss on ignition was calculated.
Gas chromatography
Routinely, two types of quantitative gas chromatographic analyses were performed:

1. Gas Chromatography with Flame Ionisation Detection (GC-FID)
   A Varian 3400 GC equipped with a 50 m x 0.32 i.d. cross-linked methyl silicone (0.25 µm film thickness) fused-silica capillary column (Varian cp-sil 8-CB) was used for GC-FID analysis, using Helium as the carrier gas. Operating conditions were as follows; head pressure 70kPa, total flow 35 ml/min, purge flow 1 ml/min, injector temperature 290°C, detector temperature 310°C. Temperature program; initial oven temperature 50°C, held for 1 minute then 25°C/min to 180°C then 2°C/min to 280°C then 10°C/min to 310°C, held for 15 minutes. One-microlitre injections were standard. Peak areas were quantified using Star Chromatography Software (Varian Australia). The internal standard 5α-cholestane was used to check extraction efficiency which was typically between 70% and 100 %. The response factor of selected sterols was calculated in relation to 5α-cholestane over a series of concentrations. Peak identification was based on comparison of retention time data with that obtained for authentic and laboratory standards and GC mass spectral data.

2. Gas Chromatography with Mass Spectrometry (GC-MS)
   Mass spectral data were acquired on a Varian 3800 GC coupled to a Saturn 2000 mass spectral detector. GC-MS operating conditions and temperature programs were the same as those for GC-FID. The mass-spectral detector operating conditions were: electron multiplier ~2000V; transfer line 1700°C, 0.7 scans/sec; mass range 100-550amu; solvent delay 5min; EI-auto. The column was a Varian cp-sil 8-CB low bleed/MS.

Sediment Characterisation
General sediment samples were collected for each site to characterise bottom substrates for grain size distribution, total organic matter and total heavy metal concentrations. Each time, three replicate samples were retrieved similarly to those for sterol analysis using a corer from the boat instead of diver-operated syringes. For each sample, grain size fractions were determined for seven fractions (> 2000, 2000-1000, 1000-500, 500-250, 250-125, 125-63, and > 63 µm) in triplicates. Total organic matter was determined as for sterol sediment samples.

Boat Counts
Each time a sampling or reference site was visited, vessels at anchor, intending to anchor or in the process of anchoring up were counted within limits set by distinctive landmarks (e.g. channel markers, land marks). Open tinnies and tenders to larger vessels were not included during such counts. All persons on-board the research vessel were asked to count vessels, and the average number to the nearest five was used (e.g. 0, 5, 10, 15, ...). This rounding scheme acknowledged that the vessels counted during the limited time that researchers collected samples was only an indication of a general level of activity.

The overall number of recreational vessels using anchor sites in eastern parts of Moreton Bay during long weekends and holidays were determined by aerial survey. Vessels were photographed from a light aircraft and later counted by staff of MSQ.

Statistical Analysis
All statistical analyses were performed on SPSS v.10. Following recommendations contained in the ANZECC guidelines for Fresh and Marine Water Quality, central tendencies for bacterial counts were presented as medians and log 10 transformed for further analysis.
Chapter 3

RESULTS

Characterisation of Anchor and Reference Sites

All sites investigated in this study differed markedly in regard to their environmental settings and the way in which they were used by members of the recreational boating community. The following provides a detailed description for each site used for this study.

Myora

This anchor site is created by a rocky outcrop on the eastern site of Rainbow Channel, which forms a north-pointing inlet with mangroves and mudflats along the shoreline of North Stradbroke Island. Oyster leases cover most of the mudflats around the inlet. The outcrop and the mudflats are only exposed during low tide. During high tide, water from the South Passage between Moreton Island and North Stradbroke Island can move over the outcrop and into the anchorage with an average depth of around three to four metres.

The bottom sediments are mostly sand with some organic matter and, occasionally, parts of mollusc shell debris. Dense seagrass beds, interrupted by a few bare patches in between, cover the more shallow parts towards the fringing mudflats and mangroves. Discarded beer bottles, aluminium cans and bottle caps were found frequently during dives.

The number of vessels observed at this anchorage was generally low, i.e. rarely exceeding 10 vessels. Only during weekends or holiday periods with moderate to fresh south-easterly winds, vessel numbers increased to more than 30 (see Table 1).

1 Mile

Also referred to as ‘Little Ships Club Anchorage’, this anchorage also forms a north facing inlet off the main access channel to the Volunteer Marine Rescue Station on North Stradbroke Island. Several permanent swing moorings were provided, which were also used by a few commercial fishing trawlers. Overall, the anchorage is quite shallow, particularly in its northern part where some vessels fall dry at low tide. The bottom substrate consists mostly of sand with an organic matter content higher than Myora. Solid wastes (mostly beer bottles and aluminium cans) were frequently scattered between swing moorings. Most of the bottom substrate was covered by a dense layer of Caulerpa taxifolia and various seagrass species.

During most of the sampling times, approximately 25 to 30 vessels were found using swing moorings around the anchor site. At least two or three vessels were used as liveaboards. More than 50 vessels were observed during rough weather associated with fresh south-easterly winds when taking shelter at 1 Mile, one of the most protective anchorage in eastern parts of the Bay (see Table 1).

Horseshoe Bay

This anchorage is famous for its wide, sickle-shaped sandy beaches. These south facing beaches are exposed to wind swells generated by southerly or south-easterly winds (the prevailing wind direction in this region) blowing across the southern part of Moreton Bay. Overnight anchoring is only recommended in north-westerly to north-easterly winds. This anchorage is therefore predominantly used as a day-time or stopover anchorage. During favourable conditions, i.e. northerly winds or generally calm weather, more than 150 vessels were observed during holidays or long weekends. On other days, it was not uncommon to find this anchorage deserted. In other terms, this anchorage is not heavily used most of the times, but can be visited by a very large number of vessels for several hours when weather conditions are right (see Table 1).

