TRACING THE PRESENCE OF ANTHROPOGENIC DERIVED NUTRIENTS IN INTERTIDAL ECOSYSTEMS USING STABLE ISOTOPES OF CARBON ($^{12}$C:$^{13}$C) AND NITROGEN ($^{14}$N:$^{15}$N) IN THE HUNTER REGION, N.S.W, AUSTRALIA

ANGUS LACHLAN NOEL FANNING
B. ENV. SC. MGT. (HONS.)

2015

U OF N
TRACING THE PRESENCE OF ANTHROPOGENIC DERIVED NUTRIENTS IN INTERTIDAL ECOSYSTEMS USING STABLE ISOTOPES OF CARBON ($^{12}\text{C}:{^{13}\text{C}}$) AND NITROGEN ($^{14}\text{N}:{^{15}\text{N}}$) IN THE HUNTER REGION, N.S.W, AUSTRALIA

ANGUS LACHLAN NOEL FANNING
B. ENV. SC AND MGT (HONS.)
SUBMISSION DATE: 20TH NOVEMBER 2015

UNIVERSITY OF NEWCASTLE, AUSTRALIA

A thesis submitted to the discipline of Environmental Science and Management, The University of Newcastle, in partial fulfilment of the requirements of the Honours Degree of the Bachelor of Environmental Science and Management

NOVEMBER, 2015
ACKNOWLEDGEMENTS

I would like to thank all the people who contributed in many ways to the work described in this thesis. First and foremost, I must thank my academic supervisors, Dr. Troy Gaston from the University of Newcastle and Dr. Louise McKenzie from Hunter Water for their perseverance, patience, commitment and camaraderie throughout the year. I also wish to express my appreciation of their appraisals when there we achieved desired outcomes, and support and advice when things didn’t quite work out during honours – it made a world of difference.

Additionally, I wish to express sincere gratitude to both Carolyn Bellamy, and everyone in the WaterRA team, for their support throughout the project, and drive to ensure that I developed both personally, and professionally. The staff and the students of the WaterRA organisation provided ongoing drive and motivation to persevere throughout what was most certainly an action-packed and academically challenging year.

Next, my eternal gratitude must go to my Mum, Dad, and sisters, Gabrielle and Olivia. Without your unconditional love, home-cooked meals, washing of clothes and the sneaky slips of pineapple bills; I don’t know whether I could have tackled this project and thesis. The fact that you were more than happy to come and dig for near-microscopic, transparent crustaceans in the sand without even knowing what you were looking for says more than words ever will for the importance of a solid family backbone. I feel it is also appropriate to include my housemates and arguably second family members, Ryan, Zac and in particular, Jack in this section as without their constant distractions, whether it be through surfing, gaming or more than frequently just sitting on the couch and debriefing over a beer; I would have most certainly lost my mind.

Special thanks must also be extended to Dr. Stephen Keable from the Australian Museum for his insight and assistance in the identification of the isopods used in this study, as well as the University of Newcastle staff members such as Dr. Margaret Platell, Dr. Anthony Martin, Amy Gumbleton and Jake McCubbin who assisted greatly in accommodating the logistics of fieldwork and lab work in considerably tight timeframes. I would also like to thank the many volunteers and undergraduate students from the University, who gave up days that could have been potentially spent lying on the beach or recovering from hangovers in bed, to rather drag themselves out in the wee hours of the morning to spend a day scrambling across the rocky shores and the beaches of the Hunters coastline. A special mention must go to Jack Albert, Dan Hewitt, Gene van der Westhuizen and Mel Brown who were silly enough to not learn the first time, and came on numerous expeditions. Although sampling was often in adverse conditions battling the wind and wave surges to pick up even just a single cunjevoi, none of you ever complained.

It has been the accumulation of these gestures that have made a world of difference in this experience they call ‘Honours’, and it gives me faith that the world has such passionate, selfless, and motivated individuals.

“Unless someone like you cares a whole awful lot, nothing will get better, it’s not”

- The Lorax
ABSTRACT

Intertidal marine environments are highly valued for their ecosystem services, yet it is often unclear whether productivity within sandy beaches and rocky shores is driven by nutrients derived from terrestrial, marine or in situ sources. Three ocean outfalls along the Hunter coastline, N.S.W., discharge nutrient rich, secondary treated effluent offshore and at depth to the marine environment. Additionally, substantial quantities of suspended material and nutrients are provided to these environments by the Hunter River during outwelling plumes. Excessive nutrient loads on intertidal areas can result in an array of ecological impacts, directly driving and changing ecosystem function; yet the source and fate of nutrients advected to intertidal areas of the Hunter are relatively unknown. Stable isotope analysis is an effective ecological method in investigating nutrient sources and pathways in marine ecosystems. Isotopic ratios of nutrients assimilated into organism tissues enable distinct sources to be traced in highly variable and mixed environments such as intertidal shores. As such this study utilised stable isotopes to trace nutrients derived from these key sources and determine their spatial and temporal fate in intertidal beaches and rocky shores.

Multiple bioindicators with differing turnover rates were collected across 32 sites along the Hunter’s coastline, from Lake Macquarie to Port Stephens. Duel carbon (δ13C) and nitrogen (δ15N) stable isotope analysis of Pipis (Donax deltoides) and Ulva (Ulva lactuca) were used as appropriate time-integrated tracers of nutrient advection across intertidal shores. Evidence from these organisms suggest that food webs of intertidal sandy beaches and rocky shores of the region are predominately derived from marine sources. However, after significant rainfall events, terrestrial and anthropogenic derived nutrients can provide a substantial nutrient subsidy to the intertidal food web. Enrichment of bioindicators on intertidal shores adjacent to ocean discharging wastewater treatment works indicates that treated effluent may be advected back on shore, where it is assimilated and utilised within the food web. Also, plumes emanating from the Hunter River during extreme wet conditions can provide nutrient subsidy to intertidal shores as indicated by a depletion in δ13C and δ15N associated with terrestrial-derived material. Results of this study indicate that the relative significance of external nutrient (terrestrial and anthropogenic) sources to coastal systems is strongly related to climatic (rainfall) and oceanographic (onshore advection) events.
Furthermore, pipi’s have been identified as an excellent indicator of nutrient source to intertidal systems providing a baseline for future assessment of the Hunter coastal region.

Cover Photo: Angus Fanning, Dudley Beach, NSW, Australia. 2015.
**TABLE OF CONTENTS**

1. **Background** ........................................................................................................................................... 1
   1.1 Nutrient Sources of Coastal Systems ................................................................................................. 2
       1.1.1 Marine inputs ............................................................................................................................. 2
       1.1.2 Estuarine Inputs .......................................................................................................................... 3
       1.1.3 Anthropogenic Inputs ................................................................................................................. 4
   1.2 Intertidal Shores ................................................................................................................................. 5
       1.2.1 Value, Use and Management of Intertidal Shores ..................................................................... 6
       1.2.2 Rocky Shores .............................................................................................................................. 7
       1.2.3 Sandy Beaches ............................................................................................................................ 9
   1.3 Detecting and Tracing sewage-derived Nutrients In Intertidal Environments ................................. 10
   1.4 Stable Isotope Analysis .................................................................................................................... 11
       1.4.1 Nitrogen \(^{15}\text{N}:^{14}\text{N}\) ................................................................................................................. 11
       1.4.2 Carbon \(^{13}\text{C}:^{12}\text{C}\) ...................................................................................................................... 13
       1.4.3 Time Integrated Measure ............................................................................................................ 14
   1.5 Aims and Objectives ......................................................................................................................... 15
2. **Methodology** ......................................................................................................................................... 15
   2.1 Site Description ................................................................................................................................... 16
   2.2 Collection of Samples ....................................................................................................................... 21
   2.3 Physical Indicators ............................................................................................................................ 23
   2.4 Biological indicators ......................................................................................................................... 23
   2.5 Laboratory Analysis ......................................................................................................................... 25
       2.5.1 Physical Indicator Analysis ........................................................................................................ 25
       2.5.2 Stable Isotope Preparation ........................................................................................................ 27
   2.6 Data Analysis ..................................................................................................................................... 28
       2.6.1 Physical Characteristics .............................................................................................................. 28
       2.6.2 Stable Isotope Analysis .............................................................................................................. 29
3. **Results** .................................................................................................................................................. 29
   3.1 Physical Characteristics .................................................................................................................... 29
       3.1.1 Water Quality ............................................................................................................................. 30
       3.1.2 Organic Matter ........................................................................................................................... 36
       3.1.3 Chlorophyll-a ............................................................................................................................ 37
       3.1.4 Granulometry ............................................................................................................................. 41
3.2 Stable Isotope Analysis .................................................................................. 44
3.2.2 Pipis ........................................................................................................... 44
3.2.3 Isopods ...................................................................................................... 49
3.2.4 Nerita ........................................................................................................ 53
3.2.5 Barnacle .................................................................................................. 56
3.2.6 Ulva ........................................................................................................... 59
4. Discussion ......................................................................................................... 63
  4.1 Dominant Nutrient Sources ....................................................................... 63
  4.2 Conclusion .................................................................................................. 69
References ............................................................................................................. 70
Appendices ............................................................................................................ 78
TABLE OF FIGURES

Figure 1: Hunter River plume event during the East Coast Low event assessed in this study. Photo: T. Gaston, Hunter River, N.S.W., Australia. 23rd April, 2015 ........................................ 4
Figure 2: Overview of Sampling Sites across the Hunter Region, N.S.W, Australia ............... 18
Figure 3: (Insert 1): Port Stephen sampling locations, N.S.W, Australia............................ 18
Figure 4 (Insert 2): Newcastle sampling locations, N.S.W, Australia .............................. 19
Figure 5 (Insert 3): Lake Macquarie sampling locations, N.S.W, Australia ........................ 19
Figure 6: Rainfall and effluent flows from: A: Boulder Bay, B: Burwood Beach and C: Belmont Wastewater Treatment Works (WWTW’s) for the sampling period. Rainfall statistics were sourced from Bureau of Meteorology Weather Stations (BoM) at Nelson Head, Nelson Bay (A); Nobby’s Signal Station, Newcastle East (B) and Catherine Street, Swansea (C) as per Table 1................................................................. 20
Figure 7: Sampling events in relation to 2015 rainfall conditions from reference location Nobby’s Signal Station, Newcastle East (BoM Station: 61055) ........................................ 23
Figure 8: Sandy beach stations diagram, Zenith Beach, N.S.W, Australia. Image Adapted from: Google Earth, 2015 ............................................................... 23
Figure 9: Principal Component Analysis (PCA) output for analysis of water quality (temperature and salinity) and sediment characteristics (chlorophyll-α, mean grain size, sediment organic matter and %mud) for a) Event and b) Site .................................................. 30
Figure 10: MODIS satellite imagery of Event 3, Hunter River plume including sites within zone of influence. Imagery: MODIS, 23rd April 2015 ................................................................. 32
Figure 11: Mean (±SE) temperature and salinity profile change at site NOBD (Nobby’s Dog Beach) before and after the East Coast Low (Event 3) ........................................ 33
Figure 12: Mean thematic temperature (⁰C) interpolations across sampling locations by event..... 34
Figure 13: Mean thematic salinity interpolation across sampling locations by event .................. 35
Figure 14: Mean thematic Sediment Organic Matter (%) interpolation across sampling locations by event ............................................................... 39
Figure 15: Mean sediment Chlorophyll-α (g/cm²) thematic interpolation across sampling locations by event ................................................................. 40
Figure 16: Mean Sediment Grain Size (µm) thematic interpolation across sampling locations by event (Typical Wet – Event 2, and Typical Dry – Event 4) ................................................................. 43
Figure 17: Mean %Mud composition interpolation across sampling locations by event (Typical Wet – Event 2, and Typical Dry – Event 4) ................................................................. 43
Figure 18: Stable carbon (δ¹³C) and nitrogen (δ¹⁵N) isotope composition of Pipi (Donax deltoides). A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), D: Event 4 (Typical Dry). Values are mean ± SE ................................................................. 46
Figure 19: Stable a) carbon (δ¹³C) and b) nitrogen (δ¹⁵N) isotope thematic interpolation of Pipi (Donax deltoides). Values are mean ± SE ................................................................. 46
Figure 20: Carbon isotope (δ¹³C) and D. deltoides size regression bi-plots for significant (p<0.05) results by sites within events ................................................................. 48
Figure 21: Nitrogen isotope (δ¹⁵N) and D. deltoides size regression bi-plots for significant (p<0.05) results by sites within events ................................................................. 48
Figure 22: Stable carbon (δ¹³C) and nitrogen (δ¹⁵N) isotope composition P. concinna . A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), D: Event 4 (Typical Dry). Values are mean ± SE ................................................................. 51
Figure 23: Stable \( a) \) carbon (\( \delta^{13}C \)) and \( b) \) nitrogen (\( \delta^{15}N \)) isotope thematic interpolation of Isopod (\( Pseudolana concinna \)). Values are means. ................................................................. 51

Figure 24: Isotope (\( \delta^{15}N \) and \( \delta^{13}C \)) and \( P. concinna \) size regression bi-plots for significant (\( p<0.05 \)) results by sites within events. \( a) \) Nitrogen isotope (\( \delta^{15}N \)) v. \( P. concinna \) size at Dudley, Event 1, \( b) \) Carbon isotope (\( \delta^{13}C \)) v. \( P. concinna \) size at Zenith Beach, Event 3 and \( c) \) Carbon isotope (\( \delta^{13}C \)) v. \( P. concinna \) size at Zenith Beach, Event 4. ................................................................. 52

Figure 25: Stable carbon (\( \delta^{13}C \)) and nitrogen (\( \delta^{15}N \)) isotope composition of Nerita (\( Nerita atramentosa \)). \( A) \) Event 1 (Typical Dry), \( B) \) Event 2 (Typical Wet), \( C) \) Event 3 (East Coast Low / Extreme Wet), Event 4 (Typical Dry) ............................................................. 55

Figure 26 Stable \( a) \) carbon (\( \delta^{13}C \)) and \( b) \) nitrogen (\( \delta^{15}N \)) isotope thematic interpolation of Nerita (\( Nerita atramentosa \)). Values are means.................................................................................. 55

Figure 27: Stable carbon (\( \delta^{13}C \)) and nitrogen (\( \delta^{15}N \)) isotope composition of Barnacle (\( Tesseropora rosea \)). \( A) \) Event 1 (Typical Dry), \( B) \) Event 2 (Typical Wet), \( C) \) Event 3 (East Coast Low / Extreme Wet), \( D) \) Event 4 (Typical Dry). Values are mean ± SE......................................................... 58

Figure 28: Stable \( a) \) carbon (\( \delta^{13}C \)) and \( b) \) nitrogen (\( \delta^{15}N \)) isotope thematic interpolation of Barnacle (\( Tesseropora rosea \)). Values are means.................................................................................. 58

Figure 29: Stable carbon (\( \delta^{13}C \)) and nitrogen (\( \delta^{15}N \)) isotope composition of Ulva (\( Ulva lactuca \)). \( A) \) Event 1 (Typical Dry), \( B) \) Event 2 (Typical Wet), \( C) \) Event 3 (East Coast Low / Extreme Wet), \( D) \) Event 4 (Typical Dry). Values are mean ± SE......................................................... 62

Figure 30: Stable \( a) \) carbon (\( \delta^{13}C \)) and \( b) \) nitrogen (\( \delta^{15}N \)) isotope thematic interpolation of Ulva (\( Ulva lactuca \)). Values are means.................................................................................. 62
TABLE OF TABLES

Table 1: Bureau of Meteorology Weather Stations used for rainfall and temperature condition reference ........................................................................................................................................... 21

Table 2: Summary of physical and biological parameters collected for laboratory analysis in both ecosystems across study sites ........................................................................................................................................... 25

Table 3: Differences between Typical Dry (Event 4) and Typical Wet (Event 2); and Typical Dry (Event 4) and Extreme Wet (Event 4) for mean sediment Chlorophyll-α (mg/cm³) and Organic Matter (%). ↑ indicates significant difference and direction of difference (p<0.001), whereas ↑ indicates increasing or decreasing difference and direction of difference .................................................. 38

Table 4: D. deltoides regression summary table for carbon (13C) and nitrogen (15N) isotopes and size ........................................................................................................................................... 47

Table 5: P. concinna regression summary table for carbon (13C) and nitrogen (15N) isotopes and size ........................................................................................................................................... 52
DECLARATION OF ORIGINALITY

I declare that this thesis does not contain any material which has been submitted by me previously for any degree or diploma to any University, and to the best of my knowledge it does not contain any material previously published or written by another person, except where due reference is made in the text.

Angus Lachlan Noel Fanning
20th November, 2015
1. BACKGROUND

Humans are changing the environment in many ways. In the several centuries since the industrial revolution, population growth and urbanisation have placed increasing demands on both terrestrial and aquatic ecosystems (Smith-Evans and Dawes 1996, Carpenter et al. 1998, Galloway et al. 2003). A major and ongoing anthropogenic threat to water quality and ecosystem health in intertidal marine environments are excessive nutrient loads, primarily derived from fertiliser use and contamination from human and animal wastes (Kendall 1998, Costanzo et al. 2001, Howarth et al. 2002). Although nutrients generally promote primary production within aquatic ecosystems, over-enrichment can have detrimental impacts such as eutrophication. One of the most widespread and significant sources of stress on intertidal areas can be attributed to nutrient enrichment and the consequential changes to habitat, population dynamics, food web structure and the cycling of nutrients (Lee and Olsen 1985, Carpenter et al. 1998, Costanzo et al. 2001, Fraschetti et al. 2006, Halpern et al. 2007). In temperate intertidal ecosystems such as sandy beaches and rocky shores, nitrogen is a limiting factor for primary production, driving bottom-up ecosystem function (Nixon 1995, Vitousek et al. 1997, Howarth 2004). Considering the fundamental linkages intertidal areas provide in connecting and cycling nutrients and material between terrestrial and marine ecosystems, as well as the series of economic and social benefits they provide humans; understanding nitrogen sources and inputs to intertidal communities is integral to guiding effective and appropriate coastal management strategies (Menge et al. 1997, Wheeler et al. 2012).

