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**Spatio-temporal patterns of faunal distribution
along a near-pristine estuarine edge
environment**

Thesis submitted by

Krista Elizabeth Stegemann, BSc

in November 2013

for the degree of

Master of Science (Research)

in the School of Marine and Tropical Biology

James Cook University

Statement on the Contribution of Others

Every reasonable effort has been made to acknowledge research contributors as well as to gain permission and acknowledge the owners of copyright material. I would be pleased to hear from any contributor or copyright owner who has been omitted or incorrectly acknowledged.

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Declaration on Ethics

This research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council (NHMRC) Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th Edition, 2004 and the Qld Animal Care and Protection Act, 2001. The proposed research study received animal ethics approval from the JCU Animal Ethics Committee Approval Number #A1781.

Signature:

Date: 26 November 2013

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Abstract

Estuarine edges are important areas of estuarine systems, with high levels of both economic and ecological value. However, variable conditions created by factors such as changing tides, runoff, changing levels of habitat connectivity, shifting bathymetric conditions and fluctuating amounts of submerged structure make these areas extremely dynamic. Despite these difficult conditions, estuarine edges are important habitats for many estuarine fauna. However, the way they are utilised is not well understood. The response to temporal changes and the way in which edge fauna react to spatial changes is unclear. The overall aim of the study was to gain a better understanding of the spatial and temporal changes in the distribution of nekton along the margins of estuaries and thus to obtain clearer insight into the way these organisms utilise these habitats and respond to their varying conditions. The specific approach was to investigate the temporal changes in the distribution of edge organisms over the tidal cycle and to determine how site-specific habitat characteristics, such as the presence of structure, algal growth, rugosity, depth and current flow, may be influencing faunal changes seen in distribution.

First, a new approach for sampling estuaries was needed in order to give a new perspective on these systems and to investigate changes over a period of time. Underwater videography was such a technique and was used throughout the study. As this is a novel method for these systems, protocols were first developed to ensure it was a valid approach for this study and that biases were limited.

Utilising this camera technique to sample along a tropical estuarine bank in Deluge Inlet, Queensland, Australia, temporal patterns of change were observed in estuarine nekton over the tidal cycle. Abundances were greatest at times of higher tides, just as water began to flood or drain from the fringing mangrove forest, suggesting that nekton were aggregating outside the forest at these times due to their utilisation of its resources when it is inundated

with water at high tides. However, abundances decreased at low tides, suggesting that not all nekton that use the estuarine edge at higher water levels remain there at low tide when the mangrove forest is unavailable. Estuarine fauna thus utilise the connections available between habitats within the estuary, although where these fauna move to at low tide is still unclear.

While there was an obvious temporal response to tidal changes, site-specific habitat characteristics also influenced nekton distribution along the bank. The greatest max. N values were observed at two hot spot areas of the bank, where linear flow was disrupted by incoming tributaries to create turbulent currents. These broad-scale current conditions probably substantially contributed to the high abundances in these areas. However, the examination of current as a separate variable along the bank found that it did not have a highly substantial influence, and so other factors probably also influenced distribution. Depth was also important in structuring distribution, especially within the hot spot areas, although depth preference varied between taxa. The remaining variables that were investigated (algal growth, presence of structure and rugosity) had little influence over nekton distributions in this study. Overall, results suggest that it is probably a complex interaction of many factors that determine distribution.

This study was effective in determining the spatial and temporal distribution patterns of estuarine nekton in a dynamic edge habitat. The study emphasises the complex nature of these faunal movements and the utilisation of habitat connectivity, as well as the importance of gaining a detailed understanding of the changes within an estuarine edge and how they influence organisms. Gaining insight into these systems allows for improved management and conservation efforts in these important areas.