The bottom substrate contains more coarse sand and mollusc shell debris than any other site investigated in this study. Patchy seagrass areas were common at a depth between one and three to four meters. The organic content was lower than for the other two anchor sites in Moreton Bay.

Tipplers

Overall, Tipplers was probably the most frequently visited anchorage in the whole Moreton Bay and Broadwater region. Every day, a number of tourist vessels carry visitors from the Gold Coast to Tipplers Island Resort. To
the south of the resort, a number of recreational boating clubs have established facilities (camping, showers, toilets, etc.) for their members and their guests. The anchorage itself is basically a narrow channel running in a north-east to south-west direction between the Never Fail Islands and South Stradbroke Island. As such, the area provides protection against winds from most directions. Even during weekdays, about 15 to 30 vessels can be regularly observed anchoring at Tipplers. This spot is also very popular with holidaymakers that hired or own a houseboat.

Mangrove vegetation covers most of the Never Fail Islands while casuarinas and other coastal woodland species grow along the beaches of South Stradbroke Island. Immediately to the north of Tipplers, an oyster lease separates this anchorage from Dux Anchorage.

The bottom substrate at Tipplers revealed the highest organic matter content of all sites investigated in this study. A thin layer of fine organic material covered a layer of sandy substrate bare of any algae or seagrass. At the average depth of about three meters, the visibility at this site was usually less than one metre, which probably prevented growth of any larger marine flora.

**Marine Stadium**

This anchorage was constructed, as was Wave Break Island, at the same time as the Gold Coast Seaway (see Figure 4). The shape of this inlet is an elongated ‘U’ with a 280 m wide opening facing south. In 1999, part of the middle section of the inlet was dredged as part of the Gold Coast beach replenishing program. The shores are mostly sandy beaches aligned with casuarinas and other foredune species planted as part of the EPA’s beach erosion protection program.

The bottom substrate at this location is mostly sand or coarse sand with little organic matter. A few pockets of seagrass exist, but most of the sediment is bare of any marine flora.

In some parts, the Marine Stadium is quite shallow. The middle section has an average depth of two to three metres and provides protection against wind from all directions. Even during normal weekdays, 10 or more vessels were regularly observed at this anchorage. The highest numbers of vessels, i.e. more than 70, were counted during the Christmas and Easter Holidays. Such densities could only be achieved by mooring vessels in several rafts of one to four vessels alongside a larger vessel.

**Banana Banks (Moreton Bay South)**

This site was located between the northern tip of Banana Banks and another shallow area to the east. This location was chosen as a reference site for a number of reasons:

- it was considered distant enough from land on the western side of the Bay to eliminate any direct effects from land-based sources;
- the depth in this area was similar to those noted for anchor sites; and
- the bottom substrate was sand covered by a mix of algae and seagrass – again similar to what was observed at popular anchor sites.

The only recreational vessels observed at this site on one or two occasions were small open aluminium craft with recreational fishermen aboard.

**Squire Island (Jumpinpin Bar)**

A second reference site was considered necessary to determine general background levels of trace metals at the node between Moreton Bay and the Broadwater, i.e. near Jumpinpin Bar. The overall water quality in this area was considered to be the result of clean water coming across the bar during the incoming tide, and water moving out of the southern parts of Canaipa Passage and along the northern tip of South Stradbroke Island during the outgoing tide.

The only site in this area that was not regularly used as an anchor site or a fishing spot was a sand bank east of Squire Island. Occasionally, small trailerable craft were observed in and around the area (see Table 9). The bottom substrate was again mostly sand with some patches of algae and seagrass.

**Wavebreak Island**

The third and last reference site was located at a sandbank south of Wavebreak Island near the Gold Coast Seaway. This location was chosen to represent the mixing of water coming down the Nerang River and past the Marina Mirage and SeaWorld complex during the outgoing tide, and clean ocean water moving in through the seaway during the incoming tide. Treated effluent discharged from the diffuser pipes of the Gold Coast sewage discharge scheme was not regarded as having a major effect on bacterial or sterol concentrations because (a) effluent was only released with the outgoing tide, (b) most bacteria were killed during chlorine sterilisation at the
treatment plant, and (c) most sterols were degraded and/or settled into the sludge during the secondary phase of the effluent treatment process.

The bottom substrate was mostly coarse sand with little organic matter and no seagrass or algae cover. Closer inspections on scuba revealed that strong currents in that location constantly moved parts of the top layer of the bottom substrate. The only recreational vessels observed in this area were small tinnies used by recreational fishermen to collect yabbies from the nearby sandbank.