A significant component of nitrogen enrichment can be attributed to the anthropogenic inputs of sewage, both raw and treated (Lee and Olsen 1985, Nixon 1995, Costanzo et al. 2001). Until the early 1980’s primary and secondary municipal wastes were disposed through shoreline outfalls causing significant health and environmental impacts (Otway 1995, Koop and Hutchings 1996, Philip and Pritchard 1996). Sustained and episodic impacts of near-shore disposal were linked to reduced water quality, the contamination of fish and alterations to a range of species abundances and diversities (McLean et al. 1991, Smith and Simpson 1992, Zmarzly et al. 1994, Otway 1995). In an effort to minimize the
human health and environmental impacts of near-shore effluent disposal, many outfalls in Australia were extended and placed at depth to dilute the effluent stream. Designed to provide a cost-effective method of disposal by promoting submergence within the water column (Boehm et al. 2002), ocean outfalls minimise noticeable pollution to the marine environment (Otway 1995, Boehm et al. 2002). Several oceanographic studies however, have documented cases where neutral buoyancy within the pyconocline did not provide a barrier to retaining disposed wastewater, particularly during periods where the water column was weakly stratified and during upwelling events (Pineda 1991, 1994, 1999, Boehm et al. 2002). As such, potential exists for products derived from the effluent plume, such as nutrients, to be advected onshore (Boehm et al. 2002). Considering the potential of nutrient advection to nearshore areas, particularly in conjunction with existing anthropogenic inputs; the relative sources and respective contributions of nutrients to intertidal ecosystems can be difficult to discern. The use of stable isotopes provides an effective method to investigate the sources and trophic pathways of nutrients in complex aquatic ecosystems (Peterson and Fry 1987, Lindau et al. 1989, Cabana and Rasmussen 1996, Costanzo et al. 2005).

1.1 NUTRIENT SOURCES OF COASTAL SYSTEMS

1.1.1 Marine inputs

Marine borne nutrients, namely nitrogen and carbon, can be derived from a range of both natural and anthropogenic sources in either soluble or particulate forms. Phytoplankton, carrion, stranded algae and detrital matter derived from decaying seagrasses and other marine macrophytes all provide fundamental natural inputs of organic matter when advected onto intertidal systems (McLachlan and Brown 2006c, Lercari et al. 2010, Bergamino et al. 2011). Additionally, interstitial microphytobenthos found within the photic zone of subtidal sediments can also contribute significantly to biologically available particulate organic material (Evrard et al. 2010). For intertidal shores, particularly in the case of sandy beaches, which are essentially devoid of in-situ primary production; such organic accumulations are likely to support habitat and food availability, and may have ecological effects to ecosystems beyond the immediate shoreline via the transfer of nutrients (Polis et al. 1997, Ince et al. 2007). Depending on the location however, terrestrial and estuarine ecosystems can provide a substantial portion of overall organic matter to the intertidal food web, offsetting the
absence of primary production within the intertidal zone, and contributions sourced from marine environments (McLachlan and Brown 2006c, Maria et al. 2011).

1.1.2 Estuarine Inputs

Riverine and estuarine outwelling events contain substantial quantities of suspended material and nutrients, and are considered globally as a significant cross-boundary transfer of organic matter (Schulz et al. 1994, Gaston et al. 2004, Ince et al. 2007, Connolly et al. 2009). Outwelling events, predominantly triggered by extensive rainfall within inland and coastal catchments, provide large volumes of brackish water containing significant quantities of nutrients and sediment to near-shore areas (Schlacher and Connolly 2009, Schlacher et al. 2009, Schlacher et al. 2015) (Figure 1). Excessive primary production from seagrass beds and near-shore macrophytes within estuarine and riverine ecosystems, often leads to a surplus of organic biomass that is delivered to broader marine environments during these flushing events (Bergamino et al. 2011). As a result, plumes enrich coastal waters through the transfer of inorganic nutrients across trophic boundaries to energy deficient habitats, leading to enhanced biological production within these areas (Gaston et al. 2006, Ince et al. 2007, Vanderklift and Wernberg 2008). Estuarine plumes are therefore considered to provide a key functional link, coupling terrestrial and aquatic landscape elements in providing a trophic subsidy to the receiving oligotrophic marine environment (Connolly et al. 2009, Schlacher and Connolly 2009). Over the past decades however, human activities have greatly intensified the delivery of nutrients, particularly reactive nitrogen, to estuarine and coastal environments which is altering ecosystem production and structure across the food web (Vitousek et al. 1997, Galloway et al. 2003).
Figure 1: Hunter River plume event during the East Coast Low event assessed in this study. Photo: T. Gaston, Hunter River, N.S.W., Australia. 23rd April, 2015.

1.1.3 Anthropogenic Inputs

Inputs of anthropogenic nitrogen are estimated to have increased several-fold due to greater human activity within, and surrounding estuarine and coastal areas (Vitousek et al. 1997, Seitzinger and Kroeze 1998, Howarth et al. 2002). Organic enrichment from point-sources such as residential and industrial effluent discharges, as well as diffuse sources such as urban and agricultural runoff have collectively added significant nutrient inputs to most coastal environments (Sweeney et al. 1980, Sara et al. 2006, Vizzini and Mazzola 2006, McClelland et al. 2014). As a result of these substantial modifications to the nitrogen cycle, both beneficial and detrimental changes have transpired to a range of terrestrial and aquatic ecosystems (Galloway et al. 2003). Although residence time of nitrogen within coastal ecosystems is generally short in comparison to terrestrial ecosystems due to their dynamic nature and increased denitrification rates, the period in which nutrients persist in these environments can have a profound impact on the coastal ecosystem and its functioning (Vitousek and Howarth 1991, Galloway et al. 2003).
Temperate coastal areas around Australia have limited availability of reactive nitrogen (Vitousek and Howarth 1991, Nixon 1995, Galloway et al. 2003). As such, additional nitrogen inputs in moderation can boost primary production, filtering throughout the food web to promote production across all trophic levels. Excessive additional nitrogen, as a consequence of anthropogenic activities however, can have detrimental impacts on near shore communities (Jorgensen 1996, Vitousek et al. 1997, Galloway et al. 2003). The extent and dispersal of anthropogenic nutrients however depends both on the quantity and source of the waste, and physical features of the receiving environment such as the geographical setting and hydrodynamic regime (Sara et al. 2006, Sampaio et al. 2010).

In the context of the Hunter coastline, New South Wales, the influence of additional anthropogenic nutrients may have on the intertidal food web is unknown. Furthermore, managing and monitoring the potential advection of biological pollutants derived from effluent outfalls and other diffuse sources to intertidal shores is considered as a major environmental challenge due to the dynamic nature of both the ocean, contributing estuaries and receiving environments (Nixon 1995).

1.2 INTERTIDAL SHORES

Intertidal shores are determined by the amplitude and frequency of the tides, the configuration of the shoreline and seafloor, and relative exposure to waves and broader atmospheric-oceanic processes (Petraitis et al. 2008). They provide a continental transition zone between the open ocean and terrestrial water sheds, forming an environment that supports diverse habitats and abundant terrestrial and aquatic biota that are both influenced, and limited by flows of energy and biomass from the broader marine environment (Colombini et al. 2011). Large quantities of organic matter and nutrients derived from the land, ocean and atmosphere are processed across intertidal shores; as such they are one of the most biogeochemically active areas on earth (Eyre et al. 2011). Additionally, they provide a series of economic and social benefits to humans such as fishing, harvesting, and an array of recreational activities; leading to the common conception of intertidal shores as one of Australia’s most highly valued natural assets (Schlacher et al. 2008, Gonçalves et al. 2013).
1.2.1 Value, Use and Management of Intertidal Shores

Australia has one of the longest coastlines, and as a result, largest intertidal shorelines of any country (34,218 km, excluding external territories; ABS 2004, Maguire et al. 2011). The use and intrinsic value placed on these areas is thereby inherently derived geographically due to their vast expanse and accessibility, as well as socially through the development of the Australian beach culture; a paradigm driven by phenomena such as the “sea-change” movement (Gurran 2008, Schlacher and Thompson 2012). Whilst recreational activities associated with intertidal shores vary geographically and are dependent on the demographics, attitudes and accessibility of individual users; fishing and surfing are generally popular, in addition to walking, swimming, picnicking, camping, bait/shell collection and where permitted, driving of off-road vehicles (ORV’s) (Schlacher and Morrison 2008, Defeo et al. 2009, Maguire et al. 2011, Schlacher and Thompson 2012). Although evidence supports the positive influence these recreational interactions can have on individual wellbeing and fulfilment (de Vries et al. 2003, Wheeler et al. 2012), such activities and the ongoing human presence within these areas are also recognised to impact, and potentially exacerbate pressures already imposed on intertidal areas (Brown and McLachlan 2002, Defeo and McLachlan 2005, Schlacher et al. 2007, Defeo et al. 2009, Schlacher et al. 2014).

Population growth, increased leisure time and greater affluence will continue to intensify the demand for both the development, and use of sandy beach and rocky shore environments (Defeo et al. 2009, Schlacher and Thompson 2012, Nel et al. 2014, Schlacher et al. 2015). As such, current coastal development practices primarily consider intertidal areas as geophysical assets of the landscape, and as a result corresponding environmental management is often focussed at addressing the physical issues that directly affect ongoing development capabilities and potential (Nordstrom 2003). This frequently discounts the irreplaceable ecosystems these areas support and the corresponding ecosystem services they provide such as water filtration, leisure and ecotourism, contribution to coastal fisheries, and nutrient cycling (Schlacher and Thompson 2012). As such, it is integral for ongoing management of coastal ecosystems to consider both the broad physical top-down anthropogenic pressures...
as a result of processes such as habitat destruction, as well as bottom-up ecological impacts (e.g. nutrient pathways) when assessing anthropogenic influences on intertidal shorelines.

1.2.2 Rocky Shores

Within New South Wales, intertidal shores consist predominately of wave-dominated sandy beaches bounded by metamorphic rocky shores and headlands (Masselink 2010). Overall, rocky shores comprise approximately 40% of Australia’s coastline and can adopt a wide range of shapes and profiles (Bulleri et al. 2005, Masselink 2010). Zonation across these dynamic ecosystems is determined by a range of physical, chemical, and biological processes, which when combined with the properties of the rock substrate, define the biological assemblages of individual rocky coastlines (Laurand and Riera 2006, Petraitis et al. 2008, Masselink 2010). The intertidal is considered as the most energetic zonation of rocky shores due to its exposure to waves, tides, currents and biological activity, in addition to those factors influencing other zones such as terrestrial runoff and wind (Underwood and Kennelly 1990, Masselink 2010).

Wave surge and tidal cycles have profound effects on exposing organisms to extreme conditions. High energy wave action can damage and sweep away organisms, favouring less mobile and more robust individuals (Petraitis et al. 2008). Whereas the lowest summer tides, which generally occur in the middle of the day expose organisms to extremely high temperatures, preferring only species that can cope under these adverse conditions (Underwood and Chapman 1998, Petraitis et al. 2008). These examples and other broad-scale, top-down controls have been considerably studied in the assessment of rocky intertidal communities; however, the effects of bottom-up influences such as nutrients and productivity are comparatively less understood (Menge et al. 1997, McQuaid and Payne 1998, Menge et al. 2003, Hill and McQuaid 2008).

Nutrient availability determines primary productivity in the form of algal growth, composition and coverage, defining the overall structure and dynamics within rocky shore communities (Bosman et al. 1987, Menge et al. 1997, Vinueza et al. 2006). Unlike terrestrial habitats, which rely predominately on local primary production to maintain internal trophic
functioning, rocky intertidal assemblages rely not only on the primary production sourced from within their food webs, but also biological matter from the broader marine environment that is advected onshore (Heymans and McLachlan 1996, Petraitis et al. 2008). Due to the diversity and abundance of communities across rocky shorelines and the exchanges they provide in linking terrestrial and broader marine activities, it is necessary to understand how nutrients from extensive sources both influence, and assimilate throughout these regions (Laurand and Riera 2006, Guerry et al. 2009). Similarly to intertidal rocky shores, the intertidal zone of sandy beach ecosystems also relies heavily on external organic inputs in facilitating ecosystem function.

1.2.3 Sandy Beaches

Sandy beaches are the largest coastal ecosystem on earth, covering approximately 70% of ice-free coastal interfaces (Brown and McLachlan 2002, McLachlan and Brown 2006a). Despite their extent, they mostly lack biological structure and consequently have limited in-situ primary production (Brown 2000, Brown and McLachlan 2002, McLachlan and Brown 2006b, Schlacher et al. 2009). As a result, they rely heavily on material and nutrients imported from external sources (McLachlan and Brown 2006c, Schlacher and Connolly 2009). Wave, aeolian and tidal processes enable the interchange of nutrients between the terrestrial and the marine environment, via the mixing of sand and biological matter (Scapini et al. 2003). In turn, these processes dictate the morphological environment of sandy beaches, defining species diversity, biomass and community structure as a result (Brown and McLachlan 2002, Defeo and McLachlan 2005, Defeo et al. 2009, Masselink 2010). As the biological structure of beaches is determined by a range of physical environmental conditions, they are extremely dynamic across both space and time (Defeo and McLachlan 2005). Understanding the flow of biological materials through the intertidal beach system is therefore essential for considering broader scale influences on these areas, particularly regarding the temporal and spatial influence of anthropogenic inputs (Brown and McLachlan 2002, McLachlan and Brown 2006c, Schlacher et al. 2007, Defeo et al. 2009).

The primary production that occurs within sandy shore ecosystems is largely subsidised by nutrients derived from external sources such as offshore marine autotroph production (Mellbrand et al. 2011). Algal wrack and kelp that is washed up and stranded on
Intertidal shores provide an important food source for intertidal crustaceans and insects, whereas filter feeders such as Pipis (*Donax deltoides*) generally rely on suspended particles such as diatoms within the surf-zone (Kirkman and Kendrick 1997, Colombini et al. 2011). Additionally, high tides and wave action wash marine carrion onto the shore, which is consumed by a range of aquatic crustaceans and scavenging gastropods. During lower tides, terrestrial animals and birds may scavenge across the intertidal area (Brown and McLachlan 2002, Defeo et al. 2009). At a microbial scale, bacteria and meiofauna convert organic material in the sandy sediments to mineralized nutrients, which are either utilized locally within the system, or returned to sea; highlighting the complex interrelationship and dependence sandy shores have with the broader marine environment (Brown and McLachlan 2002, Ince et al. 2007). Transfer of organic matter between these ecosystems, however, varies temporally with weather events, and spatially between and within intertidal areas (Gonçalves et al. 2013). In instances where anthropogenic nutrients such as treated sewage effluent are discharged to the nutrient-limited marine environment, a degree of mixing and dispersal may potentially transfer additional nutrients to beaches and adjacent rocky shores (Boehm et al. 2002, Gartner et al. 2002).

Although detecting the influence of coastal effluent dispersal and advection to coastal areas is an important consideration for coastal management, its effectiveness in ecological studies can often be hindered by natural variability in community composition and structure (Hewitt et al. 2005). Furthermore, recognizing the ecological implications of this nutrient advection may be inhibited by a limited understanding of the uptake and assimilation of effluent constituents between organisms, and across varying localities (Dudley and Shima 2010). It is therefore necessary to employ methods enabling both spatial and temporal tracing of effluent advection, whilst concurrently providing an appreciation of any ecological influence additional inputs may have.

1.3 DETECTING AND TRACING SEWAGE-DERIVED NUTRIENTS IN INTERTIDAL ENVIRONMENTS

Temporal and spatial distributions of sewage-derived effluent are commonly assessed using parameters such as dissolved nutrients, bacteria, organic matter compositions, salinity,
phytoplankton biomass and less commonly in some instances, through the use of dye fluorescence and fluorescent whitening agents (Sweeney et al. 1980, Lindau et al. 1989, Smith-Evans and Dawes 1996, Costanzo et al. 2001, Gartner et al. 2002, Hayashi et al. 2002, Geary and Davies 2003, Laurand and Riera 2006). These methods provide useful techniques for determining the physical extent of effluent dispersal in the marine environment, however, provide limited perception to the biological uptake and influence of these nutrients on the receiving ecosystems that may rely on their inputs (Vandover et al. 1992, Costanzo et al. 2001). They are generally laborious, time consuming and costly to collect and analyse. Additionally, in well-mixed environments such as intertidal shores, tracers can be lost quickly through dilution and mixing and as a result provide only an instantaneous indication of effluent dispersal (Gartner et al.; 2002).