Table of Contents

Statement on the Contribution of Others	i
Declaration on Ethics	v
Acknowledgements	vii
Abstract	ix
List of Tables	xiii
List of Figures	xv
Chapter 1: General Introduction	1
Chapter 2: An Evaluation of Camera Methods for Investigating the Identification, Distribution and Temporal Patterns of Estuarine Fishes	5
2.1 Introduction.....	5
2.2 Materials & Methods.....	9
2.2.1 <i>Initial Technique Development</i>	14
2.2.1.1 <i>Camera Systems</i>	16
2.2.2 <i>Camera Position in the Water Column</i>	19
2.2.3 <i>Lateral Camera Placement</i>	21
2.2.4 <i>Data Analysis</i>	22
2.3 Results & Discussion.....	23
2.3.1. <i>Initial Technique Development</i>	24
2.3.2 <i>Camera Position in the Water Column</i>	26
2.3.3 <i>Lateral Camera Placement</i>	32
2.3.4 <i>Conclusions</i>	35
Chapter 3: Assessing the Validity of Videography Techniques for Sampling Nekton in Tropical Estuaries through Comparison with Cast Netting Procedures	37
3.1 Introduction.....	37
3.2 Materials & Methods.....	39
3.2.1 <i>In-Field Sampling</i>	39

3.2.2 <i>Data Analysis</i>	40
3.3 Results.....	41
3.4 Discussion.....	44
Chapter 4: Temporal Changes in Species Distribution Along Shallow Edge Habitats in a Near-Pristine Tropical Estuary.....	51
4.1 Introduction.....	51
4.2 Materials & Methods.....	53
4.2.1 <i>In-Field Sampling</i>	53
4.2.2 <i>Data Analysis</i>	55
4.3 Results.....	57
4.4 Discussion.....	62
Chapter 5: The Impact of Site-Specific Habitat Characteristics on Nekton Distribution Along Shallow Edge Habitats of a Near-Pristine Tropical Estuary.....	65
5.1 Introduction.....	65
5.2 Materials & Methods.....	68
5.2.1 <i>In-Field Sampling</i>	68
5.2.2 <i>Data Analysis</i>	69
5.3 Results.....	70
5.4 Discussion.....	75
Chapter 6: General Discussion.....	79
6.1 Key Findings.....	79
6.2 Potential Drivers of Habitat-Use Patterns.....	81
6.3 Implications for Connectivity.....	83
6.4 Implications for Conservation and Management.....	84
6.5 Challenges & Directions for Future Research.....	85
References.....	87

List of Tables

Table 2.1 Summary of potential issues and concerns associated with underwater camera methodology, as well as the methods and solutions to address them. Issues must be addressed and validated before the successful use of cameras in tropical estuaries is possible.....	10
Table 2.2 Species assemblage list from floating and bottom cameras (collected as described in section 2.2.2). Numbers indicate the proportion of samples (n) in which each species was recorded for each gear type.....	28
Table 3.1 Characteristics of taxa sampled by camera methods. Taxa only sampled by cameras are in the upper portion of the table; those also sampled by cast netting are located in the lower portion and are indicated by (*). (Hyland <i>et al.</i> 1984; Davis 1988; Bade 1989; Robertson and Duke 1990a; Sheaves 1993; Bagarinao 1994; Stewart and Jones 2001; Meyer <i>et al.</i> 2007; Froese and Pauly 2011; O’Toole <i>et al.</i> 2011; Jardine <i>et al.</i> 2012; Corcoran <i>et al.</i> 2013).....	46
Table 3.2 Characteristics of taxa sampled only by cast nets. Only taxa with a frequency of occurrence >0.003 (sampled more than once) were included. (Moynihan 1983; Robertson and Duke 1990b; Salini <i>et al.</i> 1990; Minami and Tanaka 1992; Xiao and Greenwood 1992; Hyndes <i>et al.</i> 1997; Newman <i>et al.</i> 1997; von Byern and Marwoto 2009; Froese and Pauly 2011).....	47