Table 1: Characterisation of anchor and reference sites

<table>
<thead>
<tr>
<th>Name</th>
<th>Area [ha]</th>
<th>Depth [m]</th>
<th>Entrance width [m]</th>
<th>Bottom substrate</th>
<th>Boat use patterns</th>
<th>Frequency of high boat nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myora</td>
<td>~ 30</td>
<td>4-6</td>
<td>~ 150 (1100*)</td>
<td>Sand w/ some mud, seagrass</td>
<td>protected in SE-winds, 0-10 boats weekdays, &lt; 30 weekends</td>
<td>low</td>
</tr>
<tr>
<td>1 Mile</td>
<td>~ 25</td>
<td>1.5-2</td>
<td>~ 50 (900*)</td>
<td>Sand w/ some mud, dense seagrass and algae</td>
<td>protected in SE-winds, 20-30 boats weekdays (permanent swing moorings), ~ 40 (30+) weekends</td>
<td>common</td>
</tr>
<tr>
<td>Horse-shoe Bay</td>
<td>~ 85</td>
<td>1-6</td>
<td>~ 2200</td>
<td>Mostly coarse sand, seagrass, algae</td>
<td>protected in NE-NW winds, 0-20 boats weekdays, &lt; 80 (150+) weekends</td>
<td>very low (only few days w/ NE-NW winds)</td>
</tr>
<tr>
<td>Tipplers</td>
<td>~ 20</td>
<td>1-5</td>
<td>2 × 120 (400*)</td>
<td>Fine sand and organic matter</td>
<td>protected in E, SE, E–winds, 15-30 boats weekdays, &lt; 120 (160+) weekends</td>
<td>common</td>
</tr>
<tr>
<td>Marine Stadium</td>
<td>~ 25</td>
<td>1-4</td>
<td>~ 280</td>
<td>Mostly coarse sand</td>
<td>protected in all conditions, 5-10 boats weekdays, &lt; 50 (70) weekends</td>
<td>common</td>
</tr>
<tr>
<td>Moreton Bay South</td>
<td>n.a.</td>
<td>2-3</td>
<td>n.a.</td>
<td>Mostly sand, seagrass</td>
<td>occasionally used by 1-2 recreational fishermen</td>
<td>never</td>
</tr>
<tr>
<td>Canaipa Passage South</td>
<td>n.a.</td>
<td>1-2</td>
<td>n.a.</td>
<td>Sand w/some mud, sparse seagrass</td>
<td>occasionally used by 1-5 recreational fishermen</td>
<td>extremely rare</td>
</tr>
<tr>
<td>Wave-break Island</td>
<td>n.a.</td>
<td>2-3</td>
<td>n.a.</td>
<td>Bare sand (coarse)</td>
<td>occasionally boat anchored in nearby channel</td>
<td>rare</td>
</tr>
</tbody>
</table>

Note: * = entrance width at high tide

Overall Vessel Numbers in Eastern Parts of Moreton Bay at Different Times of the Year

The number of vessels counted during aerial surveys between Tipplers and Amity Point varied substantially, from 96 vessels on a weekday afternoon to 657 vessels between 10 am and 11:30 am on Easter Sunday (Table 2). Not all of these vessels would anchor overnight at the location where they were spotted: counts between 9 am and 5 pm are likely to include day trippers or vessels, which may still move to another anchorage for the night. Overall, however, data from aerial surveys supported observations made during sampling of bacterial water quality indicators for the main study. Anchor sites in the Moreton Bay/Broadwater area can be classified into four principal categories:

1. Minor importance, usually 1 to 2 vessels and up to 20(30) vessels during holidays (Amity, Deanbilla Bay, Slipping Sands, etc.);
2. Commonly used, usually 10 to 30 vessels and up to 40 (50+) vessels during holidays (Myora, 1 Mile, Marine Stadium, etc.);
3. Popular but infrequent use, usually 0 to 5 vessels but up to 120 (180) vessels during holidays and favourable weather conditions (Horseshoe Bay);
4. Popular but frequent use, usually 20 to 40 vessels but up to 120 (160) vessels during holidays (Tipplers).

Table 2: Aerial vessel counts conducted by staff of Maritime Safety Qld, April 2002 – April 2003

<table>
<thead>
<tr>
<th>Date (time)</th>
<th>Use period</th>
<th>Southern Moreton Bay (Amity Point – Tipplers)</th>
<th>Western site of Moreton Island</th>
<th>Weather</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Apr 2002 (3:30-4:30pm)</td>
<td>1st weekday after Easter holidays</td>
<td>65</td>
<td>31</td>
<td>Cloudy, SE 10-15kn</td>
</tr>
<tr>
<td>6 May 2002 (8:45-10:15am)</td>
<td>Labour Day long weekend</td>
<td>216</td>
<td>39</td>
<td>Cloudy, SE 15kn</td>
</tr>
<tr>
<td>10 Jun 2002 (10:30-12:00noon)</td>
<td>Queen’s Birthday long weekend</td>
<td>314</td>
<td>85</td>
<td>No rain, W-NW 10kn</td>
</tr>
<tr>
<td>12 Oct 2002 (4:00-5:30 pm)</td>
<td>1st weekend after school holidays</td>
<td>240</td>
<td>26</td>
<td>No rain, S-SE-SSE 15-20kn</td>
</tr>
<tr>
<td>2 Jan 2003 (3:20-4:50 pm)</td>
<td>Summer holidays</td>
<td>416</td>
<td>154</td>
<td>Isolated showers, NE-NW 10-15kn</td>
</tr>
<tr>
<td>19 Apr 2003 (10:00-11:00 am)</td>
<td>Easter Saturday</td>
<td>598</td>
<td>59</td>
<td>No rain, S-SE 10-15kn</td>
</tr>
</tbody>
</table>

The use of popular anchor sites was further investigated by Schleich (2001). This study included, amongst others, two of the sites investigated here: Tipplers and the Marine Stadium. Vessels were counted at these two sites (and Runaway Bay Marina) every three to four days, or even daily during the Easter Holiday weekend (see Figure 4). Again, the data collected by Schleich demonstrated a general increase of vessels during weekends at both anchor sites, and a massive, sudden increase during the Easter Holiday weekend at Tipplers. Such dramatic changes in boat numbers were not observed for the Marine Stadium.