Stable isotopes are increasingly used in ecological research as chemical tracers of the sources, pathways and fate of organic matter in both terrestrial and aquatic systems (Peterson and Fry 1987, Ponsard and Arditi 2000, Robinson 2001, Vizzini and Mazzola 2003, Darnaude et al. 2004). They differ from radioactive isotopes in that they do not decay with time (Peterson and Fry 1987, Savage 2005). The abundance of naturally occurring stable isotopes of nitrogen and carbon have been effectively utilised to trace the spatial and temporal influence of a range of contributing sources across aquatic environments (Tucker et al. 1999, Costanzo et al. 2001, Waldron et al. 2001, Rogers 2003, Gaston and Suthers 2004, Savage 2005, Bannon and Roman 2008). They provide a measure of trophic positioning, that integrates the assimilation of nutrient flow within organisms through various pathways over given timescales (Post 2002). Recurring isotopic patterns identified within primary consumers over time can indicate imperative nutrient contributions to food webs, whereas significant temporal variation, such as what may be associated with seasonal or weather related events may indicate a variety of sources that support trophic function at different timescales (Post 2002, Bergamino et al. 2011). As such, stable isotopes have the ability to simultaneously capture both complex trophic interactions whilst tracking energy flow through ecological communities within intertidal shores (Peterson and Fry 1987, Cabana and Rasmussen 1996, Post 2002, Bergamino et al. 2011).
1.4 STABLE ISOTOPE ANALYSIS

1.4.1 NITROGEN (\(15\text{N}:14\text{N}\))

There are two known naturally occurring atomic forms of nitrogen, distinguishable by the number of neutrons within their relative atom shell (Costanzo et al. 2001). The heavier and less common form (0.3663\%), containing an extra neutron is referred to as nitrogen 15, versus the lighter and more common isotope (99.6337\%) of nitrogen which contains seven protons and neutrons, known as nitrogen 14 (Costanzo et al. 2001). They are commonly expressed as \(15\text{N}\) and \(14\text{N}\) respectively (Owens 1987, Costanzo et al. 2001). Stable isotopes of nitrogen are conventionally reported in \(\delta\) notation with respect to atmospheric nitrogen, standardised by the use of calibrated organic standards (Mariotti 1983):

\[
\delta^{15}\text{N} (\text{‰}) = \frac{\left[15\text{N}:14\text{N}_{\text{sample}} - 15\text{N}:14\text{N}_{\text{standard}}\right]}{15\text{N}:14\text{N}_{\text{standard}}} \times 1000
\]

Various sources of nitrogen to coastal areas often have distinguishable ratios of \(15\text{N}:14\text{N}\), providing a means to differentiate between sources of anthropogenic nutrient enrichment and naturally derived sources such as marine detritus (Deniro and Epstein 1981, Heaton 1986, Peterson and Fry 1987, Savage 2005). Groundwater, which has nitrate solitarily derived from atmospheric deposition, tends to have \(\delta^{15}\text{N}\) values ranging from 2‰ to 8‰, whereas nitrate contained in human or animal faeces and wastes tends to have \(\delta^{15}\text{N}\) values ranging from 10‰ to 20‰ (Kreitler 1979, Kroeger et al. 2006). Broad scale, anthropogenic diffuse sources such as runoff from synthetic fertilisers have been shown to have reduced \(\delta^{15}\text{N}\) values (-3 to 3‰) (Heaton 1986, Bowen and Valiela 2008), whereas point discharges such as untreated or primary treated sewage carries an isotopically depleted signal (2 - 5‰) relative to marine (~6‰), and estuarine derived organic matter (13‰) (Sweeney et al. 1980, Savage and Elmgren 2004, Fry 2006, Dudley 2007). In contrast to this, secondary treated sewage, or treated effluent, has a notably elevated \(\delta^{15}\text{N}\) signal (~20-30‰). This is due to the treatment process selectively discriminating the fractionation of nitrogen to utilise \(14\text{N}\) which produces \(15\text{N}\) enriched effluent (Heaton 1986, Owens 1987, Jordan et al. 1997). This enriched
signature can therefore often be distinguished from other contributing nutrient sources, and then subsequently traced through primary producers and their respective dependant food chains (Costanzo et al. 2001).

The enriched nitrogen signature is transferred to consumers with a degree of fractionation of 1.5‰-4‰ in the $^{15}$N/$^{14}$N ($\delta^{15}$N) ratio between trophic levels (Deniro and Epstein 1981, Pitt et al. 2009). Fractionation occurs across trophic levels, and as such can be utilised to examine interactions within food webs. As nutrients pass through chemical reactions in various biological systems, the process of kinetic fractionation whereby the lighter isotope generally becomes more concentrated relative to the heavier isotope leads to different quantities within each individual, and as a result is transferred throughout the food web via trophic interactions (Dudley 2007). As such, in addition to tracing nutrient flow to consumers within the food web, stable isotopes of nitrogen ($\delta^{15}$N) can provide powerful tools for estimating trophic positioning. Variation in stable nitrogen isotope ratios within organism tissues can therefore be used to identify both a broad range of energy sources such as sewage-derived organic matter within the aquatic environment, as well as how these nutrients are transpired throughout the ecosystem (Cabana and Rasmussen 1996, Tucker et al. 1999, Anderson and Cabana 2006).

Both sewage-derived particulate and dissolved inorganic nitrogen isotope values however, can vary between wastewater treatment works and processing facilities, in some instances providing $\delta^{15}$N values within the range associated with marine-derived nitrogen (Gartner et al. 2002, Savage and Elmgren 2004). As such, the practicality of using $^{15}$N as a sole tracer of sewage derived effluent across intertidal ecosystems is limited if the $\delta^{15}$N values of effluent are not prominently distinct from nitrogen sources naturally available to bioindicators (Bedard-Haughn et al. 2003, Dudley 2007, Dudley and Shima 2010). Additionally, carbon isotopes of both marine and treated effluent have been shown to converge under certain conditions, particularly when the marine environment is influenced by terrestrial and estuarine-produced organic carbon (Gartner et al; 2002). As both $\delta^{13}$C and $\delta^{15}$N have potential to vary under a range of conditions, the use of a dual isotope approach using both carbon and
nitrogen isotopes is considered to provide a robust assessment in reliably differentiating patterns of effluent dispersal in aquatic and intertidal ecosystems (Dudley 2007).

1.4.2 Carbon (\(^{13}\text{C} :^{12}\text{C}\))

Primary producers have distinct carbon isotopic signatures, and as a result, analysis of the ratios of \(^{13}\text{C} :^{12}\text{C}\) (or \(\delta^{13}\text{C}\)) can enable the identification of food sources for a range of organisms (DeNiro and Epstein 1978, Ponsard and Arditi 2000). Fractionation of \(\delta^{13}\text{C}\) within primary producers is determined by either their respective photosynthetic pathways or whether they assimilate carbon predominately from the atmosphere or the aquatic environment (Gaston et al. 2006, Connolly et al. 2009, Bergamino et al. 2011). As such, C\(_3\) and C\(_4\) plants have distinguishable isotopic ratios, which are reflected in the composition of organisms that derive their carbon from either of these photosynthetic types (Smith and Epstein 1971, DeNiro and Epstein 1978). Furthermore, the \(\delta^{13}\text{C}\) ratios between aquatic organisms do not overlap with those of terrestrial organisms, providing the ability to differentiate between terrestrial and marine carbon sources for near-shore communities, such as intertidal shores (Craig 1953, Degens et al. 1968, DeNiro and Epstein 1978). The isotopic composition of organisms’ body tissues reflects the isotopic composition of its diet, with an enrichment factor on average of 1-2‰ relative to the diet (Craig 1953, DeNiro and Epstein 1978, Rogers 2003, Fukumori et al. 2008, Schlacher and Connolly 2009). Analysis utilising isotope ratios of carbon can therefore determine the relative contribution of derived energy sources to the diet of the organism, as well as estimate the individuals place within food webs by supporting enrichment values attained from nitrogen isotope analysis (Craig 1953, DeNiro and Epstein 1978, Peterson and Fry 1987). An important consideration in interpreting these values however is the role of fractionation processes as isotopes are assimilated and transformed through food chains.

1.4.3 Time Integrated Measure

Fractionation occurs across tissues within organisms and is dependent on the organism, their metabolic rate and cell breakdown and replacement times (Deniro and Epstein 1981, Lorrain et al. 2002, Laurand and Riera 2006, Pitt et al. 2009). The various turnover rates and fractionation between tissues of organisms studied therefore provides the potential to
determine both short and medium term energy sources assimilated into respective tissues. Within algae, nitrogen is generally turned over quickly throughout the entire organism by nutrient uptake via their thalli, often reaching equilibrium with ambient dissolved inorganic concentrations in approximately a week (Fernandes et al. 2009). Turnover in animals however is often considerably slower, and varies to a greater degree both between species and individual tissues, typically ranging from weeks to months (Lepoint et al. 2002, Gaston and Suthers 2004). Muscle tissues tend to have lower turnover rates within relatively sessile intertidal organisms, thus indicating isotopic characteristics of a long-time period and consequently press sources of pollution such as the ongoing disposal of treated sewage effluent (Lorrain et al. 2002, Weems et al. 2012). Lipids on the other hand are useful in detecting shorter term exchanges of food sources, indicative of rapid alterations such as abrupt or seasonal nutrient sources (Weems et al. 2012).

By using a combination of nitrogen and carbon isotopic signatures assimilated through trophic interactions on intertidal shores, the biologically available and therefore ecologically significant sources of nutrients can be detected and mapped across coastal ecosystems (Peterson and Fry 1987, Costanzo et al. 2001). Stable isotope analysis is an effective methodology for determining the temporal and spatial influence of both point and diffuse nutrient sources on intertidal ecosystems.

1.5 AIMS AND OBJECTIVES

The aim of this study is to identify the dominant nutrient sources on the intertidal areas of sandy beaches and rocky shores across an approximate 80 kilometre stretch of coastline in New South Wales, Australia. Three ocean outfalls discharge treated effluent at depth to the marine environment within this area in conjunction with three large estuarine systems (Lake Macquarie, Hunter River and the Port Stephens estuary), all of which potentially provide a nutrient subsidy to nearby intertidal areas. Using stable isotope signatures of carbon ($^{12}$C:$^{13}$C) and nitrogen (N$^{14}$:N$^{15}$), the relative contribution of multiple nutrient sources can be discerned. The key objectives of the study are to use stable isotopes of carbon ($^{12}$C:$^{13}$C) and nitrogen (N$^{14}$:N$^{15}$) to:

- Determine the dominant nutrient sources that contribute to the food webs of intertidal ecosystems;
- Determine whether anthropogenically derived nutrients, such as those derived from treated effluent, are advected onto and utilised within intertidal ecosystems;
- Determine the spatial extent of influence from treated effluent and estuarine sourced nutrients, and;
- Estimate the temporal contribution of nutrients to the food web over short and medium timeframes using different species / components of the food web.

2. METHODOLOGY

2.1 SITE DESCRIPTION

The study was conducted at thirty-two sites, incorporating twenty sandy beaches and twelve rocky shores across the Hunter region, New South Wales (Figures 2-5). The Hunter has a semi-diurnal tidal cycle generally ranging between 0.5 and 2 metres for low and high tides respectively. Sites were selected on the basis of proximity from key known anthropogenic influences, such as the Hunter River, and three wastewater treatment works (WWTW’s) which discharge secondary treated effluent at depth via outfalls to the marine environment.

The Hunter River estuary is classified as a geomorphologically mature, wave-dominated estuary, similar to other larger river systems on the mid-north coast of NSW such as the Manning and Macleay Rivers (BMT WBM 2015). Tides within the estuary reflect the same characteristics as the relative ocean tidal cycle, with semi-diurnal unequal tides, comprising strong fortnightly variations between spring and neap stages of the tidal cycle (BMT WBM 2015). Extensive clearing of the Hunter catchment over the past two centuries has resulted in unnaturally high sediment loads to the river, leading to an imbalance of geological forces acting on the channel (BMT WBM 2015). As such the area receives direct runoff from a number of urban catchments, with the majority of development concentrated around Newcastle and the surrounding suburbs in the lower reaches of the estuary. These catchments contribute diffuse sources of sediment, nutrients and additional pollutants to the Hunter River estuary (MHL 2003). Point-source discharges are also prominent throughout the estuary; including 19 WWTW’s which collectively discharge approximately
40ML/day of treated effluent to the Hunter River waterway in some manner. During periods of low rainfall, there is potential that these point-source anthropogenic inputs may provide significant portions of base flow within the system. As a result, constituents such as nutrients may be advected onto near-shore environments during outgoing tides (Connolly et al. 2009). Similarly, during wet weather it is assumed that a large proportion of diffuse nutrient sources may be advected to coastal intertidal shores such as sandy beaches and rocky shores via the estuarine plume.

Boulder Bay WWTW (Figure 3) is the smallest of three ocean outfalls discharging in the Hunter catchment, and treats wastewater from Tomaree Peninsula in Port Stephens. Due to intensified holiday loading, discharges can range from averages of 9ML/day, to volumes of 13ML/day during summer peak or wet weather periods (Figure 4). Treated effluent is discharged to the marine environment at depths of approximately 20 metres, by a 500 metre outfall and multi-port diffuser. Unlike the adjacent shorelines of Burwood Beach and Belmont Beach which are comprised of a sandy beach and dune system; rock platforms and cliff faces encompass the majority of the adjacent shoreline with small, isolated beaches comprised of boulders and gravel.

Burwood Beach WWTW (Figure 4) is the largest of the ocean disposal treatment works, servicing approximately 185,000 people in the greater area of Newcastle, which equates to effluent discharges on average of 40-48ML/day. Secondary treated effluent is dispersed through nine ocean outfall diffuser heads that open as the rate of discharge increases, which during wet weather can reach volumes of 200-300 ML/day (Figure 4). In addition to treated effluent discharge, Burwood Beach WWTW releases approximately 2ML/day of waste-activated sludge through a separate pipe diffuser which extends with the effluent outfall approximately 1.5km offshore at a depth of 22 metres.

Belmont WWTW (Figure 5) is smaller in volumetric comparison, servicing 115,000 people with daily effluent discharges on average of 30ML/day (Figure 4). Similarly to Burwood Beach, effluent is subjected to secondary treatment and discharged through a 1.5km ocean outfall, however waste activated sludge is dewatered, dried and transported offsite for beneficial agriculture reuse. Belmont receives wastewater from both the eastern side, as well as the western side of Lake Macquarie, receiving treated effluent from Edgeworth and Toronto WWTW’s via an effluent pipeline underlying the lake.
Figure 2: Overview of Sampling Sites across the Hunter Region, N.S.W, Australia

Figure 3: (Insert 1): Port Stephen sampling locations, N.S.W, Australia
Figure 4 (Insert 2): Newcastle sampling locations, N.S.W, Australia

Figure 5 (Insert 3): Lake Macquarie sampling locations, N.S.W, Australia
Figure 6: Rainfall and effluent flows from: A: Boulder Bay, B: Burwood Beach and C: Belmont Wastewater Treatment Works (WWTW’s) for the sampling period. Rainfall statistics were sourced from Bureau of Meteorology Weather Stations (BoM) at Nelson Head, Nelson Bay (A); Nobby’s Signal Station, Newcastle East (B) and Catherine Street, Swansea (C) as per Table 1.
2.2 COLLECTION OF SAMPLES

Targeted event based sampling was carried out from January 2015 to July 2015, and was triggered by rainfall events recorded from three Bureau of Meteorology (BoM) automated rainfall stations representative of the sampling area (Table 1). As a result of rainfall variability during this period, sampling was undertaken at all sites in both dry (<40mm in a week), and wet (<40mm within a 24 hour period) conditions as well as extremely wet conditions associated with a severe east coast low (~100mm within 24 hour period; Figure 7). Additionally, a preliminary scaled-down assessment was carried out in January 2015, which contributed to a second dry weather event that has been included within the data set.

Table 1: Bureau of Meteorology Weather Stations used for rainfall and temperature condition reference

<table>
<thead>
<tr>
<th>Location</th>
<th>BoM Station Number</th>
<th>Representative Areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catherine Street, Swansea</td>
<td>61977</td>
<td>Swansea, Blacksmiths, Belmont, Pelican, Redhead, Dudley</td>
</tr>
<tr>
<td>Nobby's Signal Station, Newcastle East</td>
<td>61055</td>
<td>Burwood, Merewether, Bar Beach, Newcastle, Nobby's, Stockton</td>
</tr>
<tr>
<td>Nelson Head, Nelson Bay</td>
<td>61054</td>
<td>Birubi, Boulder Bay, Fingal Bay, One Mile, Box, Zenith</td>
</tr>
</tbody>
</table>

The intertidal zone of both sandy beach and rocky shore locations were sampled for all events. Site information was recorded for physical conditions such as weather (rainfall, temperature and wind direction), ocean conditions (tidal phase and surf size), and time at each sampling site. Physical and biological indicators were from both sandy beach and rocky shores.
Figure 7: Sampling events in relation to 2015 rainfall conditions from reference location Nobby’s Signal Station, Newcastle East (BoM Station: 61055)
2.3 PHYSICAL INDICATORS

Sediment organic matter content and chlorophyll-α were determined at each beach. Additionally, sediment grain size (granulometry) was analysed for typical dry and typical wet conditions.

Sediment cores were collected from three stations at 10m intervals (parallel to the swash zone) on each beach (Figure 8). For assessing granulometry, 3 x 80mL samples and for organic matter content, 3 x 30mL samples were collected. For chlorophyll-α content, sediment was scrapped from the surface (1-5mm) in the swash zone (3 x 5mL). A calibrated multi-parameter water quality meter (Horiba U-50 Multi-parameter water checker) was used at approximately 1-2 metre depth to determine *in-situ* water properties of temperature (°C) and salinity adjacent to each sandy beach station (n=3).