List of Figures

- Figure 2.1** Map of sampling sites in North Queensland, Australia, showing locations of Mendel Creek, Deluge Inlet, Waterfall Creek and Fisher’s Creek off Hinchinbrook Channel; Stuart Creek and Gordon Creeks are located off the Ross River in Townsville, Queensland, Australia.....15
- Figure 2.2** Floating camera setup: a) An example of how the floating camera setup is deployed in water; b) A demonstration of how cameras are housed within the floating setup; c) & d) A closer view of the floating housing with camera and protective foam mat.....17
- Figure 2.3** Bottom camera setup: a) An example of how the bottom camera setup is deployed in water; b) An above view of the weighted aluminium base and plastic fin with camera located at one end; c) A closer view of the weighted base and plastic fin with camera mounted on a small platform.....18
- Figure 2.4** Map of study sites with approximate positions of the two sampling locations at Deluge Inlet and Mendel Creek.....20
- Figure 2.5** Diagram of the configuration of floating and bottom cameras used to assess camera setup and position in the water column.....21
- Figure 2.6** Species richness in relation to video analysis time, providing information as to appropriate video length. Data is from bottom cameras used in the first experiment in this study (section 2.2.2) and portrays species richness values at their appropriate times throughout each video (i.e. some videos may be represented by multiple points). Only videos with a species richness of ≥ 1 were used. Dashed line provides visual representation of the plateau in species richness at approximately 10 minutes into analysis.....25
- Figure 2.7** Max. N values by camera type (df=1, F=44.008, p=0.000). Vertical bars denote 0.95 confidence intervals.....27
- Figure 2.8** Species richness of floating (a) and bottom (b) cameras at various depths.....31
- Figure 2.9** Presence of organisms at varying positions from shore; a) Mean species richness data from the first experiment (see section 2.2.2). Unfilled points represent bottom camera data, filled points represent floating cameras. Vertical bars denote standard error; b) Mean species richness of bottom cameras from the second experiment (see section 2.2.3). Vertical bars denote standard error; c) One-way ANOVA plot of mean max. N (df=5, F=0.86331, p=0.508). Vertical bars denote 0.95 confidence intervals.....33
- Figure 3.1** Frequency of occurrence of taxa found in (a) cast net and (b) camera data. Frequency of occurrence is represented by the proportion of the number of samples in which each taxon is present out of the total number of samples (n) for cast nets (n=360) and cameras (n=98).....43
- Figure 4.1** Location of eighteen zones for camera placement along a bank in Deluge Inlet, Queensland, Australia.....54

Figure 4.2 Map of replicate sampling sites: Mangrove Point near Hinchinbrook Island, Australia, and Stuart Creek, near Townsville, Australia.....	55
Figure 4.3 Non-metric multidimensional scaling analysis (nMDS) of Deluge species occurrence data. Species observed in >5% of samples and with R ² values of >0.4 are represented in the figure. Stress is approximately 0.1. Twelve points represent data from five tidal stages (early run out, mid run out, late run out, early run in, mid run in) over three sampling days.....	58
Figure 4.4 Multivariate regression tree (mCART) investigating species max. N over tidal stage (ero: early run out; mro: mid run out; lro: late run out; eri: early run in; mri: mid run in) and location (D: Deluge Inlet; MP: Mangrove Point; SC: Stuart Creek). Only the 10 species that were present in >5% of all samples were used in analysis. Max. N data were fourth root transformed; barplots show the multivariate species mean at each node, and the numbers in parentheses are the number of replicates.....	60
Figure 4.5 Mean max. Ns of the five most prevalent species observed in video samples in Deluge Inlet through the tidal cycle (ero: early run out; mro: mid run out; lro: late run out; eri: early run in; mri: mid run in). Note that y-axis scales vary among species. Error bars denote 1 standard error.....	61
Figure 5.1 Map of Deluge Inlet, indicating the placement of the 18 zones used for sampling. Zones were also grouped for analyses to reflect bank type: ‘A’, zones 1-3; B, 4-6; C, 7-9; D, 10-12; E, 13-15; F, 16-18.....	69
Figure 5.2 Mean max. N of the five most prevalent species across 18 zones sampled in Deluge Inlet. Zone numbers and bars are coloured to reflect type of bank: blue: ‘A’, zones 1-3, light red: B, 4-6, green: C, 7-9, light blue: D, 10-12, dark red: E, 13-15 and light green: F, 16-18. Bars depict mean max. N over three sampling days (14/06/12, 27/08/12, & 12/10/12). Error bars denote standard error.....	72
Figure 5.3 Multivariate classification and regression tree (mCART) investigating max. N in Deluge Inlet as it relates to habitat type, classified by: type of bank (A, B, C, D, E, F), depth, current flow (linear or turbulent), structure (present or absent), algal growth (present or absent) and rugosity (flat or uneven). Only the 11 species that were present in >5% of samples were used in analysis. Max. N data were fourth-root transformed; barplots show the multivariate species mean at each node, and the numbers in parentheses are the number of replicates.....	74