Overall, the various data presented in this section confirmed that on weekdays, the number of recreational vessels at popular anchor sites in the Moreton Bay/Broadwater system is low. Only during weekends, particularly long weekends or during holidays, the number of vessels out at anchor sites double or for sites like Tipplers or Horseshoe Bay, increase more than ten-fold.
Pilot Study: *E. coli* concentrations during intensive sampling at Marine Stadium during winter and summer periods

Transect data collected for the Marine Stadium in 2000 and 2001 revealed that recreational vessels can contribute significantly to concentrations of faecal bacterial indicator organisms when including data from the entire sampling period (see Figure 5). Closer statistical analysis (multiple regression, see Table 3) revealed that not only boats, but also temperature and salinity had an effect on bacterial counts at an $\alpha$ level of 0.10. The highest levels of 260 presumptive *E. coli*/100 ml were collected on 28 December when high boat numbers coincided with a short, but heavy rain event. Furthermore, higher boat numbers were only observed for an extensive sampling over the summer school holidays (see Figure 5). Analyses of this sub sample for the summer period revealed no significant influence of recreational vessels on the level of *E. coli* concentrations at the Marine Stadium – irrespective of excluding or including the point of extreme leverage on 28 December.
### Table 3: Multiple Regression Analysis of presumptive E. coli concentrations at Marine Stadium (all data)

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Unstandardised Coefficients</th>
<th>Std. Error</th>
<th>Standardised Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>B</td>
<td>1434.969</td>
<td>506.681</td>
<td>2.832</td>
<td>.011</td>
</tr>
<tr>
<td>(Constant)</td>
<td>Std. Error</td>
<td>506.681</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of vessels</td>
<td>3.749</td>
<td>.865</td>
<td>1.054</td>
<td>4.334</td>
<td>.000</td>
</tr>
<tr>
<td>Water temp.</td>
<td>-7.488</td>
<td>4.591</td>
<td>-.427</td>
<td>-1.631</td>
<td>.120</td>
</tr>
<tr>
<td>Salinity (EC)</td>
<td>-39.020</td>
<td>15.873</td>
<td>-.356</td>
<td>-2.458</td>
<td>.024</td>
</tr>
<tr>
<td>Rain 72h prior</td>
<td>4.677</td>
<td>3.370</td>
<td>.179</td>
<td>1.388</td>
<td>.182</td>
</tr>
<tr>
<td>Tide difference</td>
<td>11.157</td>
<td>23.782</td>
<td>.062</td>
<td>.469</td>
<td>.645</td>
</tr>
</tbody>
</table>

a Dependent Variable: MEDIAN of presumptive median E. coli CFU

It is however worth noting that bacterial indicator levels exceeded ANZECC/ ARMCANZ (2000) guidelines (<150 FCF organisms/100 ml) recommended for primary contact at least once, and that they came up to two-thirds of this guideline value almost twice during the one month of sampling in summer.

**Faecal Coliform and E. coli Concentration at Popular Anchor Sites in Southern Moreton Bay and the Gold Coast Broadwater**

In general, faecal coliform and E. coli levels at anchor and reference sites in southern Moreton Bay and the Broadwater were quite low. The point furthest from any dry land, the reference site near the Banana Banks, revealed the lowest bacteria levels all year round, i.e. usually zero for sample volumes of up to 500ml (Figure 6). In contrast, the highest levels were observed at 1 Mile Anchorage during the first sampling event in 2001 when most agar dishes were overgrown and only those with lower sample volumes revealed enumerable counts. This, however, occurred only once. For the remainder of the sampling period, levels of presumptive faecal coliforms or presumptive E. coli never exceeded 80 CFU/100 ml, even with more than 100 vessels present at Tipplers Passage.

![Figure 6: Average ± 1 s.e. of median faecal coliform (clear bars) and presumptive E. coli (dotted bars) concentrations at five anchor sites (white bars) and three reference sites (grey bars) in 2001/02](image-url)
**Presumptive faecal coliforms (FC)**

Further statistical analysis of bacterial counts was based on the assumption that time periods of 2-4 weeks between each sampling event were considered sufficient to treat each event as an independent measure. Data for presumptive faecal coliform levels in the Moreton Bay and Broadwater area were severely negatively skewed. Subsequent analyses were therefore restricted to log10 transformed data.

As indicated by Figure 6, faecal coliform levels at anchor sites, i.e. sites subject to high boat use intensities, appeared generally higher than those measured at reference sites. A more detailed ANOVA correcting for water temperature, tide difference and wind speed supported this observation (see Table 4).

### Table 4: Multifactorial ANOVA for FCF concentrations in Moreton Bay

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>9.607</td>
<td>4</td>
<td>2.402</td>
<td>9.920</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>.130</td>
<td>1</td>
<td>.130</td>
<td>.538</td>
<td>.466</td>
</tr>
<tr>
<td>Water temperature</td>
<td>6.330</td>
<td>1</td>
<td>6.330</td>
<td>26.142</td>
<td>.000</td>
</tr>
<tr>
<td>Tide difference</td>
<td>3.952</td>
<td>1</td>
<td>3.952</td>
<td>16.324</td>
<td>.000</td>
</tr>
<tr>
<td>Wind speed</td>
<td>1.811</td>
<td>1</td>
<td>1.811</td>
<td>7.480</td>
<td>.008</td>
</tr>
<tr>
<td>Reference vs. anchor sites</td>
<td>2.645</td>
<td>1</td>
<td>2.645</td>
<td>10.926</td>
<td>.001</td>
</tr>
<tr>
<td>Error</td>
<td>17.190</td>
<td>71</td>
<td>.242</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54.694</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>26.797</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a  R Squared = .359 (Adjusted R Squared = .322)

A visual comparison between vessel numbers and median FC counts showed no clear trend between these two parameters (Figure 7), yet a bivariate correlation analysis between vessel numbers and median FCF counts (transformed and non transformed) indicated a strong correlation with \( p < 0.001 \).

![Figure 7: Median faecal coliform concentrations at boat anchorages in Moreton Bay and the Gold Coast Broadwater](image)

In a second step, a multiple regression including water temperature, tide difference, boat numbers and wind speed as independent variables clearly demonstrated that boat numbers had a significant effect on the level of faecal coliforms measured at anchorages in Moreton Bay and the Broadwater (see Table 5). This result essentially remained the same even after removing all sample points with zero FC counts. Water temperature, on the other hand, also showed a strong influence on bacterial levels in the water column. The regression coefficient...
for this variable was positive, which indicated that the number of faecal coliform bacteria increased with increasing water temperatures.