![Figure 8: Sandy beach stations diagram, Zenith Beach, N.S.W, Australia. Image Adapted from: Google Earth, 2015](image)

2.4 BIOLOGICAL INDICATORS

A variety of species were sampled as bioindicators to represent multiple trophic levels and turnover rates across both sandy beach and rocky shore ecosystems (Table 2). Species were determined by an abundance and distribution pre-assessment to ensure availability of most organisms across the study site. Pre-assessment results indicated sandy beach
assemblages were characteristic of exposed reflective beaches along south-eastern New South Wales (Dexter, 1984). Similarly, species assemblages across rocky shores within the study site were consistent with those typical of the east-Australian temperate coastline (Underwood and Kennelly 1990, Underwood and Chapman 1998, Bulleri et al. 2005, Ferguson et al. 2015). As such, species targeted for collection on beaches were the intertidal scavenging isopod *Pseudolana concinna* and the common beach bivalve *Donax deltoides*. The two feeding mechanisms of these species are assumed to provide an indicator of both the instantaneous contribution of nutrients advected onshore (*D. deltoides*), as well as those sourced from marine carrion and other allochthonous sources (*P. concinna*). Due to the relatively specific detail involved in the identification of isopods, reference specimens (n=10) of *P. concinna* were confirmed by the Australian Museum, Sydney and deposited in the collection (Reg. No: P.98302). Where possible, Pipis (*Donax deltoides*) were collected for a range of sizes (n=10) at each sandy beach location to determine the influence of size variation on isotope ratios.

Across rocky shores, the barnacle *Tesseropora rosea*, grazing gastropod *Nerita atramentosa*, and chlorophyte algae *Ulva latuca* were collected where available. Similarly to the sandy beaches, organisms were selected to indicate the contribution and origin of nutrients over a short timeframe (*T. rosea* and *U. latuca*) and the fate of those nutrients throughout the food web (*N. atramentosa*).
Table 2: Summary of physical and biological parameters collected for laboratory analysis in both ecosystems across study sites.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of Samples (n)</th>
<th>Intertidal Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sandy Beach</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rocky Shore</td>
</tr>
<tr>
<td><strong>Physical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Quality (°C, Salinity)</td>
<td>3</td>
<td>√</td>
</tr>
<tr>
<td>Chlorophyll-α (mg/cm³)</td>
<td>3</td>
<td>√</td>
</tr>
<tr>
<td>Organic Matter Content (%)</td>
<td>3</td>
<td>√</td>
</tr>
<tr>
<td>Granulometry (µm)</td>
<td>3</td>
<td>√</td>
</tr>
<tr>
<td><strong>Biological (Stable Isotope Analysis)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isopods (<em>Pseudolana concinna</em>)</td>
<td>5</td>
<td>√</td>
</tr>
<tr>
<td>Pipis (<em>Donax deltoides</em>)</td>
<td>5¹</td>
<td>√</td>
</tr>
<tr>
<td>Ulva (<em>Ulva lactuca</em>)</td>
<td>3</td>
<td>√</td>
</tr>
<tr>
<td>Rose-coloured barnacle (<em>Tesseropora rosea</em>)</td>
<td>3</td>
<td>√</td>
</tr>
<tr>
<td>Nerita (<em>Nerita atramentosa</em>)</td>
<td>3</td>
<td>√</td>
</tr>
</tbody>
</table>

All collected samples were placed in labelled zip-lock bags and put on ice, transported to the laboratory where they were frozen until analysis.

2.5 LABORATORY ANALYSIS

2.5.1 PHYSICAL INDICATOR ANALYSIS

2.5.1.1 SEDIMENT ORGANIC MATTER

As per Parsons (1984) methodology, organic matter content of sediment was determined by the Loss on Ignition method (LOI) using 30mg replicates of sediment that were dried and ground by mortar and pestle before being placed into a labelled, pre-weighed crucible. Samples were dried in a muffle furnace for 24 hours at 105°C and reweighed to determine the oven-dried weight. Samples were returned to the muffle furnace for a further 24 hours at 430°C, removing the organic carbon from the sample and leaving only the residual mineral component, providing the ‘loss on ignition weight’. Organic matter content (%) was then calculated by the following formula:

¹ n≥10 for analysis of size and isotope ratios within Sandy Beach sites
2.5.1.2 CHLOROPHYLL-A EXTRACTION

Chlorophyll-α was extracted and analysed by adding 10mL of 90% acetone to each 5mL of sediment collected from each site (n=3) (Parsons, 1984). The sample was then covered in aluminium foil and refrigerated for 60 minutes before being placed into a centrifuge and spun at 3,000rpm for five minutes. The extract was then be decanted into a 1.3cm cuvette and measured against 90% acetone blanks at wavelengths of 750nm, 665nm, 647nm and 630nm. The absorbance recorded at 750nm was subtracted from each wavelength, and concentration of chlorophyll species determined by the following formula:

\[
\text{Chl-} \alpha = (11.85E_{665} - 1.54E_{647} - 0.08E_{630})
\]

\[
\text{Chl-b} = (21.03E_{647} - 5.43E_{665} - 2.66E_{630})
\]

\[
\text{Chl-c} = (24.52E_{630} - 1.67E_{665} - 760E_{647})
\]

Chlorophyll within the extract was calculated as the concentration of the pigments per volume of the substrate by the following calculation:

\[
\text{C(mg)} \times V \\
\text{Chl-} \alpha (\text{mgcm}^3) = \frac{\text{C(mg)} \times V}{L(\text{cm}) \times \text{Volume of Sediment}}
\]

Where:

- \(C\) = Concentration of the chlorophyll calculated previously
- \(V\) = Volume of extract in mL (10mL)
- \(L\) = Cuvette Length (1.3cm)
- Volume of sediment = 5mL

2.5.1.3 SEDIMENT GRANULOMETRY

Mean grain size and mud composition were determined for both typical wet (Event 2) and typical dry (Event 4) conditions using a graded sieve stack. A 30mg sample of dried sediment (24 hours at 65°C) was shaken through mesh sizes of 4mm, 2mm, 1mm, 0.5mm, 0.25mm,
0.125mm and 0.063mm respectively for 5 minutes. Retained sediments within each sieve were weighed and recorded to determine size composition and profile for each location for both typical wet and dry conditions.

2.5.2 STABLE ISOTOPE PREPARATION

2.5.2.1 MACROFLORA AND MACROFAUNA

Sandy beach and rocky shore fauna were measured with Vernier callipers and recorded for individual length and width (±0.01mm). Muscle tissue was removed from *T. rosea* and *D. deltoides*, before being rinsed in a petri-dish with deionised water to exclude any calcareous fragments. Similarly, individual *N. atramentosa* were removed of their shell and operculum before being rinsed with deionised water to remove any remaining calcareous material. *U. lactuca* samples were examined and washed in deionised water to remove any attached microfauna that may be present.

All samples were dried for 24 hours at 65°C. Dried samples were then ground to a fine powder using a mortar and pestle before being scrapped into a glass vial where 1-2mg was be weighed into a tin capsule for SIA.

2.5.2.2 STABLE ISOTOPE ANALYSIS

Biological indicators were sent in tin capsules to UC Davis Stable Isotope Laboratory, California for nitrogen and carbon stable isotope analysis. A PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer was used. Both facilities assessed samples relative to international standards of atmospheric N₂ and Pee Dee Belemite Limestone Carbonate for nitrogen and carbon analysis respectively. Ratios of carbon and nitrogen established from isotope analysis are expressed in conventional delta (δ) notation and were determined by the following calculation:

\[ \delta X (\text{‰}) = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \]
2.6 DATA ANALYSIS

2.6.1 PHYSICAL CHARACTERISTICS

To determine factors driving physical differences (if any) across sandy beaches, water quality (temperature and salinity), and sediment characteristics (Chlorophyll-α, organic matter, grain size and % mud) were analysed by Principal Component Analysis (PCA) in PRIMER using Euclidean distance. Values were not required to be transformed.

2.6.1.1 WATER QUALITY

Water temperature and salinity were analysed by a one-way univariate analysis (ANOVA) in SPSS 22® for fixed factors: site and time, to calculate similarity between all locations and events. Salinity values were not analysed or presented for event 4 however due to faulty readings. Where values were found to differ significantly by either factor in ANOVA, Tukey’s HSD (THSD) post hoc testing was performed to identify the source(s) of difference(s). Mean temperature and salinity were spatially analysed and presented as thematic maps using inverse distance weighting (IDW) in MapInfo Professional 12.5®.

2.6.1.2 SEDIMENT ORGANIC MATTER AND CHLOROPHYLL-A

Sediment organic matter and chlorophyll-α were initially determined by calculation of mean (±) and standard error (SE) in Microsoft Excel®. One-way univariate analysis (ANOVA) was conducted for chlorophyll-α concentrations and organic matter content in SPSS 22® for fixed factors: site and time to calculate individual similarity matrices. When values were found by ANOVA to differ significantly (p < 0.05), Tukey’s HSD (THSD) post hoc testing was performed to identify the source(s) of difference(s). Mean sediment organic matter and chlorophyll-a were spatially analysed and presented as thematic maps using inverse distance weighting (IDW) in MapInfo Professional 12.5®.

2.6.1.3 GRANULOMETRY

Mean particle size of each sample was calculated using pan weights and the Microsoft Excel® add-in “GradiStat”. Two-way analysis of variance (ANOVA) was conducted in SPSS® for fixed
factors: site and time. When values were found to differ significantly ($p < 0.05$), Tukey’s HSD post hoc testing was performed to identify significant differences.

2.6.2 Stable Isotope Analysis

Isotope data was tested for homogeneity of variance (Cochran’s test) and transformed if variances were heterogeneous. Two-way analysis of variance (ANOVA) of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) isotope ratios for pipis, isopods, Ulva and barnacles were analysed for the fixed factors site and time in SPSS11®. Where values were found to differ significantly ($p < 0.05$), Tukey’s HSD post hoc testing was performed to identify significant differences. Mean isotope values were spatially presented for each species analysed in a thematic 3-way plot in Surfer 10®.

Linear regressions were used to examine the relationship between stable isotope ratio and individual size of Pipis (*D. deltoides*) and isopods (*P. concinna*).

3. RESULTS
3.1 PHYSICAL CHARACTERISTICS

Principal component analysis (PCA) on water quality and sediment characteristics at all sandy beaches for typical dry (Event 2) and typical wet (Event 4) conditions indicates that almost all of the variation in beaches by event and site was due to mean grain size (PC1: 99.3%; Figure 7).
3.1.1 WATER QUALITY
3.1.1.1 TEMPERATURE

There was significant seasonal difference in water temperature between Summer (Event 1 and Event 2), Autumn (Event 3) and Winter (Event 4), with average temperatures across beaches cooling from 24.12 ± 0.56°C in January, to 21.97 ±0.92°C in April, and 18.25 ±0.40°C in July ($F_{3, 212} = 228.25$, $p < 0.001$) (Figure 10).

Temperatures varied significantly between sites for all events. Water generally increased in temperature up the coast in summer for both dry and wet events, with sites in
the south of the study area ranging from 22.05 ± 0.03°C and 22.28 ± 0.10°C, warming to 24.69 ± 0.05°C and 27.57 ± 0.02°C in the northernmost sites for Events 1 and 2 respectively (F\textsubscript{11,24} = 59.586, p<0.001; F\textsubscript{18,38} = 19.634, p<0.001). For the east coast low (Event 3), temperature remained relatively constant across sites south of the Hunter River, ranging 1.67°C between Swansea (SWA) and Nobby’s (NOB) with the exception of Newcastle (NEW) which had significantly lower temperature of 20.94 ± 0.00°C (F\textsubscript{19,40} = 288.980, p<0.001). In contrast, sites within and north of the Hunter River were significantly cooler, with average temperatures from Stockton Little Beach (STOL) to Zenith Beach (ZEN) of 20.93 ± 0.02°C, compared to 23.11 ± 0.06°C across the sites south of the Hunter River (p<0.001). These sites were all located within the zone of influence of a large estuarine plume which encompassed all sites north of the Hunter River to varying extents in Event 3 (Figure 10). Temperatures within Event 4 were significantly different across sites, however these differences were driven primarily by specific sites such as Dudley (DUD), Burwood (BUR) and Fingal (FIN) beaches which had significantly cooler temperatures of 17.76 ± 0.01°C, 17.92 ± 0.01°C and 17.99 ± 0.03°C respectively compared to surrounding sites (p<0.001). Similar to Event 3, beaches located within or immediately north of the Hunter River (Nobby’s Dog (NOBD), Stockton Little (STOL), Stockton (STO) and Birubi South (BIRS) had notably reduced temperatures compared to other sites within the study area for Event 4 (p<0.001).

3.1.1.2 SALINITY

Salinity remained relatively similar across the study area for both typical dry (Event 1), typical wet (Event 2) and the extreme wet weather (Event 3) conditions (F\textsubscript{2, 102} = 1.880, p=0.158) (Figure 11). No results were available for Event 4 (Typical Dry).

Within an event, sites differed significantly during wet weather, in both typical (Event 2) and extreme (Event 3) wet weather conditions (p<0.001). Pelican (PEL) and Redhead (RED) beaches were both significantly reduced in salinity (26.02 ± 0.02 and 26.35 ± 0.06 respectively) compared to all other sites in typical wet conditions (Event 2; p<0.01). Similarly, during extreme wet conditions associated with the east coast low (Event 3), Nobby’s Dog Beach (NOBD) and Stockton Little Beach (STOL) within the Hunter River had significantly reduced salinity conditions compared to other sites with averages of 25.83 ± 4.48 and 7.62 ± 0.03
respectively (p<0.001). There were no significant salinity variations between sites during typical dry conditions (Event 1; p>0.05).

Salinity within sites, between events also differed significantly (F_{19,102}=8.552, p<0.001). Belmont (BEL), decreased significantly in salinity in extreme wet conditions (Event 3) compared to typical dry and wet conditions (Event 1 and Event 2 respectively; p<0.001). Similarly, Nobby’s Dog Beach (NOBD) and Stockton Little Beach (STOL), located within the mouth of the Hunter River estuary, also decreased significantly in salinity after extreme wet weather compared to other events (Event 3; p<0.001).

Temperature and salinity values from the Hunter River, as determined by Nobby’s Dog Beach (NOBD), indicate a significant shift from before the peak of the east coast low storm event (Event 3) to conditions after (p<0.001; Figure 9).

![Figure 10: MODIS satellite imagery of Event 3, Hunter River plume including sites within zone of influence. Imagery: MODIS, 23rd April 2015](image)
Figure 11: Mean (±SE) temperature and salinity profile change at site NOBD (Nobby’s Dog Beach) before and after the East Coast Low (Event 3).
Figure 12: Mean thematic temperature (°C) interpolations across sampling locations by event.
Figure 13: Mean thematic salinity interpolation across sampling locations by event.
3.1.2 Organic Matter

Average organic matter composition within surface sediments increased seasonally across the study area with 3.11 ± 0.55% in summer (Event 2), to 3.39 ± 0.23% in autumn (Event 3) and 4.00 ± 0.38% in winter (F$_{2, 122}$=11.427, p<0.001) (Event 4; Figure 12). Organic matter varied temporally and spatially amongst beaches in the study area, however significant differences were highly localised (F$_{38, 122}$= 9.853, p<0.001). While beaches differed in organic matter content over time and between sites, overall the amount of organic material was generally in the range typical of sandy beaches (0-5%), with the exception of a number of sites at Port Stephens in Event 4 (Figure 12).

Sites varied significantly within typical wet (Event 2), extremely wet (Event 3) and typical dry (Event 4) conditions in winter, however no significant difference was evident in organic matter content across sites for typical dry conditions in summer (Event 1; F$_{12,26}$=1.722, p>0.05). In typical wet conditions (Event 2), differences across the study area were driven by Stockton Beach (STO) located immediately north of the Hunter River which had significantly elevated organic matter (8.61 ±2.87%) compared to all other sites which ranged between 1.21% and 4.34% (p<0.001). In extreme wet weather (Event 3), variability between sites was similar and generally remained within the same range of values in Event 2 (1.61% to 4.64%) with the exception of Nobby’s Dog Beach which had significantly elevated organic matter content (5.71%; p<0.001). Blacksmiths Beach (BLA) was significantly lower than all other sites in extreme wet conditions (Event 3) with organic matter content of 1.61% (p<0.001). Similarly, Blacksmiths Beach was also significantly lower in sediment organic matter compared to all other sites in typical dry conditions (Event 4; p<0.001). Both One Mile (ONE) and Fingal Bay (FIN) were significantly elevated in organic matter in Event 4 (10.95% and 11.62%) compared to other sites (p<0.001).

Significant differences also were evident at specific sites across events. Swansea (SWA), One Mile (ONE) and Fingal Bay (FIN) locations were all elevated during typical dry conditions (Event 4) when compared to wet conditions (Events 2 and 3; Table 3; F$_{60, 122}$ = 10.898, p<0.001). Additionally, organic matter content at Swansea increased 3-fold from typical dry conditions in summer (2.21 ± 0.48%) to winter (6.88 ± 0.48%). Stockton Beach (STO) on the other hand had elevated sediment organic matter content during typical wet
conditions (Event 2) (8.61 ±2.87%) in comparison to extreme wet and typical dry events (4.17 ±
0.29% and 2.25 ±0.37% respectively; p<0.001).

Blacksmiths (BLA), Belmont (BEL) and Pelican (PEL) beaches had relatively consistent
and lower sediment organic matter content compared to other sites, with average
concentrations of 1.90% ±0.27, 2.16% ±0.17 and 1.73% ±0.13 across all events respectively
(Figure 12).

3.1.3 Chlorophyll-a

Surface sediment chlorophyll-α concentrations varied significantly both temporally and
 spatially across the study area (Figure 13). Generally, average chlorophyll-α concentrations
were greater across the study area in wet weather conditions (Events 2 and 3) (0.65µg/cm³
±0.11 and 0.62µg/cm³ ±0.016) compared to dry conditions (Events 1 and 4) (0.37µg/cm³
±0.05 and 0.33µg/cm³ ±0.10; p<0.001).

Birubi South (BIRS) and Fingal Bay (FIN) both increased significantly between typical
dry (Event 4) and typical wet (Event 2) conditions (0.37 ± 0.09µg/cm³ to 0.82 ±0.06µg/cm³
and 0.46 ± 0.12µg/cm³ to 2.98 ± 0.33 µg/cm³ respectively; p<0.001). Extreme wet weather
(Event 3) conditions significantly increased chlorophyll-α concentrations for Pelican (PEL),
Belmont (BEL) and Burwood (BUR) beaches compared to typical dry (Events 1 and 4) and
typical wet conditions (Event 3) (p<0.001; p<0.05; p<0.05 respectively).

During wet weather (Events 2 and 3), Fingal Bay had markedly elevated
concentrations relative to all other sites with averages of 2.98 ± 0.33 µg/cm³ and 1.91 ± 0.29
µg/cm³, compared to averages across all other sites which were more than 2-fold less at
0.52 ± 0.10 µg/cm³ and 0.56 ± 0.16 µg/cm³ for Events 2 and 3 respectively (F20,42=2.379,
p<0.009). Stockton Little Beach (STOL) on the other hand had the lowest chlorophyll-α
concentrations (0.07 ±0.01 and 0.19 ±0.13) across all sites for both typical (Event 2) and
extreme wet (Event 3) conditions respectively (p<0.05).
Table 3: Differences between Typical Dry (Event 4) and Typical Wet (Event 2); and Typical Dry (Event 4) and Extreme Wet (Event 4) for mean sediment Chlorophyll-α (mg/cm$^3$) and Organic Matter (%).

↑ indicates significant difference and direction of difference (p<0.001), whereas ↑ indicates increasing or decreasing difference and direction of difference.

<table>
<thead>
<tr>
<th>Site</th>
<th>Chlorophyll-α (mg/cm$^3$)</th>
<th>Organic Matter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Typical Dry → Typical Wet</td>
<td>Typical Dry → Extreme Wet</td>
</tr>
<tr>
<td>SWA</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BLA</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>PEL</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BEL</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>RED</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>DUD</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BUR</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>MER</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BAR</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>NEW</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>NOB</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>NOBD</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>STOL</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>STO</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BIRS</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BIR</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>ONE</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>FIN</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>BOX</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>ZEN</td>
<td>↑</td>
<td>↓</td>
</tr>
</tbody>
</table>

37
Figure 14: Mean thematic Sediment Organic Matter (%) interpolation across sampling locations by event.
Figure 15: Mean sediment Chlorophyll-a (g/cm³) thematic interpolation across sampling locations by event.
3.1.4. GRANULOMETRY

3.1.4.1 GRAIN SIZE

A comparison of Events 2 and 4 (typical wet and typical dry events) indicated significant variation in mean grain sizes both spatially and temporally ($F_{1,80} = 7.255, p<0.001$). Across both events, grain sizes generally ranged between 400 µm and 750 µm, with the exception of Birubi and Birubi South (BIR and BIRS), One Mile Beach (ONE) and Fingal Bay (FIN) in Port Stephens which had smaller average grain sizes (291.6 ± 13.5µm, 271.0 ±12.6µm and 328.1 ±16.3µm respectively; Figure 14). Grain size within sites generally increased from typical wet (Event 2) to typical dry conditions (Event 4) for all sites. Swansea (SWA) and Dudley (DUD) had the greatest significant increase in sediment grain size, increasing between typical wet (Event 2) and typical dry conditions (Event 4) by 74% and 57% respectively ($p<0.05$). Similarly, both Belmont (BEL) and Bar (BAR) beaches increased significantly by 24% from Event 2 to Event 4 ($p<0.05$ and $p<0.01$), whereas Burwood Beach (BUR) increased significantly by 30% ($p<0.01$). Stockton Little Beach (STOL) within the Hunter River, and the beaches Stockton (STO) and Birubi South (BIRS) immediately north of the mouth of the Hunter River however decreased from typical wet (Event 2) to typical dry conditions (Event 4). Stockton Beach (STO) decreased significantly by 38% in mean grain size between wet (Event 2) and dry (Event 4) events ($p<0.01$), whereas both Stockton Little (STOL) and Birubi South (BIRS) decreased by 10% and 7% respectively. Grain size also decreased at Fingal and Zenith beaches in Port Stephens, by 16% and 6% between wet (Event 2) and dry (Event 4) events, however was not considered statistically reduced.

There was no significant relationship between sediment organic matter and grain size in typical wet and typical dry conditions across all sites ($r=-0.304, p<0.0001, n=120$).

3.1.4.2. MUD CONTENT

Sediment mud content was relatively low (~0.01%) across the study area for Events 2 and 4 (typical wet and typical dry events), and did not vary significantly between the two conditions ($F_{1,80}=3.740, p>0.05$; Figure 17). Mud composition in sediments however did vary significantly
between sites within an event (p<0.001). During typical wet conditions (Event 2) mud content was elevated at Nobby’s Dog Beach (NOBD) within the mouth of the Hunter River relative to other sites (p<0.001). In typical dry conditions (Event 4), mud content was only elevated at Fingal Bay (FIN) (0.18 ±0.095%) relative to other sites within the same event (0.02 ±0.004%) (p<0.001). Small increases in sediment mud content were also evident at sites Redhead (RED) (0.01 ±0.003% to 0.03 ±0.007%) and Stockton Little Beach (STOL) (0.01 ±0.004%), however these increases were marginal and not significant.
Figure 16: Mean Sediment Grain Size (µm) thematic interpolation across sampling locations by event (Typical Wet – Event 2, and Typical Dry – Event 4)

Figure 17: Mean %Mud composition interpolation across sampling locations by event (Typical Wet – Event 2, and Typical Dry – Event 4)
3.2 STABLE ISOTOPE ANALYSIS

3.2.2 PIPIS

3.2.2.1 CARBON

Pipis (*D. deltoides*) were collected from all sandy beach locations except Nobby’s Dog Beach (NOBD) and Stockton Little Beach (STOL) within the Hunter River. There was significant variability in δ\(^{13}\)C both temporally and spatially (*F*\(_{15, 247}\)=9.056, p<0.001), ranging from -15.55 ‰ to -19.59‰ (Figure 17a). During extreme wet conditions (Event 3), sites varied significantly with notable differences in the north of the study area (Port Stephens; Figure 19c and 20a). Box Beach (BOX) and Zenith Beach (ZEN) had significantly reduced δ\(^{13}\)C values (-18.42 ± 0.28‰ and -18.35 ± 0.34‰) compared to Birubi (BIR) and Birubi South (BIRS) in the south of Port Stephens (-16.38 ± 0.13‰ and -16.39 ± 0.13‰ respectively; p<0.05). A similar trend was reflected between sites in typical dry conditions (Event 4; Figure 18d and 19a) in which Birubi South (BIRS) and Birubi (BIR) had significantly elevated δ\(^{13}\)C values (-16.18 ± 0.32‰ and -16.38 ± 0.11‰) compared to all other sites within the event (p<0.05). Whereas Zenith Beach (ZEN), located at the northernmost site of the study area, had a significantly reduced δ\(^{13}\)C value (-18.51 ± 0.24‰; p<0.05). Swansea Beach (SWA), the southernmost site in the study area also had a significantly reduced carbon signature for Event 4 compared to all other sites (-18.57 ± 0.04‰; p<0.05; Figure 18; Figure 19a).

Carbon signatures also varied significantly within a site, between events. Both Birubi (BIR) and Birubi South (BIRS) were significantly depleted in \(^{13}\)C in typical wet conditions (Event 2; -17.83 ± 0.28‰ and -17.73 ± 0.18‰) compared to both extreme wet (Event 3; -16.39 ± 0.13‰ and -16.18 ± 0.16‰) and typical dry (Event 4; -16.53 ±0.24‰) conditions (p<0.05; Figure 18 and 19a). Similarly, Stockton Beach (STO) was also significantly depleted during typical wet conditions (Event 2; -18.20 ± 0.11‰) compared to typical dry (Event 1; -17.89 ± 0.21‰ and Event 4; -17.52 ± 0.18‰) and extreme wet conditions (Event 3; -17.98 ± 0.11‰; p<0.05; Figure 18 and 19a).
3.2.2.2 NITROGEN

Similarly to carbon, nitrogen isotopic values ($\delta^{15}$N) within Pipis varied significantly between both event and site ($F_{15, 247}=11.525, p<0.001$). Nitrogen isotope values varied between sites within typical wet (Event 2; Figure 18b), extreme wet (Event 3; Figure 18c) and typical dry conditions in winter (Event 4; Figure 18d). One Mile Beach (ONE) was significantly depleted in $\delta^{15}$N compared to other sampled sites for both typical wet (Event 2; Figure 18b) and extreme wet (Event 3; Figure 18c) conditions (7.59 ± 0.16‰ and 7.93 ± 0.11‰ respectively; $p<0.05$; Figure 19b). Conversely, Pelican Beach (PEL) was significantly enriched (8.78 ± 0.10‰) in typical wet conditions (Event 2; Figure 18b and 19b) compared to other sites within the same event ($p<0.05$, Figure 19b). Zenith Beach (ZEN) and Fingal Bay (FIN) were depleted compared to other sites in wet weather events (7.95 ± 0.17‰ and 7.96 ± 0.17‰ for Event 2, and 7.96 ± 0.15‰ and 8.26 ± 0.22‰ for Event 3, respectively; Figure 19b). Both Birubi (BIR) and Birubi South (BIRS) beaches had significantly elevated $\delta^{15}$N signatures (89.13 ±0.11‰ and 9.37 ± 0.18‰, respectively) compared to other sites in the extreme wet weather event (Event 3; $p<0.05$; Figure 18c and 19b). Similarly, Belmont (BEL) and Nobby’s Beach (NOB) were also significantly enriched (8.85 ± 0.02‰ and 8.86 ± 0.01‰, $p<0.05$; Figure 19b). Within typical dry conditions (Event 4), Birubi South (BIRS) was elevated significantly compared to all other sites (9.81 ± 0.37‰), whereas Zenith (ZEN), Box (BOX) and Stockton (STO) beaches had significantly depleted values (8.02 ± 0.15‰, 8.14 ± 0.09‰, 8.11 ± 0.11‰ respectively; $p<0.05$; Figure 18d and 19b).

$\delta^{15}$N signatures also varied significantly within sites, between events (Figure 18 and 19b). In typical wet weather (Event 2) both Birubi (BIR) and Birubi South (BIRS) had significantly reduced $\delta^{15}$N values (8.18 ± 0.34‰ and 8.03 ± 0.13‰) compared to extreme wet weather (Event 3; 9.13 ± 0.11‰ and 9.37 ± 0.18‰) and typical dry weather conditions (Event 4; 8.96 ± 0.08‰ and 9.81 ± 0.37‰; $p<0.05$; Figure 19b). Conversely, Burwood Beach (BUR) and Fingal Bay (FIN) were both significantly enriched in nitrogen (8.61 ± 0.08‰ and 9.10 ± 0.12‰) for typical dry (Event 4) compared to other events ($p<0.001$; Figure 19b).
Figure 18: Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of Pipi (*Donax deltoides*). A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), D: Event 4 (Typical Dry). Values are mean ± SE.

Figure 19: Stable a) carbon ($\delta^{13}C$) and b) nitrogen ($\delta^{15}N$) isotope thematic interpolation of Pipi (*Donax deltoides*). Values are means.
3.2.2.3 STABLE ISOTOPE COMPOSITION AND SIZE

There was a significant positive relationship between $^{13}$C stable isotope values and pipi length across eight sites (Figure 18). Pipi length explained approximately 73 to 90% of the variation in stable carbon isotope ratios at sites (for example, Birubi beach (BIR) during Event 2: $F_{1,11}=82.227$, $p<0.001$, $r^2 = 0.901$; Figure 21a, Table 4). Similarly, there was a significant positive relationship between $^{15}$N and pipi length explaining 66 to 76% of the variation in stable nitrogen isotope composition at four sites (Table 4, Figure 21). Fingal Bay (FIN) in typical wet (Event 2) and extremely wet conditions (Event 3), and Birubi Beach South (BIRS) in extremely wet conditions all had a significant positive relationship with stable nitrogen isotope composition (Table 4, Figure 21). However, Zenith Beach (ZEN) in typical dry conditions (Event 4) had a significant negative relationship between size and $^{15}$N ($F_{1,9}=15.487$, $p=0.004$, $r^2 = 0.659$; Figure 21d).

<table>
<thead>
<tr>
<th>Site</th>
<th>Event</th>
<th>Isotope</th>
<th>df</th>
<th>F</th>
<th>p</th>
<th>$r^2$</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 (BIR)</td>
<td>2</td>
<td>$^{13}$C</td>
<td>1, 11</td>
<td>82.227</td>
<td>0.000</td>
<td>0.901</td>
<td>19a</td>
</tr>
<tr>
<td>20 (ZEN)</td>
<td>2</td>
<td>$^{13}$C</td>
<td>1, 9</td>
<td>26.079</td>
<td>0.001</td>
<td>0.765</td>
<td>19b</td>
</tr>
<tr>
<td>5 (RED)</td>
<td>3</td>
<td>$^{13}$C</td>
<td>1, 8</td>
<td>18.9714</td>
<td>0.003</td>
<td>0.730</td>
<td>19c</td>
</tr>
<tr>
<td>18 (FIN)</td>
<td>3</td>
<td>$^{13}$C</td>
<td>1, 9</td>
<td>55.269</td>
<td>0.000</td>
<td>0.874</td>
<td>19d</td>
</tr>
<tr>
<td>19 (BOX)</td>
<td>3</td>
<td>$^{13}$C</td>
<td>1, 9</td>
<td>27.418</td>
<td>0.001</td>
<td>0.774</td>
<td>19e</td>
</tr>
<tr>
<td>20 (ZEN)</td>
<td>3</td>
<td>$^{13}$C</td>
<td>1, 9</td>
<td>39.810</td>
<td>0.000</td>
<td>0.833</td>
<td>19f</td>
</tr>
<tr>
<td>7 (BUR)</td>
<td>4</td>
<td>$^{13}$C</td>
<td>1, 9</td>
<td>25.770</td>
<td>0.001</td>
<td>0.763</td>
<td>19g</td>
</tr>
<tr>
<td>20 (ZEN)</td>
<td>4</td>
<td>$^{13}$C</td>
<td>1, 9</td>
<td>21.179</td>
<td>0.002</td>
<td>0.726</td>
<td>19h</td>
</tr>
<tr>
<td>18 (FIN)</td>
<td>2</td>
<td>$^{15}$N</td>
<td>1, 11</td>
<td>22.001</td>
<td>0.001</td>
<td>0.688</td>
<td>20a</td>
</tr>
<tr>
<td>15 (BIRS)</td>
<td>3</td>
<td>$^{15}$N</td>
<td>1, 9</td>
<td>25.535</td>
<td>0.001</td>
<td>0.761</td>
<td>20b</td>
</tr>
<tr>
<td>18 (FIN)</td>
<td>3</td>
<td>$^{15}$N</td>
<td>1, 9</td>
<td>18.842</td>
<td>0.002</td>
<td>0.702</td>
<td>20c</td>
</tr>
<tr>
<td>20 (ZEN)</td>
<td>4</td>
<td>$^{15}$N</td>
<td>1, 9</td>
<td>15.487</td>
<td>0.004</td>
<td>0.659</td>
<td>20d</td>
</tr>
</tbody>
</table>
Figure 20: Carbon isotope ($\delta^{13}C$) and *D. deltoides* size regression bi-plots for significant (p<0.05) results by sites within events.

Figure 21: Nitrogen isotope ($\delta^{15}N$) and *D. deltoides* size regression bi-plots for significant (p<0.05) results by sites within events.
3.2.3 ISOPODS

3.2.3.2 CARBON

Isopod (*P. concinna*) carbon isotope values (δ¹³C) were only significantly different between sites in the study area ($F_{14,149}=5.762$, $p<0.001$). Sites within events varied significantly in all cases (Figure 22, Figure 23a). In typical dry conditions (Event 1) carbon values reduced in a gradient scale up the coastline from Swansea (SWA) in the south (-16.39 ± 0.67‰) to Bar Beach (BAR) in the north (-20.25 ± 0.49‰; Figure 23a). Swansea was significantly enriched in carbon compared to other sites within Event 1, as was Redhead (RED) however to a lesser degree (-17.30 ± 0.60‰; $p<0.001$, Figure 22a). Additionally, Dudley (DUD), Burwood (BUR) and Merewether (MER) all had similar δ¹³C values, whereas Bar Beach (BAR) located 500 metres further north of Merewether had a significantly different carbon signature (-20.25 ± 0.49‰; $p<0.001$, Figure 23a). In typical wet conditions (Event 2) Belmont (BEL) was significantly enriched in ¹³C compared to other sites (-16.00 ± 0.71‰; $p<0.05$), whereas both Merewether (MER) and Bar Beach (BAR) were significantly depleted (-19.14 ±0.18‰ and -19.51 ±0.81‰ respectively; $p<0.05$, Figure 22b). In typical dry conditions in winter (Event 4), One Mile Beach (ONE) was significantly enriched in ¹³C compared to all other sites both within the event as well as across all events (-13.11 ±0.20‰) ($p<0.001$; Figure 21a).