**Table 5: Multiple regression analysis of level of faecal coliforms measured at anchorages in Moreton Bay and the Broadwater**

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.648</td>
<td>.420</td>
<td>.387</td>
<td>.468074</td>
</tr>
</tbody>
</table>

Coefficients

a Predictors: (Constant), wind speed, vessel numbers, tide difference, water temperature

This result is somewhat contrary to the traditional view that higher temperatures accelerate the die-off of faecal indicator bacteria (e.g. El-Sharkawi et al. 1989; Craig, Fallowfield & Comar 2001). On the other hand, results by Burkhardt et al. (2000) suggest that warmer summer waters increase bacterial metabolism and therefore resistance to UV irradiation. In this regard, results from this study certainly warrant further investigation.

**Presumptive E.coli**

Data for presumptive *E.coli* were dominated by zero counts, i.e. occasions when no fluorescent colony forming units could be detected. In response, data were analysed at two levels: a) for presence or absence of presumptive *E.coli* during occasions when recreational vessels were present (= anchored) or absent, and b) for a relationship between the numbers of vessels and concentrations of *E.coli* at times when *E.coli* counts were greater than zero (see Tables 6 & 7).

**Table 6: Presence/Absence of *E.coli* counts and recreational vessels**

<table>
<thead>
<tr>
<th>0 vessels</th>
<th>&gt; 0 vessels</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 <em>E.coli</em>/100ml</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>&gt; 0 <em>E.coli</em>/100ml</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>29</strong></td>
<td><strong>47</strong></td>
</tr>
</tbody>
</table>

**Table 7: Multiple regression analysis of *E.coli* concentration at anchor and reference sites when counts were greater than zero (n= 35)**

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
<th>Std. Error of the Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.573</td>
<td>.328</td>
<td>.263</td>
<td>.429691</td>
</tr>
</tbody>
</table>

Coefficients

a Predictors: (Constant), 72h rain, number of vessels, wind speed

a Dependent Variable: Log10+1 median fluorescent CFU/100ml (presumptive *E. coli*)
A further noteworthy result is the difference in bacterial counts for the Marine Stadium for the period of intensive sampling during 2000/01, and later less frequent sampling during 2001/02. Even though the first period focused on \textit{E}.\textit{coli} and the second reported presumptive faecal coliforms and \textit{E}.\textit{coli} (see Figure 6), values for both bacteria groups were lower during the second sampling period. A possible explanation for this discrepancy was the fact that sampling in 2001/02 occurred during a period of lowest rainfall on record for the Gold Coast region. This lack of rain could have very well reduced bacterial loads from land-based sources such as urban runoff and sediment erosion in the upper catchments and therefore reduced ambient bacterial concentrations in the water column during that year. On the other hand, this unusual weather pattern might have helped to create environmental conditions that are normally very hard to reference; the domination of rain on background levels of bacterial indicator organisms.

\textbf{Summary of microbiological examination of water quality}

Accordingly, bacterial concentrations at all anchor sites never exceeded ANZECC guidelines for primary contact, i.e. a median of no more than 150 FCF/100ml (5 samples over one month, with 4 out of 5 containing less than 600 CFU/100ml).

\textbf{Bacterial Loads in Sediments in the Intertidal Area of Two Anchor Sites (Myora and the Marine Stadium)}

Several studies in the US, the UK and Australia \cite{Byappanahalli & Fujioka 1998; Craig et al. 2001; Obiri-Danso & Jones 2000; Solo-Gabriele et al. 2000} indicated that faecal coliforms and \textit{E}.\textit{coli} can survive and even multiply in sediments of marine and brackish water bodies, particularly in areas where predation by, or competition with, naturally occurring organisms is reduced. Tidally influenced water bodies can provide large areas of such habitats with lower predation and/or competition along their shores between the average low tide and high tide marks.

Figure 8 shows results of repeated sampling at the northern-most and southern-most anchor sites of this study. Although bacterial concentrations vary depending on whether the area was sampled shortly after heavy rain (2003) or after a weeklong dry period (2003), levels rarely dropped to less than one presumptive faecal coliform CFU per gram wet weight. More importantly, levels of re-suspendable, viable bacteria exceeded 5 or even 35 FCs per gram sediment (wet weight) in different sections of the intertidal area. It is therefore reasonable to assume that these bacteria could be washed out during windy conditions or as a result of a boat wake hitting the shore. These results could explain why differences in tide levels had some influence on bacterial counts at anchor and reference sites in southern Moreton Bay and the Broadwater. It also provides an argument for the on average higher bacterial counts at the two near shore reference sites (Squire Island near Jumpinpin Bar and Wavebreak Island, Figure 6).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure8.png}
\caption{Faecal coliform concentrations in intertidal sediments at Myora and Marine Stadium}
\end{figure}
Coprostanol to 5α-cholestanol Ratios at Popular Anchor Sites in Southern Moreton Bay and the Gold Coast Broadwater

A relatively recently developed method of determining the principal source of faecal material released with sewage inputs relies on measuring concentrations of naturally occurring and introduced sterols in water and sediments (Nichols et al. 1993; Leeming et al. 1996; Bull et al. 2002). The distribution of introduced sterols, i.e. sterols found in faeces, is caused by a combination of diet, an animal's ability to synthesise its own sterols and the intestinal microbiota in the digestive tract. In this aspect humans are no different. The principal human faecal sterol is coprostanol (5β(H)-cholestan-3β-ol), which is only excreted in trace amounts by birds and dogs, for example. By analysing the sterol signature or 'fingerprint' in a water or sediment sample, it is therefore possible to determine the contribution of faecal matter from different sources. In this way, sterol signatures are less ambiguous than bacterial indicators such as E.coli, which can be shed by many warm blooded animals.