Variance of carbon values within sites, across events was also significant for both Swansea (SWA) and Redhead (RED) beaches. Carbon signatures in typical dry conditions in summer (Event 1) were significantly enriched (-16.39 ±0.60‰, and -17.30 ± 0.72‰ respectively) compared to all other events in both sites ($F_{2,21}=26.256$, $p<0.001$; $F_{2,19}=9.882$, $p=0.001$, Figure 23a). No further analysis of variance within sites across events was possible due to lack of replication at sites.

3.2.3.3 NITROGEN

Nitrogen isotopes (δ¹⁵N) varied within isopods across the study area between both event and site ($F_{11,149}=5.898$, $p<0.001$; Figure 23b).
Within typical dry conditions in summer (Event 1), Swansea (SWA) and Redhead (RED) were significantly depleted in nitrogen values compared to all other sites (8.54 ±0.31‰ and 9.37 ±0.35‰ respectively, p<0.05, Figure 22a, Figure 23b). Typical dry conditions in winter (Event 4) however had significantly depleted nitrogen values at both One Mile Beach (ONE) and Birubi Beach (BIR) compared to other sites (7.51 ±0.81‰ and 9.91 ±0.18‰ respectively, p<0.05, Figure 22b, Figure 23b). Isopods at Nobby’s Beach (NOB) were enriched in nitrogen (12.55 ±0.36‰) compared to other sites in Event 4 however were not statistically significant (Figure 22d). There were no significant differences in nitrogen values across sites in wet conditions (Event 2 and Event 3; p>0.05; Figure 22). Across all events, One Mile Beach (ONE) was significantly depleted in nitrogen values (7.51 ±0.81‰) compared to all other locations which ranged between (9.91 ±0.18‰ and 11.54 ±0.27‰; Figure 23b).

Similarly to carbon, nitrogen (δ¹⁵N) signatures varied within sites, across events for both Swansea (SWA) and Redhead Beaches (RED; p<0.001; Figure 23b). In typical dry conditions in summer (Event 1) nitrogen was significantly depleted across both sites compared to other events (8.55 ±0.31‰ and 9.37 ±0.36‰; p<0.05; Figure 23b). On the other hand, typical dry conditions in winter (Event 4) were significantly enriched across both sites compared to other events (12.07 ±0.26‰ and 11.14 ±0.49‰; p<0.001). Burwood Beach (BUR) also varied across events with significantly depleted ¹⁵N in typical dry conditions in winter (Event 4; 10.50 ±0.23‰), and significantly enriched values in typical wet conditions (Event 2; 11.46 ±0.32%; p<0.05; Figure 23b). There were no other notable temporal variations in isopod nitrogen values at other sites.
Figure 22: Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition $P.\ concinna$. A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), D: Event 4 (Typical Dry). Values are mean $\pm$ SE.

Figure 23: Stable a) carbon ($\delta^{13}C$) and b) nitrogen ($\delta^{15}N$) isotope thematic interpolation of Isopod ($Pseudolana concinna$). Values are means.
3.2.3.3 STABLE ISOTOPE COMPOSITION AND SIZE

There was a significant positive relationship between $^{13}$C and isopod length at Zenith Beach in typical dry conditions (Event 4; $F_{1,6}=7.054$, $p=0.045$, $r^2=0.585$, Figure 24a), whereas Zenith Beach (ZEN) in extreme wet (Event 3) conditions, however, had a significant negative relationship between size and $^{13}$C ($F_{1,10}=15.842$, $p=0.003$, $r^2=0.638$; Figure 22b). Similar to $^{13}$C, there was a significant positive relationship between $^{15}$N stable isotope values and isopod length at Dudley (DUD) in typical dry conditions (Event 1; Figure 24c) with isopod length explaining approximately 73% of the variation in stable carbon isotope ratios (Event 1; $F_{1,9}=15.899$, $p=0.007$, $r^2 = 0.726$, Table 5).

Table 5: *P. concinna* regression summary table for carbon ($^{13}$C) and nitrogen ($^{15}$N) isotopes and size

<table>
<thead>
<tr>
<th>Site</th>
<th>Event</th>
<th>Isotope</th>
<th>df</th>
<th>$F$</th>
<th>$p$</th>
<th>$r^2$</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 (ZEN)</td>
<td>3</td>
<td>$^{13}$C</td>
<td>1, 10</td>
<td>15.842</td>
<td>0.003</td>
<td>0.638</td>
<td>a</td>
</tr>
<tr>
<td>20 (ZEN)</td>
<td>4</td>
<td>$^{13}$C</td>
<td>1, 6</td>
<td>7.054</td>
<td>0.045</td>
<td>0.585</td>
<td>b</td>
</tr>
<tr>
<td>6 (DUD)</td>
<td>1</td>
<td>$^{15}$N</td>
<td>1, 9</td>
<td>15.899</td>
<td>0.007</td>
<td>0.726</td>
<td>c</td>
</tr>
</tbody>
</table>

Figure 24: Isotope ($\delta^{15}$N and $\delta^{13}$C) and *P. concinna* size regression bi-plots for significant ($p<0.05$) results by sites within events. a) Nitrogen isotope ($\delta^{15}$N) v. *P. concinna* size at Dudley, Event 1, b) Carbon isotope ($\delta^{13}$C) v. *P. concinna* size at Zenith Beach, Event 3 and c) Carbon isotope ($\delta^{13}$C) v. *P. concinna* size at Zenith Beach, Event 4.
3.2.4 **NERITA**

3.2.4.2 CARBON

Nerita (*N. atraentosa*) varied significantly in $^{13}$C values between rocky shore sites and events ($F_{23,109}$=21.379, $p<$0.001).

Within typical wet conditions (Event 2) both Zenith Beach (ZEN) and Fingal Bay (FIN) were significantly depleted in $^{13}$C values compared to other sites (-15.97 ±0.29‰ and -15.35 ±0.37‰, $p<$0.05; Figure 26, Figure 27a). Newcastle Beach (NEW) was also relatively depleted compared to other sites, however to a lesser degree than Fingal and Zenith beaches (-13.89 ±0.67‰, $p<$0.05; Figure 26b). Nobby’s Beach (NOB) and Boulder Bay (BOU) were significantly enriched in $^{13}$C (-11.18 ±0.37 ‰ and -10.67 ±0.28‰) compared to other sites in typical wet conditions (Event 2; $p<$0.05; Figure 26a). In extreme wet conditions (Event 3), Fingal Bay (FIN) and Zenith Beach (ZEN) were significantly depleted compared to other sites, similar to typical wet conditions ($p<$0.05; Figure 26c, Figure 27a). Swansea Beach (SWA) was also significantly depleted to other sites within event 3, however to a lesser degree (-14.58 ±0.26‰, $p<$0.05; Figure 26c). In typical dry conditions in winter (Event 4), $^{13}$C values were most depleted at both Swansea Beach (SWA) and Zenith Beach (ZEN), the most southward and northward sites in the study area respectively (-15.32 ±1.02‰ and -15.23 ±0.16 ‰; $p<$0.05; Figure 26d, Figure 27a). Dudley Beach (DUD) was the only site significantly enriched in $^{13}$C during typical dry conditions (-10.98 ±0.43; $p<$0.05; Event 4; Figure 26d, Figure 27a)

Both Burwood (BUR) and Zenith (ZEN) rocky shores increased significantly in $^{13}$C between extreme wet conditions (Event 3; -13.38 ±0.10‰ and -16.70 ±0.41‰) and typical dry conditions (Event 4; -11.64 ±0.08 and -15.23 ±0.15‰; $p<$0.05; Figure 27a). Boulder Bay (BOU) on the other hand decreased significantly in $^{13}$C from 10.67 ±0.29‰ in typical wet conditions (Event 2) to -13.31 ±0.69‰ in typical dry conditions (Event 4; $p<$0.05, Figure 27a).

3.2.4.3 NITROGEN

*N. atramentosa* varied in nitrogen isotope signatures significantly across sites and events ($F_{23,109}$=3.462, $p<$0.001; Figure 25, Figure 26b).
Dudley (DUD) and Burwood (BUR) were both significantly depleted (7.87 ±0.47‰ and 8.07 ±0.20‰) in $^{15}$N compared to other sites in typical dry conditions in summer (Event 1, $p<0.05$; Figure 25a, Figure 26b). Similarly Bar Beach (BAR) was depleted in $^{15}$N, however to a lesser degree than Dudley and Burwood beaches (Figure 25a). Burwood Beach (BUR) was also significantly depleted (8.07 ±0.39‰) in typical wet conditions (Event 2) compared to other sites, with the exception of Newcastle (NEW) and Swansea (SWA) locations, which were also significantly depleted compared to other sites however to a lesser degree (8.79 ±0.67‰; 8.89 ±0.41‰, $p<0.001$, Figure 25b, Figure 26b). Within extreme wet conditions (Event 3), Bar Beach (BAR) was the only site with a significantly depleted $^{15}$N signature compared to other sites (7.49 ±0.63‰, $p<0.05$, Figure 26b). There were no significantly enriched $^{15}$N values at any sites across typical dry (Event 1), typical wet (Event 2) or extremely wet conditions (Event 3; Figure 25, Figure 26b). Nobby’s Dog Beach (NOBD), within the mouth of the Hunter River and Birubi Beach (BIR) both had significantly enriched signatures (9.43 ±0.16‰ 9.39 ±0.11‰) within typical dry conditions in winter (Event 4) compared to other sites ($p<0.05$, Figure 25d, Figure 26b). Similarly to typical dry conditions in summer and typical wet conditions (Event 1 and 2 respectively), Burwood Beach (BUR) was the most depleted location in typical dry conditions in winter (8.45 ±0.29‰), however this value was not significant ($p>0.05$, Figure 25d).

Bar Beach (BAR) was significantly depleted in $^{15}$N values in the extreme wet weather event (Event 3; 7.48 ±0.63‰) compared to all other events (Event 1; 8.80 ±0.13‰, Event 2; 9.24 ± 0.10% and Event 4; 9.00 ±0.17‰, $p<0.01$; Figure 25c, Figure 26b). No other significant temporal $^{15}$N variation was evident across sites.
Figure 25: Stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope composition of Nerita (Nerita atramentosa). A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), Event 4 (Typical Dry).

Figure 26: Stable a) carbon ($\delta^{13}$C) and b) nitrogen ($\delta^{15}$N) isotope thematic interpolation of Nerita (Nerita atramentosa). Values are means.
3.2.5 BARNACLE

3.2.5.2 CARBON

Carbon signatures ($\delta^{13}$C) varied significantly in *T. rosea* both temporally and spatially ($F_{23,79}$=2.837, $p<0.001$; Figure 27, Figure 28). Across the study area, typical wet conditions (Event 2) were significantly depleted in $^{13}$C compared to typical dry conditions in winter (Event 4, $p<0.001$; Figure 27b, Figure 28a). Sites varied significantly within typical wet (Event 2), extreme wet (Event 3) and typical dry weather in winter (Event 4).

In typical wet conditions (Event 2), Nobby’s Dog Beach (NOBD) within the Hunter River estuary had significantly enriched values compared to other sites (-14.97 ± 0.78‰; $p<0.05$). Within extreme wet conditions (Event 3) Boulder Bay (BOU) was the only site significantly enriched (-16.69 ± 0.03‰), whereas Redhead (RED) correspondingly was the only site significantly depleted (-18.88 ± 0.14‰; $p<0.001$; Figure 27c, Figure 28a). In typical dry conditions in winter (Event 4), Swansea (SWA) was significantly enriched in $^{13}$C compared to other sites (-17.91 ± 0.64‰; $p<0.001$; Figure 27d).

$^{13}$C values varied significantly between events for sites Dudley (DUD), Bar (BAR), Nobby’s Dog Beach (NOBD), and Fingal Bay (FIN) (Figure 27a). Typical wet conditions (Event 2) had elevated $^{13}$C at Dudley Beach (17.08 ± 0.23‰) compared to typical dry conditions (Event 1; -18.88 ± 0.23‰ and Event 4; -18.56 ± 0.08‰; $p<0.01$; Figure 27b, Figure 28a). Similarly, typical wet conditions elevated $^{13}$C at Nobby’s Dog Beach (NOBD; -14.97 ± 0.78‰) relative to all other conditions ($p<0.001$; Figure 27b, Figure 28a). At Bar Beach (BAR), $^{13}$C decreased incrementally throughout the year from -17.44 ± 0.13‰ in Event 1, to -18.37 ± 0.11‰ in Event 3 and -19.06 ± 0.11‰ in Event 4 ($p<0.001$; Figure 28). Both typical and extreme wet conditions (Event 2 and 3) at Fingal Bay (FIN) had significantly enriched $^{13}$C (-17.99 ± 0.13‰ and -18.02 ± 0.11‰) compared to typical dry conditions (Event 4; -18.86 ± 0.13‰; $p<0.05$; Figure 27d, Figure 28a).

3.2.5.3 NITROGEN

Nitrogen varied significantly across both events and sites in the study area ($F_{23,79}$=3.117, $p<0.001$). Sites within events also varied significantly for all events (Figure 29).
Within typical dry conditions (Event 1) both Dudley (DUD) and Burwood (BUR) were significantly depleted in $^{15}$N (10.51 ± 0.16‰ and 10.53 ± 0.11‰) compared to Swansea (SWA) and Redhead (RED) (11.87 ± 0.23‰ and 11.50 ± 0.27‰; p<0.01; Figure 27a, Figure 28b). Similarly, in typical wet conditions (Event 2), Redhead (RED) was significantly enriched (12.33 ± 0.04‰) compared to Newcastle (NEW), Nobby’s Beach (NOB), Zenith Beach (ZEN) and Boulder Bay (BOU; p<0.05; Figure 27b, Figure 28b). Extreme wet weather had less variability in comparison to typical dry (Event 1) and typical wet (Event 2) conditions, with all sites within the range of 1‰ (Figure 27c, Figure 28b). Bar Beach (BAR) was the most $^{15}$N depleted site in event 3, and was significantly reduced compared to Redhead (RED), Dudley (DUD) and Birubi (BIR) beaches as well as Fingal (FIN) and Boulder Bay (BOU; Figure 27c). Correspondingly, Birubi Beach (BIR) was the most enriched location in extreme wet conditions (Event 3; 11.84 ± 0.13‰). Similarly to extreme wet conditions, Bar Beach (BAR) was the most depleted nitrogen signature in typical dry conditions (Event 4) (10.50 ±0.25‰), and was significantly depleted compared to Swansea (SWA) and Burwood (BUR) beaches (11.69 ± 0.29‰ and 11.57 ± 0.05‰ respectively, p<0.05, Figure 27d, Figure 28b). Conversely, within typical dry conditions (Event 4), Swansea was the most enriched site, with significant enrichment compared to Bar Beach (BAR) and Fingal Bay (FIN, p<0.05, Figure 27d, Figure 28b).

$^{15}$N values also varied significantly within sites across events for Redhead (RED), Dudley (DUD), Burwood (BUR) beaches and Fingal (FIN) Bay (Figure 28, Figure 29b). Redhead (RED) had elevated $^{15}$N during typical wet conditions (Event 2; 12.33 ± 0.04‰) compared to typical dry conditions (Event 1; 11.50 ± 0.27‰ and Event 4; 11.41 ± 0.11‰, p<0.05, Figure 28b). Dudley Beach (DUD) and Burwood Beach (BUR) were both significantly depleted in typical dry conditions in summer (Event 1; 10.51 ± 0.11‰ and 10.53 ± 0.16‰ respectively) compared to other events (p<0.001; Figure 27, Figure 28a). Typical dry conditions (Event 4) at Fingal Bay (FIN) indicated significantly depleted $^{15}$N (10.66 ± 0.29‰) compared to extreme wet conditions (Event 3; 11.58 ± 0.06‰; p<0.05; Figure 27d, Figure 28).
Figure 27: Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of Barnacle (Tesseropora rosea). A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), D: Event 4 (Typical Dry). Values are mean ± SE.

Figure 28: Stable a) carbon ($\delta^{13}C$) and b) nitrogen ($\delta^{15}N$) isotope thematic interpolation of Barnacle (Tesseropora rosea). Values are means.
3.2.6 ULVA

3.2.6.2 CARBON

_U. lactuca_ $^{13}$C signatures varied significantly across both event and site ($F_{19,66}=4.886$, $p<0.001$). $^{13}$C of sites within events were significantly different across all conditions (Figure 29).

In typical dry conditions (Event 1), Redhead (RED) was the most depleted site (-18.86 ± 0.25‰) and was significantly lower than the most enriched site, Nobby's Beach (NOB) (-16.62 ± 0.29‰; $p<0.01$; Figure 29a, Figure 30a). Similarly to typical dry conditions, Redhead (RED) was the most depleted site in typical wet conditions (Event 2; -18.45 ± 0.05‰), however was only significantly different to the most enriched location, Zenith Beach (-11.51 ± 2.43‰, $p<0.01$, Figure 29b, Figure 30a). Extreme wet conditions as a result of the east coast low (Event 3) had greater variability in carbon signatures across sites than any other event, with mean $^{13}$C ranging from -16.21‰ to -9.21‰ (Figure 29c). Both Redhead (RED) and Bar Beach (BAR) were the most depleted sites in Event 3 (-15.99 ± 0.59‰ and -16.21 ± 0.06‰), and were significantly different to all sites north of Newcastle (NEW) with the exception of Nobby's Beach (NOB), which was also depleted (-14.92 ± 0.06‰; $p<0.001$; Figure 29c, Figure 30a). Both Fingal Bay (FIN) and Birubi Beach (BIR) were significantly elevated in $^{13}$C compared to all other sites (-9.21 ± 0.02‰ and -10.14 ± 0.52‰ respectively; $p<0.001$; Figure 30a). In typical dry conditions in winter (Event 4), Nobby's Beach (NOB) was significantly enriched compared to all other sites (-12.57 ± 0.71‰) excluding Swansea (SWA) and Birubi Beach (BIR) which were also elevated in $^{15}$N, however to a lesser degree (-14.87 ± 1.30‰ and -14.93 ± 0.26‰; $p<0.001$; Figure 29d).