Introduced sterols, however, can be assimilated by microorganisms that naturally occur in the environment. A very small percentage of sterols occurring in plant material or faecal matter from local animal populations can be converted to coprostanol as part of the normal environmental degradation processes (Bull et al. 2002). Rather than using absolute concentrations of tracer sterols per se – in this case coprostanol as the most common type of sterol excreted with human faeces – it has been suggested to calculate ratios of this tracer sterol and its thermodynamically most stable, and therefore naturally occurring, intermediate (α-cholestanol) of the natural breakdown pathways of cholesterol and other sterols (Nishimura 1982). A conservative rule of thumb suggested by Leeming (pers. com. 2002), provides a ratio of coprostanol to α-cholestanol greater or equal to 0.5 as a good indication of inputs associated with gut microbiota, i.e. sewage (see also discussion in Bull et al. 2002).

Elevated coprostanol to α-cholestanol ratios (levels of 0.5 or greater) in the absence of any other major amounts of sterols at popular anchorages would point to a noticeable input of human, and therefore vessel-, derived sewage and, likewise, nutrients. Where these ratios remain below 0.5 or even 0.3, it can be assumed that faecal inputs were too low to dominate over other natural processes such as tidal flushing, natural assimilation and sediment transport. A strong indication of significant sewage inputs would be an increase in coprostanol to α-cholestanol ratios at anchor sites during or shortly after a period of high recreational boating intensities (e.g. summer holidays).

Using these ratios, results from 80 sterol fingerprints extracted from pooled samples taken at anchor and reference sites in Moreton Bay and the Broadwater indicated very low or no sewage inputs for most sampling events (see Figure 9). There were, however, a few exceptions. A ratio of 3.2 was measured in sediments at ‘One Mile’ anchorage during the first sampling event in December 2001, which coincided with very high bacterial counts but average boat numbers. This ratio continued to drop over the next three sampling times until five months later, coprostanol could no longer be detected – despite the continuous use of this site by an average of 25-30 vessels. This explains the high ratio for ‘One Mile’ anchorage shown in Figure 9.

On three other occasions, coprostanol to α-cholestanol ratios greater than 0.4 were detected once at Banana Banks, Tipplers and Wavebreak Island. For all other sampling events and locations, these ratios were low to very low. The most consistent ratios for coprostanol to α-cholestanol were found for the Marine Stadium. Actual concentrations of sterol compounds (e.g. cholesterol, stigmasterol, 24-ethylcholesterol) varied considerably between top and bottom layers of pooled sediment samples and between sampling events.
**Sediment Characteristics**

The bottom sediments at anchor and reference sites in Moreton Bay and the Gold Coast Broadwater varied considerably in regard to their total organic matter and clay contents, and their general red-ox status as indicated by a Munsell colour analysis of dried sediment (Table 8). The highest clay and organic content matter content was found at Tipplers Passage, which also coincided with the darkest grey of all colours identified in the samples collected for this study. At the opposite spectrum, pale yellow mobile sands with low organic content dominated the bottom substrate at the Marine Stadium and Wavebreak Island. Intermediate sites with some clay and between 1–2% organic matters included Horseshoe Bay and Myora anchorages. The perhaps most unexpected result was the high organic matter content at the two reference sites, Banana Banks and Jumpinpin Bar (see Table 8).

**Table 8: Selected characteristics of bottom sediments at anchor and reference sites in Moreton Bay and the Gold Coast Broadwater**

<table>
<thead>
<tr>
<th>Location</th>
<th>Grain size &lt; 63 µm (% of total weight)</th>
<th>Total organic matter (% dry weight)</th>
<th>Munsell colour analysis (dried sediment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horseshoe Bay</td>
<td>0.03 ± 0.01</td>
<td>1.05 ± 0.15</td>
<td>pale yellow – light grey</td>
</tr>
<tr>
<td>Myora</td>
<td>0.68 ± 0.03</td>
<td>1.59 ± 0.29</td>
<td>dark greyish brown</td>
</tr>
<tr>
<td>One Mile</td>
<td>3.26 ± 0.11</td>
<td>2.54 ± 0.23</td>
<td>dark greyish brown – very dark grey</td>
</tr>
<tr>
<td>Tipplers Passage</td>
<td>3.31 ± 0.75</td>
<td>5.39 ± 0.45</td>
<td>very dark grey</td>
</tr>
<tr>
<td>Marine Stadium</td>
<td>0</td>
<td>0.83 ± 0.14</td>
<td>pale yellow</td>
</tr>
<tr>
<td>Banana Banks</td>
<td>1.62 ± 0.05</td>
<td>4.26 ± 0.33</td>
<td>light grey – dark greyish brown</td>
</tr>
<tr>
<td>Jumpinpin Bar</td>
<td>0</td>
<td>2.80 ± 1.00</td>
<td>light brownish grey – dark greyish brown</td>
</tr>
<tr>
<td>Wavebreak Island</td>
<td>0</td>
<td>0.82 ± 0.11</td>
<td>light grey – pale yellow</td>
</tr>
</tbody>
</table>

A final, noteworthy observation was the presence of broken gastropod shells and possibly coral debris in sediments at Horseshoe Bay and Banana Banks. In some sections, this marine debris was very dense and made it hard to push corers into the sediment to collect undisturbed samples.
DISCUSSION

Vessel Counts

The data collected for this study (spot counts during aerial surveys and monthly visits for water quality sampling) strongly indicate that for most times of the year, the number of boats using sites in the eastern parts of Moreton Bay and the northern sections of the Gold Coast Broadwater were low. During most weekdays, only few anchor sites were used by more than 10 to 15 vessels. Only on weekends, particularly long weekends or holiday weekends with favourable boating conditions (light to moderate winds and no rain), boat numbers on the Bay and at anchorages can increase almost by an order of magnitude. In other terms, major inputs of pollutants from recreational vessels only occur sporadically or intermittently (depending on the anchorage and its exposure to adverse weather conditions), and for most of the year, pollution loads from this recreational vessels are therefore low and dispersed.