$^{13}$C varied significantly within sites across conditions with the exception of Bar Beach (BAR) and Zenith (ZEN) beaches which remained within the range of -17.69 ± 0.36‰ to -16.09 ± 0.06‰ and -16.90 ± 0.13‰ to -11.51 ± 2.43‰ respectively (Figure 29). At Redhead (RED), typical dry (Event 1) and typical wet conditions (Event 2) were statistically similar (-18.86 ± 0.07‰ and -18.44 ± 0.15‰) however were significantly depleted from both typical dry conditions in winter (Event 4; -16.49 ± 0.26‰), and extreme wet conditions (Event 3; -15.99 ± 0.59‰; $p<0.01$; Figure 30a). Dudley Beach (DUD) was significantly depleted in
typical dry conditions in summer with mean \^{13}C of -18.76 ± 0.11‰ (Event 1) compared to typical wet (Event 2; -15.29 ± 0.37‰), extreme wet (Event 3; -15.27 ± 0.86‰) and typical dry conditions in winter (Event 4; -13.62 ± 0.24‰; \(p<0.001\); Figure 29, Figure 30a). Burwood Beach (BUR) varied significantly in \^{13}C across events, with typical dry (Event 1) and typical wet (Event 2) conditions statistically similar, with mean values of -17.60 ± 0.31‰ and -17.39 ± 0.27‰ (Figure 29, Figure 30a). Typical dry conditions in winter (Event 4) however were significantly enriched compared to Event 1 and 2 (-15.25 ± 0.06‰; Figure 31a). Similarly, extreme wet weather (Event 3) resulted in significant carbon enrichment compared to all other events (-13.86 ± 0.21‰; \(p<0.001\); Figure 29c). At both Newcastle (NEW) and Birubi (BIR) beaches, extreme wet conditions (Event 3) significantly elevated carbon signatures compared to all other events (-12.84 ± 1.01‰; \(p<0.01\), and -10.13 ± 0.52‰; \(p<0.001\) respectively; Figure 29c).

3.2.6.3 NITROGEN

\(^{15}N\) varied significantly between both event and site (\(F_{19,66}=4.006, p<0.001\); Figure 29). Sites within events varied significantly across typical wet conditions (Event 2), extreme wet conditions (Event 3) and typical dry conditions in winter (Event 4). There were no significant differences in \(^{15}N\) values within typical dry conditions in summer (Event 1; \(p>0.05\); Figure 30).

Within typical wet conditions (Event 2), Dudley (DUD) was most depleted across sites in \(^{15}N\) (6.32 ± 0.14‰) and was significantly different to Redhead Beach (RED) which was most enriched for typical wet conditions (7.24 ± 0.05‰; \(p<0.01\); Figure 29b, Figure 30b). Nitrogen signatures in extreme wet weather (Event 3) were most enriched at both Newcastle (NEW) and Birubi (BIR) beaches (8.40 ± 0.11‰ and 8.26 ± 0.11‰; Figure 30b). Zenith beach (ZEN) was the most depleted site in extreme wet conditions (7.07 ± 0.38‰), however was only significantly different to both Newcastle and Birubi beaches (\(F_{8,18}=6.168, p<0.001\); Figure 29c, Figure 30b). In typical dry conditions in winter (Event 1), Nobby’s Beach (NOB) was significantly depleted (6.77 ± 0.31‰) compared to all other sites, with the exception of Zenith (ZEN) and Birubi (BIR) beaches which were also depleted in \(^{15}N\) (7.26 ± 0.16‰ and 7.39 ± 0.16‰ respectively; \(p<0.001\); Figure 29d, Figure 30b).
$^{15}$N varied significantly within sites across conditions with the exception of Zenith Beach (ZEN) which ranged within 0.53‰ for all events ($p>0.05$; Figure 29). Redhead Beach varied significantly between typical dry conditions, with significantly depleted $^{15}$N in summer opposed to winter (Event 4; $p<0.05$; Figure 30b). Both Dudley (DUD) and Burwood (BUR) beaches had similar $^{15}$N signatures between typical dry (Event 1; 6.52 ± 0.11‰ and 6.61 ± 0.15‰) and typical wet (Event 2; 6.32 ± 0.14‰ and 6.89 ±0.20‰) conditions, however varied significantly between enriched signatures during extreme wet (Event 3; 7.53 ± 0.02‰ and 7.83 ±0.08‰) and typical dry conditions in winter (Event 4; 7.64 ± 0.08‰ and 7.77 ± 0.05‰; $p<0.001$; Figure 30b). At Newcastle (NEW) both extreme wet conditions (Event 3) and typical dry conditions in winter (Event 4) were significantly enriched compared to both typical dry (Event 1) and typical wet (Event 2) conditions ($p<0.001$; Figure 30b). Birubi Beach (BIR) was enriched significantly from typical wet conditions (Event 2; 6.74 ± 0.41‰) to extreme wet conditions (Event 3; 8.26 ± 0.11‰; $p<0.05$; Figure 30b).
Figure 29: Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope composition of Ulva (Ulva lactuca). A: Event 1 (Typical Dry), B: Event 2 (Typical Wet), C: Event 3 (East Coast Low / Extreme Wet), D: Event 4 (Typical Dry). Values are mean ± SE.

Figure 30: Stable a) carbon ($\delta^{13}C$) and b) nitrogen ($\delta^{15}N$) isotope thematic interpolation of Ulva (Ulva lactuca). Values are means.
4. DISCUSSION

Through the use of multiple bioindicators with differing turnover rates, this study has provided temporal and spatial information on the nutrient sources supporting intertidal areas of the Hunter Region. Evidence from this study suggests that food webs of intertidal sandy beaches and rocky shores of the region are predominately derived from marine sources. However, after significant rainfall events, terrestrial and anthropogenic derived nutrients can provide a substantial nutrient subsidy to the intertidal food web.

4.1 DOMINANT NUTRIENT SOURCES

During typical conditions the dominant sources of nutrients along the Hunter coastline are marine derived. This is evident from *D. deltoides*, which had a relatively constant δ¹⁵N signature, comparable to the marine environment (6-7‰; Fry 2006) during both typical dry and typical wet conditions. Marine signatures were also reflected in the δ¹³C values of *D. deltoides* during both typical dry and wet conditions, which aligned closely to values from similar studies of *D. deltoides* at ‘control’ beach locations (-17.5‰ to -19.5‰; Schlacher and Connolly 2009). Similarly, *U. lactuca* reflected marine carbon (δ¹³C) and nitrogen (δ¹⁵N) signatures in typical dry conditions (Rogers, 2003). However, during extensive rainfall in April, some intertidal locations became either significantly enriched or depleted in δ¹⁵N; isotopic shifts consistent with the incorporation of anthropogenic derived nutrients. Locations where anthropogenic derived nutrients were reflected, as either an enrichment or depletion of δ¹⁵N signatures assimilated within bioindicator tissues, spatially align to sites associated with known nutrient sources including the Hunter River and ocean discharges from Wastewater Treatment Works (WWTW’s).

Stockton, One Mile, Box and Zenith beaches located in Port Stephens, had significantly depleted δ¹⁵N in *D. deltoides* tissues three months after the extreme wet weather event. This is likely attributed to the reduced δ¹⁵N derived from the Hunter River plume in April (4.3 ± 0.3‰; Ryan, 2015). Barnacles (*T. rosea*) reflect this distinctly depleted signature of the Hunter River with significantly reduced δ¹⁵N values at sites within the mouth of the estuary across the study period. During the extreme wet event the depleted
nitrogen plume reached the northern extent of the Port Stephens estuary before being transported further offshore and south by the East Australian Current (EAC; Ryan 2015). The extent of this plume event is supported both by satellite imagery, as well as physiochemical water parameters of temperature and salinity, which were significantly reduced both within and north of the Hunter River reflecting the large volume of cooler and brackish estuarine flows (Ryan, 2015). Furthermore, a reduction in grain size at sites north of the Hunter River following the extreme wet weather event indicates the distribution of small-grained fluvial inputs to these sandy beach locations (Sampio et al., 2010). Conversely, both Birubi and Birubi South beaches in Port Stephens became significantly enriched in δ¹⁵N relative to other sites during, and after the extreme wet event, as well as compared to their own respective baseline values in typical dry and wet conditions. D. deltoides at Birubi South remained enriched in δ¹⁵N three months following extreme wet weather, demonstrating both the extent and persistence of nutrient enrichment from this event. This δ¹⁵N isotopic enrichment is likely attributed from onshore advection of wrack, which is known to have values within the range of those analysed in D. deltoides muscle tissues at these sites (7.2 ± 0.4‰; Clarke, 2015). Wrack was likely ripped from offshore reefs and deposited onshore during the East Coast Low wet weather event by its associated and persistent large swells. Adveceted marine wrack is known to offset primary production within the intertidal zone of sandy beaches, contributing significantly to biologically available particulate organic material and resulting in an enriched shift in δ¹⁵N and δ¹³C isotope values when assimilated into organism tissues (McLachlan and Brown 2006c, Lercari et al. 2010, Bergamino et al. 2011). U. lactuca was also enriched in δ¹⁵N and δ¹³C at Birubi compared to all other sites in Port Stephens during this event. Enriched δ¹⁵N within U. lactuca however returned to baseline conditions by the following sampling event three months later, reflecting the shorter turnover period in macroalgae compared to pipis used in this study. This respective variance in turnover between the two species was indicated in Rogers (2003), whereby the isotopic signature of U. lactuca subjected to effluent enrichment, returned to control levels within a range of 3 weeks to 3 months, whereas suspension feeders had slower turnover, storing isotopic signatures within their bodies up to 9 months. Correspondingly, Gartner et al. (2002), showed that Ulva sp. were enriched in δ¹⁵N in as little as seven days after exposure to sewage derived effluent, and are therefore capable of discriminating additional nutrient enrichment over a relatively short timeframe. Enriched δ¹⁵N in U. lactuca at this
site therefore indicates that the dissolved inorganic nitrogen and carbon derived from decaying wrack that appears to be providing a dominant nutrient source to *D. deltoides*, may also provide a nutrient subsidy to *U. lactuca*. It is not expected that Boulder Bay WWTW could have a direct contribution to this enrichment at Birubi Beach, due to the distance and southward location relative to the Boulder Bay WWTW. However, enriched nitrogen was analysed in signatures within *D. deltoides* at Fingal Bay located immediately north of Boulder Bay following the ECL wet weather event, as such indicating a potential influence of treated effluent advected from the Boulder Bay WWTW.

Following the April storm event, Boulder Bay had discharges consistently elevated 3-4ML/day above dry base-flow conditions (6ML/day) for approximately three months, providing significantly greater volumes, and as a result greater advection potential of treated effluent to the intertidal marine environment. Gaston et al, (2006) showed that enriched plumes can be transported in nearshore marine environments to distances up to several kilometres if the quantity of flow is relatively high enough. An assessment on water quality and dispersion of nutrients from Boulder Bay WWTW over a three year period (2011-2014) indicated that nutrients within 500 metres of the outfall were occasionally elevated indicative of wastewater effluent, compared to other locations (MEAP, 2014). Considering Fingal Bay is located within this range, the consistent elevated flows from the WWTW following the April storm event, and the direction of the Hunter River effluent plume in a northerly direction; it is plausible that Boulder Bay effluent, known to be elevated in δ\textsuperscript{15}N (Gaston and Suthers 2004) was advected onshore and assimilated as enriched δ\textsuperscript{15}N into tissues of *D. deltoides* during this period. *U. lactuca* sampled from regions influenced by outfalls have been shown to be significantly enriched in δ\textsuperscript{15}N compared to uncontaminated control sites (Rogers 2013). Correspondingly, δ\textsuperscript{15}N was enriched in *U. lactuca* compared to baseline conditions immediately after the ECL event at Fingal Bay aligning with a significant elevation in sediment chlorophyll-α, a physiochemical response to increased nutrient loads, therefore reiterating the likelihood of effluent advection from Boulder Bay WWTW to this site during extreme wet weather.

Belmont Beach and Burwood Beach, located near two ocean discharging wastewater treatment works, were also relatively enriched in δ\textsuperscript{15}N following the ECL extreme wet weather event. Nitrogen enrichment in *D. deltoides* at Belmont during this period is possibly
associated with onshore advection of effluent from the Belmont Wastewater Treatment Works (WWTW), which had discharges elevated during this event (>60ML/day). The average effluent flow during this period is considered relatively conservative due to flow-meters deactivating in the later stages of the wet weather event as a result of telemetry and power outages. As such, effluent discharge may have in fact been significantly higher during this period, providing an even greater likelihood of advection to Belmont Beach approximately 1.8km shoreward. Furthermore, discharges remained consistently elevated above average volumes from the extreme wet weather event through until July as a result of ongoing wet weather. Gaston and Suthers (2004), showed that continuous discharges of municipal effluent, that are comparatively smaller than upwelling and estuarine plumes, provide a nutrient subsidy to planktonic hula fish (*A. strigatus*) within the vicinity of ocean outfalls in New South Wales. Similarly, Schlacher and Connolly (2009) showed a relationship between increased plume volumes and greater assimilation of terrestrial nutrients within *D. deltoides*; supporting the likelihood that both the greater and consistent volume of effluent discharge from Belmont WWTW may be enriching *D. deltoides* at Belmont beach during this period. Concurrently, a significant reduction in salinity was analysed at Belmont beach after the extreme wet weather event, providing a further case supporting input of a fresh, nutrient enriched source such as the Belmont WWTW or seepage from the adjacent Belmont lagoon during this period.

Burwood WWTW also had ongoing elevated discharges in the three month period following the extreme wet weather event, in some instances 4-fold greater than average dry flow conditions (>250ML/day), with correspondingly high, partially treated bypass flows. Secondary treated sewage has a notably elevated $\delta^{15}$N signal and therefore can be distinguished from other contributing nutrient sources through respective organisms (Costanzo et al, 2001). $\delta^{15}$N values within *D. deltoides* at Burwood Beach following the storm event in April were significantly enriched compared to all other events at the site. This indicates to some degree that the above average effluent discharge at Burwood WWTW, similar to the other ocean discharging wastewater treatment works, may be advecting nutrients ashore. This is a notable finding, as previous near-field effluent fate and transport modelling of Burwood Beach WWTW on present base-case conditions (discharges of 44ML/day of effluent and 4.5ML/day of biosolids) indicated a relatively localized impact on nutrients within the water column, confined
to within 500m of the ocean outfall (MEAP, 2014). However, MEAP (2014) was unable to differentiate between natural and anthropogenic-derived nitrogen. As such, this study provides an additional line of evidence, using stable isotopes within tissues of *D. deltoides*, to determine that effluent derived from the Burwood Beach WWTW can be advected onshore and assimilated into the beach foodweb.

Suspension feeders have shown distinct isotopic signatures from both sewage derived effluent and estuarine plumes compared to those collected at reference sites in a range of studies. Tucker (1999) showed a spatial gradient of enriched δ^{15}N muscle tissues within Blue Mussels (*Mytilus edulis*) which decreased with increasing distance from effluent outfalls. Whereas Schlacher and Connolly (2009), showed an enrichment of pipis (*D. deltoides*) subjected to estuarine plume events under various flow conditions. Suspension feeders such as pipis are considered as ideal bioindicators to measure carbon and nitrogen transfers across beach trophic pathways as they are the dominant macroscopic consumer of organic matter in beach benthos both in Australia and worldwide (Schlacher and Connolly, 2009). They can access nutrients from terrestrial and marine pathways both directly, through uptake of phytoplankton or particulate organic matter (POM) in the water column, or can assimilate dissolved forms deposited in the benthos via processing in the microbial loop. As such, tissue turnover times in filter-feeding bivalves such as *D. deltoides* are tissue and species specific, with relatively long turnover times of months to a year in whole body and abductor muscle tissues (Raikow and Hamilton, 2001). Studies have suggested that the size of pipis may influence both the feeding regime and the assimilation of isotopes within their respective tissues over time (Schlacher and Connolly, 2009). Although there were significant relationships between size of pipis and both δ^{15}N and δ^{13}C ratios in this study, values were within the same trophic level range (≈1-1.5‰) and therefore size was not considered as an ecologically significant impact to the study’s results. Bivalves exposed to anthropogenic or plume sources are therefore expected to provide a relatively long-period indicator of nutrient advection onto sandy beaches, integrating and averaging out the isotope signals of a given site over months (Raikow and Hamilton, 2001, Schlacher and Connolly, 2009). This long-term integration is reflected in *D. deltoides* in this study, which had delays of up to three months between nutrient subsidies associated with either effluent advection or the Hunter River plume from the April storm event and the associated signatures evident in
their tissues. *U. lactuca* on the other hand, sources nitrogen and carbon primarily from one nutrient pathway (Dissolved Inorganic Nitrogen and Dissolved Inorganic Carbon) and shows a faster turnover rate with the ability to reflect a nitrogen source within a week (Fernandes, 2012; Gartner 2002; Dudley 2007). As such, the use of *D. deltoides* and *U. lactuca* concurrently to assess anthropogenic nutrients over given timeframes when comparing between sites and relative exposures to anthropogenic nutrients is a powerful time integrated measure of both immediate advection of material to intertidal shores, as well as their persistence and assimilation overtime.