Bacterial Sewage Indicators

Overall, the range of concentrations of bacterial indicators (FC, E.coli) determined here was very similar to what has been reported in other studies for the same or nearby sites in the study area. For sites in the Gold Coast Broadwater [Tipplers, Wavebreak Island, Doug Jennings Park (= Marine Stadium)], Webb (2003) reported medians of around 10 FC/100ml near popular swimming beaches for the 2000/01 season, with some occasionally elevated concentrations after rain. Similar results were obtained by Moss and Cox (1999) during a 6-year monitoring exercise for sites near Wavebreak Island (site 5) and Marine Stadium (site 8). Beattie (2002) reported median FC concentrations of 4-5 CFU/100ml for surface waters at oyster leases near Couran and Tipplers. This compares to averaged medians of 18, 7 and 15 presumptive FC/100 ml measured over the course of this study for Tipplers, Wavebreak Island, and Marine Stadium, respectively.

The nearest sites in southern Moreton Bay analysed by Webb (2003) were all located along the western shore in the Redland Shire area. In these areas, FCF medians stayed mostly below 10 FC/100ml. This was higher compared to bacterial indicator concentrations determined in this study for Banana Banks and Horseshoe Bay, but similar to levels measured for surface waters at Myora and One Mile anchorages. Faecal coliform levels for oyster leases around Myora and Canaipa (0.9 and 1.9 FC/100ml, respectively; Beattie 2002), on the other hand, were more in line with levels measured at Banana Banks and Horseshoe Bay in this study.

Overall, concentrations of bacterial sewage indicators were generally low at popular anchor sites in Moreton Bay and the Gold Coast Broadwater and, based on these data, water quality could be considered as high. On the other hand, statistical analyses of the data collected for this study strongly indicated that the presence of recreational vessels does have an effect on the levels of sewage indicator bacteria. In this context, it is worth emphasising that the release of sewage from marine toilets may not be the only source of faecal indicator bacteria associated with recreational vessels. All anchor sites were partly surrounded by intertidal habitat—either sandy beaches, mud flats or mangrove forests. As mentioned previously (Chapter 3), viable presumptive faecal coliform and E.coli organisms can be isolated from sediments found in such intertidal areas. In theory, FC can be washed out together with other micro-organisms when intertidal sediments are stirred up by boat wakes hitting the shore. According to Macfarlane and Cox (2003), boat wakes can resuspend more sediment than ‘normal’, wind induced waves. The rate of wake induced sediment re-suspension depends on the vessel’s displacement, hull length (and type), its speed and its distance to the shoreline. Even smaller craft (ski boats) at planing speed can generate wakes with sufficient energy to cause shoreline erosion under certain conditions (Macfarlane & Cox 2003). It is therefore quite possible that a concentration of boat traffic at anchor sites can lead to increased sediment resuspension and, consequently, increased numbers of faecal indicator bacteria in adjacent surface waters. Furthermore, two of the three reference sites, Wavebreak Island and Jumpinpin Bar, were close to intertidal areas (sand banks, or sand banks and mangroves) and frequently passed by all types of recreational vessels. Faecal coliform concentrations at these sites were higher than those observed for Banana Banks, where sand banks are only exposed for a short time during low tide. At this stage, the increase in faecal indicator bacteria as a result of boat wake related re-suspension of shoreline sediments is no more than theory. This theory, however, warrants further investigation if the effect of changes to sewage discharge regulations of the Transport Operations (Marine Pollution) Act 1995 is to be monitored and audited using concentrations of faecal indicator bacteria.
Sterol Fingerprints in Sediments

Ratios of sterol concentrations indicating human sewage input confirmed observations from boat counts, and results from water quality monitoring using faecal indicator bacteria. Overall, the amounts of sewage released at popular anchorages were too low or too infrequent to leave a distinctive sterol signature indicating human sewage inputs over and beyond natural assimilation processes. The only occasion when both bacterial indicators and sterol signatures strongly indicated a major input of human sewage occurred at One Mile anchorage at the beginning of the sampling period (December 2001). These results, however, could not be repeated even though the number of boats moored at this location stayed the same or even increased during school holidays. This particular anchorage, on the other hand, is surrounded by a number of potential sources of human sewage. Beattie (2002) lists a caravan park with three septic trenches close to a drain emptying into the One Mile area, two sewage outfall pipes (at Polka Point, and at Adams Beach south of Dunwich) and another small caravan park with previously reported problems to its septic system. Any malfunctioning or any small accidents in the sewage treatment systems at these four sites could lead to a temporary release of considerable amounts of faecal material, including coprostanol and faecal coliforms. It is therefore quite likely, that the once-off pollution event observed at One Mile anchorage was caused by a problem in one of the sewage treatment systems discharging into the waters around Dunwich, and not by recreational vessels.

Results from sediment analyses, observations during dives and work by Pattiaratchi and Harris (2002) suggest that bottom sediments in eastern parts of Moreton Bay and the Gold Coast Broadwater are heterogeneous and dynamic. Although the entire system is sand dominated, grain size distribution and organic matter content varied considerably between sites. Furthermore, sand transport generated by tidal currents was apparent at the three reference sites: Wavebreak Island, Jumpinpin Bar and Banana Banks. Apart from the Marine Stadium, all other anchor and reference sites were subject to noticeable tidal currents—particularly during the last third of the outgoing tide (or the first third of the incoming tide, depending on the location). Sediment re-suspension due to wave action was occasionally observed at Horseshoe Bay. All sites apart from Wavebreak Island and Tipplers had frequent active animal burrows suggesting high rates of bioturbation. All these factors indicate a rapid turnover of water (laterally) and sediments (vertically and horizontally) at most sites investigated in this study. It is therefore questionable whether low or intermittent inputs of human sterol biomarkers can accumulate under such conditions. Or in other terms, more research is needed to determine whether sterol biomarkers are sensitive enough to indicate ecosystem changes at an early stage.

Estimates of Nutrient Inputs from Recreational Vessels in Eastern Parts of Moreton Bay

Nutrient loads associated with recreational vessels in eastern parts of Moreton Bay and the northern section of the Gold Coast Broadwater (Tangalooma to Tipplers) were calculated based on boat counts presented in Chapter 3 and the following (conservative) assumptions:

- an average of 2.5 persons per vessel (most vessels comfortably accommodate two persons (usually husband and wife), some have children on-board and others take friends);
- an average of 7 g nitrogen (N) or 1 g phosphorous (P) released per person per day; and
- little or no use of sewage pump out facilities (where holding tanks are being used, they will be emptied as soon as the vessel has left the anchorage and reached a safe speed).

During a full calendar year, recreational boating in eastern parts of Moreton Bay and the northern Broadwater adds about one tonne of N and around 150 kg of P to the system. Peak loads for this area equal to about 11 kg d⁻¹ N during an Easter weekend with favourable boating conditions leading to a maximum of about 3 kg d⁻¹ N for a site like Tipplers with 180 vessels. On ‘normal’ weekdays with moderate boating weather (winds up to 15 or 18 knots and overcast conditions) these loads drop to about 1.2 kg d⁻¹ N for the entire area between Tangalooma and Tipplers. It has to be emphasised here that these figures do not include N and P loads from showers, galley wastes, food scraps, burley and fishing bait.

Taking all these sources into consideration, annual nitrogen loads for the entire Moreton Bay region, i.e. from the Marine Stadium to Cape Moreton, would probably range between 5 and 10 tonnes per annum, depending on whether boat traffic on rivers and estuaries and near shore open waters are included.
### Table 9: Estimates of N and P inputs associated with recreational vessels in eastern Moreton Bay and the northern Gold Coast Broadwater (Tangalooma to Tipplers)

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Days</th>
<th>Average No. Vessels / Day*</th>
<th>Vessel Days</th>
<th>Person Days</th>
<th>N [kg]</th>
<th>P [kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer holidays (weekends)</td>
<td>10</td>
<td>350</td>
<td>3500</td>
<td>8750</td>
<td>61.25</td>
<td>8.75</td>
</tr>
<tr>
<td>Summer holidays (weekdays)</td>
<td>22</td>
<td>250</td>
<td>5500</td>
<td>13750</td>
<td>96.25</td>
<td>13.75</td>
</tr>
<tr>
<td>Easter weekend</td>
<td>4</td>
<td>487</td>
<td>1950</td>
<td>4875</td>
<td>34.12</td>
<td>4.87</td>
</tr>
<tr>
<td>Easter holidays</td>
<td>10</td>
<td>300</td>
<td>3000</td>
<td>7500</td>
<td>52.50</td>
<td>7.50</td>
</tr>
<tr>
<td>School holidays (weekends)</td>
<td>8</td>
<td>300</td>
<td>2400</td>
<td>6000</td>
<td>42.00</td>
<td>6.00</td>
</tr>
<tr>
<td>School holidays (weekdays)</td>
<td>20</td>
<td>200</td>
<td>4000</td>
<td>10000</td>
<td>70.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Long weekends (6)</td>
<td>18</td>
<td>360</td>
<td>6480</td>
<td>16200</td>
<td>113.40</td>
<td>16.20</td>
</tr>
<tr>
<td>Weekends (35)</td>
<td>70</td>
<td>200</td>
<td>14000</td>
<td>35000</td>
<td>245.00</td>
<td>35.00</td>
</tr>
<tr>
<td>Weekdays</td>
<td>203</td>
<td>70</td>
<td>14210</td>
<td>35525</td>
<td>248.67</td>
<td>35.52</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>365</td>
<td>151</td>
<td>55040</td>
<td>137600</td>
<td>~1000.0</td>
<td>~140.0</td>
</tr>
</tbody>
</table>

*NOTE: * includes allowance for adverse weather conditions
Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

The use of on-board facilities (toilets, galleys) of recreational vessels does not appear to be the main factor determining concentrations of bacterial indicators for faecal pollution at popular anchor sites in Moreton Bay and the Gold Coast Broadwater. In line with results from the literature, other environmental parameters such as water temperature, tide difference (flushing), or salinity also affect levels of indicator bacteria in a significant way.

On the other hand this study strongly indicated that, overall, recreational vessels contribute significantly to levels of indicator bacteria detected at popular anchor sites. Such contributions are, however, not sufficient to raise concentrations of bacterial indicator organisms to levels requiring further action as recommended under various water quality guidelines (primary contact). Elevated levels of bacterial sewage indicators are most likely a combination of (a) sewage input and (b) sediment re-suspension from boat wakes or anchor scouring and retrieval (the magnitude of (b) depending on environmental characteristics of the anchor site in question). Whatever the sewage input with the current use pattern and intensities in the southern Moreton Bay and Broadwater areas, it doesn’t appear to be persistent enough to cause a long-term shift towards more sewage related signatures of compounds in sterol fingerprints, i.e. it is not the equivalent of a point source of a small sewage treatment plant.
REFERENCES


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The Sustainable Tourism Cooperative Research Centre (STCRC) is established under the Australian Government's Cooperative Research Centres Program. STCRC is the world's leading scientific institution delivering research to support the sustainability of travel and tourism - one of the world's largest and fastest growing industries.

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