Compared to the isotopic results of *D. deltoides* and *U. lactuca*, other bioindicators analysed in the study display fewer indications of anthropogenic nutrient sources, displaying greater variance amongst both sites and events. Isopods (*P. concinna*) due to their opportunistic scavenging feeding regime, integrate a range of nutrient sources dependent on what is available at any given time (Keable, 1995). As such, nitrogen and carbon within their tissues, although consistent with available nutrient sources at respective sites, provides a less defined distribution of anthropogenic nutrients across and within events. Furthermore, the limited distribution and replicability of *P. concinna* across both site and event restricted spatial and temporal profiling, further limiting their use in addressing the key aims of this study.

It was assumed that by collecting a range of species from different functional groups and trophic levels, that an indication of whether anthropogenic isotope distributions are spatially repeated between taxa could be determined, thus indicating the fate of these nutrients throughout the food web. Although barnacles (*T. rosea*) indicate a distinct influence from the Hunter River estuary with significantly reduced δ¹⁵N values across the period of the study, turnover rates were generally too extensive to provide an indication of intermediate pulse events such as plumes and spikes in effluent discharge that this study aimed to investigate (Fry et al 2011). Similarly, the assumption in the collection of both *U. lactuca* and *N. atramentosa* simultaneously was that, dominant nutrients and pathways through the intertidal rocky shore could be discerned by sampling primary consumers adjacent to the primary producers assumed to be representative of their food source. Isotope results however showed this to be an invalid assumption, with limited correspondence in isotope values reflected between the two species. This was a similar
outcome to the study of Tucker (1999), whereby $\delta^{15}N$ values were elevated in algae (*U. lactuca*) relative to those of the assumed consumer (*M. edulis*), restricting the ability to resolve any influence of nutrient flows through the foodweb. Similarly, Oaks and Eyre (2015), found that *N. atramentosa* feed on both microalgae as well as macroalgae at different zonations of the rock platform. As a result, assimilation of $\delta^{15}N$ varied in *N. atramentosa* to those of *U. lactuca*, as grazer tissues reflected a combination of species-specific tissue turnover times of temporal nutrient availability variations for a range of algae. As such, it is likely that the relatively rapid fluctuations of $\delta^{15}N$ and $\delta^{13}C$ in *U. lactuca* that reflect the availability of nutrients and $\delta^{15}N$ values of wastewater dissolved inorganic nitrogen over relatively short time periods in this study, were averaged out by other nutrient sources when assimilated into *N. atramentosa*.

4.2 CONCLUSION

Overall, this study suggests that intertidal shores of the Hunter region are driven primarily in most instances by marine nutrient sources. In the event of extreme wet weather however, both terrestrial influences derived from the Hunter River can provide nutrients to sites north of Stockton via the effluent plume that are incorporated into the intertidal foodweb. Although discharges from wastewater treatment works are volumetrically significantly smaller than the estuarine plume, during extreme wet weather, nutrients derived from effluent discharge may advect to the Hunter coastline. Pipis (*D. deltoides*) and Ulva (*U. lactuca*) have been shown in this study to be excellent indicators of anthropogenic and terrestrial derived nutrients, providing an indication of both the long, and shorter residence times across impacted sites respectively. Although other bioindicators (*P. concinna*, *N. atramentosa* and *T. rosea*) provide an isotopic signature representative of their available nutrient sources, their isotope ratios were difficult to attribute to anthropogenic sources due to either their feeding regime, lack of distribution across the study site or tissue turnover rates.
REFERENCES


BMT WBM (2015) Hunter River estuary environmental and water quality review. Book 1, 126 Belford Street, Broadmeadow, NSW, 2292


Hill JM, McQuaid CD (2008) delta C-13 and delta N-15 biogeographic trends in rocky intertidal communities along the coast of South Africa: Evidence of strong environmental signatures. Estuarine Coastal and Shelf Science 80:261-268


Schlacher TA, Morrison JM (2008) Beach disturbance caused by off-road vehicles (ORVs) on sandy shores: Relationship with traffic volumes and a new method to quantify impacts using image-based data acquisition and analysis. Marine Pollution Bulletin 56:1646-1649


Winberg PC, Davis AR (2014) Ecological response to MPA zoning following cessation of bait harvesting in an estuarine tidal flat. Marine Ecology Progress Series 517:171-180

APPENDICES

Appendix 1: Temperature and Salinity summary table for Mean (±SE) values for all events and sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Event 1 (Typical Dry)</th>
<th>Event 2 (Typical Wet)</th>
<th>Event 3 (East Coast Low)</th>
<th>Event 4 (Typical Dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Salinity</td>
<td>Temperature (°C)</td>
<td>Salinity</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>SWA</td>
<td>22.05 ± 0.03</td>
<td>36.59 ± 1.15</td>
<td>24.29 ± 1.73</td>
<td>33.92 ± 1.23</td>
</tr>
<tr>
<td>BLA</td>
<td>23.11 ± 0.18</td>
<td>26.66 ± 9.34</td>
<td>23.19 ± 0.01</td>
<td>36.08 ± 0.75</td>
</tr>
<tr>
<td>PEL</td>
<td>24.03 ± 0.28</td>
<td>29.64 ± 4.51</td>
<td>22.93 ± 0.01</td>
<td>26.02 ± 0.33</td>
</tr>
<tr>
<td>BEL</td>
<td>24.17 ± 0.03</td>
<td>34.61 ± 0.42</td>
<td>22.87 ± 0.26</td>
<td>23.83 ± 8.01</td>
</tr>
<tr>
<td>RED</td>
<td>22.90 ± 0.06</td>
<td>29.20 ± 0.43</td>
<td>22.28 ± 0.10</td>
<td>26.35 ± 4.62</td>
</tr>
<tr>
<td>DUD</td>
<td>22.36 ± 0.03</td>
<td>37.43 ± 0.22</td>
<td>23.53 ± 0.08</td>
<td>34.94 ± 1.13</td>
</tr>
<tr>
<td>BUR</td>
<td>23.97 ± 0.03</td>
<td>30.20 ± 1.77</td>
<td>22.58 ± 0.01</td>
<td>35.73 ± 0.34</td>
</tr>
<tr>
<td>MER</td>
<td>24.50 ± 0.00</td>
<td>35.83 ± 0.29</td>
<td>23.10 ± 0.03</td>
<td>37.22 ± 0.04</td>
</tr>
<tr>
<td>BAR</td>
<td>24.77 ± 0.09</td>
<td>35.57 ± 0.23</td>
<td>23.18 ± 0.00</td>
<td>35.78 ± 0.07</td>
</tr>
<tr>
<td>NEW</td>
<td>24.77 ± 0.07</td>
<td>36.02 ± 0.16</td>
<td>23.69 ± 0.07</td>
<td>36.01 ± 0.10</td>
</tr>
<tr>
<td>NOB</td>
<td>25.40 ± 0.25</td>
<td>33.56 ± 0.87</td>
<td>23.55 ± 0.22</td>
<td>36.51 ± 0.41</td>
</tr>
<tr>
<td>NOBD</td>
<td>25.13 ± 0.01</td>
<td>33.83 ± 0.74</td>
<td>22.63 ± 0.02</td>
<td>30.64 ± 0.02</td>
</tr>
<tr>
<td>STOL</td>
<td>25.05 ± 0.02</td>
<td>34.01 ± 0.04</td>
<td>18.64 ± 0.04</td>
<td>4.86 ± 0.04</td>
</tr>
<tr>
<td>STO</td>
<td>24.69 ± 0.05</td>
<td>35.88 ± 0.27</td>
<td>25.18 ± 0.06</td>
<td>34.90 ± 0.16</td>
</tr>
<tr>
<td>BIR</td>
<td>26.25 ± 0.00</td>
<td>34.11 ± 0.13</td>
<td>21.06 ± 0.01</td>
<td>36.74 ± 0.03</td>
</tr>
<tr>
<td>BIR</td>
<td>24.56 ± 0.01</td>
<td>36.18 ± 0.03</td>
<td>20.15 ± 0.01</td>
<td>32.20 ± 0.01</td>
</tr>
<tr>
<td>ONE</td>
<td>26.25 ± 0.00</td>
<td>35.39 ± 0.00</td>
<td>20.74 ± 0.02</td>
<td>35.39 ± 0.13</td>
</tr>
<tr>
<td>FIN</td>
<td>26.88 ± 0.01</td>
<td>33.98 ± 0.05</td>
<td>21.85 ± 0.02</td>
<td>35.46 ± 0.01</td>
</tr>
<tr>
<td>BOX</td>
<td>27.57 ± 0.02</td>
<td>32.93 ± 0.17</td>
<td>21.99 ± 0.01</td>
<td>35.03 ± 0.08</td>
</tr>
<tr>
<td>ZEN</td>
<td>27.51 ± 0.00</td>
<td>33.08 ± 0.09</td>
<td>22.00 ± 0.01</td>
<td>35.89 ± 0.03</td>
</tr>
</tbody>
</table>

*Values presented in Table 4 and Figure 7 for site ‘NOBD’ ‘Event 3’ are those analysed before the peak of the East Coast Low.

**Salinity values were omitted for Event 4 (Typical Dry) as values were deemed above a range representative of temperate coastal waters.
Appendix 2: Mean (±SE) sediment Chlorophyll-α and Organic Matter summary table for all events and sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Event 1 (Typical Dry)</th>
<th>Event 2 (Typical Wet)</th>
<th>Event 3 (East Coast Low)</th>
<th>Event 4 (Typical Dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll-α (mg/cm³)</td>
<td>Organic Matter (%)</td>
<td>Chlorophyll-α (mg/cm³)</td>
<td>Organic Matter (%)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>SWA</td>
<td>0.66 ± 0.05</td>
<td>2.21 ± 0.48</td>
<td>0.37 ± 0.05</td>
<td>1.84 ± 0.21</td>
</tr>
<tr>
<td>BLA</td>
<td>0.47 ± 0.07</td>
<td>2.37 ± 0.16</td>
<td>0.70 ± 0.03</td>
<td>1.93 ± 0.65</td>
</tr>
<tr>
<td>PEL</td>
<td>0.14 ± 0.01</td>
<td>1.88 ± 0.14</td>
<td>0.19 ± 0.10</td>
<td>2.53 ± 0.24</td>
</tr>
<tr>
<td>BEL</td>
<td>0.26 ± 0.02</td>
<td>1.80 ± 0.26</td>
<td>0.30 ± 0.13</td>
<td>1.22 ± 0.06</td>
</tr>
<tr>
<td>RED</td>
<td>0.60 ± 0.03</td>
<td>3.06 ± 0.71</td>
<td>0.40 ± 0.09</td>
<td>1.96 ± 0.01</td>
</tr>
<tr>
<td>DUD</td>
<td>0.25 ± 0.12</td>
<td>4.51 ± 1.61</td>
<td>0.35 ± 0.07</td>
<td>1.79 ± 0.07</td>
</tr>
<tr>
<td>BUR</td>
<td>0.15 ± 0.01</td>
<td>3.13 ± 0.34</td>
<td>0.10 ± 0.05</td>
<td>3.67 ± 0.67</td>
</tr>
<tr>
<td>MER</td>
<td>0.38 ± 0.05</td>
<td>2.64 ± 1.02</td>
<td>0.24 ± 0.03</td>
<td>2.91 ± 0.13</td>
</tr>
<tr>
<td>BAR</td>
<td>0.43 ± 0.03</td>
<td>3.98 ± 0.35</td>
<td>0.82 ± 0.17</td>
<td>1.99 ± 0.32</td>
</tr>
<tr>
<td>NEW</td>
<td>0.33 ± 0.05</td>
<td>1.74 ± 0.30</td>
<td>0.22 ± 0.09</td>
<td>1.80 ± 0.52</td>
</tr>
<tr>
<td>NOB</td>
<td>0.42 ± 0.04</td>
<td>2.47 ± 0.19</td>
<td>0.41 ± 0.02</td>
<td>2.48 ± 0.74</td>
</tr>
<tr>
<td>NOBD</td>
<td>0.36 ± 0.07</td>
<td>3.96 ± 0.69</td>
<td>0.39 ± 0.11</td>
<td>3.91 ± 0.14</td>
</tr>
<tr>
<td>STOL</td>
<td>1.19 ± 0.01</td>
<td>4.35 ± 0.33</td>
<td>0.83 ± 0.01</td>
<td>3.51 ± 0.16</td>
</tr>
<tr>
<td>STO</td>
<td>0.22 ± 0.06</td>
<td>3.06 ± 0.82</td>
<td>0.07 ± 0.05</td>
<td>8.61 ± 2.87</td>
</tr>
<tr>
<td>BIRS</td>
<td>1.34 ± 0.17</td>
<td>3.41 ± 0.89</td>
<td>0.53 ± 0.29</td>
<td>3.68 ± 0.17</td>
</tr>
<tr>
<td>BIR</td>
<td>0.82 ± 0.06</td>
<td>3.96 ± 1.07</td>
<td>0.38 ± 0.03</td>
<td>4.64 ± 0.14</td>
</tr>
<tr>
<td>ONE</td>
<td>0.57 ± 0.24</td>
<td>4.13 ± 0.32</td>
<td>0.68 ± 0.42</td>
<td>3.99 ± 0.42</td>
</tr>
<tr>
<td>FIN</td>
<td>2.98 ± 0.33</td>
<td>3.08 ± 0.91</td>
<td>1.91 ± 0.29</td>
<td>3.70 ± 0.12</td>
</tr>
<tr>
<td>BOX</td>
<td>0.43 ± 0.19</td>
<td>4.22 ± 0.76</td>
<td>1.13 ± 0.20</td>
<td>2.56 ± 0.34</td>
</tr>
<tr>
<td>ZEN</td>
<td>1.00 ± 0.13</td>
<td>2.57 ± 0.11</td>
<td>0.48 ± 0.05</td>
<td>2.06 ± 0.32</td>
</tr>
</tbody>
</table>
Appendix 3: Mean (±SE) sediment grain size and mud composition for all events and sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Event 2 (Typical Wet)</th>
<th>Event 4 (Typical Dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain Size</td>
<td>%Mud</td>
</tr>
<tr>
<td></td>
<td>Mean  ± SE</td>
<td>Mean  ± SE</td>
</tr>
<tr>
<td>SWA</td>
<td>365.6804 ± 13.8283</td>
<td>0.01% ± 0.002%</td>
</tr>
<tr>
<td>BLA</td>
<td>391.4947 ± 9.2703</td>
<td>0.01% ± 0.000%</td>
</tr>
<tr>
<td>PEL</td>
<td>698.1913 ± 4.5021</td>
<td>0.01% ± 0.001%</td>
</tr>
<tr>
<td>BEL</td>
<td>605.6804 ± 15.1896</td>
<td>0.00% ± 0.001%</td>
</tr>
<tr>
<td>RED</td>
<td>429.0357 ± 12.3768</td>
<td>0.01% ± 0.003%</td>
</tr>
<tr>
<td>DUD</td>
<td>449.734 ± 20.9845</td>
<td>0.00% ± 0.001%</td>
</tr>
<tr>
<td>BUR</td>
<td>495.6189 ± 5.2545</td>
<td>0.01% ± 0.001%</td>
</tr>
<tr>
<td>MER</td>
<td>475.015 ± 28.8479</td>
<td>0.01% ± 0.003%</td>
</tr>
<tr>
<td>BAR</td>
<td>443.3783 ± 2.1247</td>
<td>0.02% ± 0.003%</td>
</tr>
<tr>
<td>NEW</td>
<td>410.7677 ± 10.1467</td>
<td>0.01% ± 0.002%</td>
</tr>
<tr>
<td>NOB</td>
<td>380.9733 ± 10.7001</td>
<td>0.00% ± 0.000%</td>
</tr>
<tr>
<td>NOBD</td>
<td>401.0114 ± 5.4437</td>
<td>0.05% ± 0.004%</td>
</tr>
<tr>
<td>STOL</td>
<td>464.0274 ± 76.5482</td>
<td>0.01% ± 0.002%</td>
</tr>
<tr>
<td>STO</td>
<td>682.7778 ± 53.0583</td>
<td>0.02% ± 0.004%</td>
</tr>
<tr>
<td>BIRS</td>
<td>332.0958 ± 18.6311</td>
<td>0.01% ± 0.002%</td>
</tr>
<tr>
<td>BIR</td>
<td>244.6904 ± 12.0902</td>
<td>0.02% ± 0.001%</td>
</tr>
<tr>
<td>ONE</td>
<td>266.4558 ± 11.5255</td>
<td>0.01% ± 0.004%</td>
</tr>
<tr>
<td>FIN</td>
<td>356.0108 ± 12.3852</td>
<td>0.01% ± 0.003%</td>
</tr>
<tr>
<td>BOX</td>
<td>486.7473 ± 0.981375</td>
<td>0.01% ± 0.001%</td>
</tr>
<tr>
<td>ZEN</td>
<td>499.5301 ± 31.3535</td>
<td>0.00% ± 0.000%</td>
</tr>
</tbody>
</table>