Chapter 1 General Introduction

1.1 Introduction

Tropical estuaries are dynamic and variable systems (Sheaves 2006). Located at the interface between terrestrial and marine environments, estuaries are susceptible to influences from both land and sea, particularly in the margins of these coastal habitats (Elliott and McLusky 2002). Terrestrial impacts can include direct effects by runoff (due to both natural and anthropogenic causes), which can deliver a range of materials into the system (e.g. sediment, nutrients, woody snags, etc.)(Windom 1975; Hopkinson and Vallino 1995; Eyre and Balls 1999; Hapeman *et al.* 2002; Ellis and Bell 2004; Davis and Koop 2006). Marine influences include tidal impacts, which can cause water level fluctuations and changing currents, also resulting in shifting sediments and changing bathymetry (Dronkers 1986; Furukawa *et al.* 1997). Tides especially influence estuarine edges, where tidal fluctuations make some resources available during high tide with access to intertidal habitats, but unavailable once water retreats (Johnston and Sheaves 2007). These factors all contribute to the highly variable nature of estuarine systems, and provide complex and ever-changing habitats which present many challenges to their resident fauna (Sheaves 2006).

Despite their challenging conditions, estuaries are important habitats for many organisms. Estuaries can provide foraging opportunities and abundant sources of refuge for fauna (Ellis and Bell 2004; Sheaves 2005), some of which utilise these areas for the majority of their lives, and many that come to fulfil certain needs at specific parts of their life cycles (Robertson and Duke 1990b; Ley *et al.* 1999; Yamashita *et al.* 2003). Tropical estuarine edges and the fringing mangrove forests that often characterise them particularly contribute to

the provision of refuge and foraging opportunities (Sheaves 2005). The mangrove root system that is inundated with water at high tides can be an important source of refuge as well as food for organisms that utilise it at these times (Laegdsgaard and Johnson 2001; Sheaves 2005). However, during low tides, this habitat is unavailable and the fauna that found refuge within it must look for alternative sources (Sheaves 2005). This may come from features of the subtidal edge habitat outside the mangrove forest, such as submerged structure that is often found on the estuarine floor (Sheaves 1992), or from refuge that is found in shallow water (Paterson and Whitfield 2000), although the refuge value of shallow water is uncertain (Sheaves 2001). As estuarine nekton utilise the connections between habitats in the estuary, they are more vulnerable when travelling through transition areas between refuges. If their movements are limited to the smaller-scale connection between the refuge of the mangrove forest and the potential refuge of the edge habitat, fauna may reduce their risk of travelling through more high-risk areas. These edge habitats may thus provide particularly important low-tide refuges for edge fauna. This suggests that any investigation of distribution patterns would find that fauna which utilise the mangrove forests at high tide then utilise the alternate refuge of shallow water by remaining in edge habitats during low tide. Abundances may thus increase just outside the mangrove forests at the time when fauna are forced out of the complex root system.

Although estuaries are important systems that play a crucial role in the life cycles of many organisms, there are still many uncertainties and gaps in the knowledge base of these systems. Much of this most likely stems from the difficult sampling conditions found in estuarine environments that limit the techniques that can be utilised in these areas (Rozas and Minello 1997). The fringing mangrove forest, in which many organisms reside during high tides (Sheaves 2005), can be difficult to access with traditional sampling methods. Additionally, high turbidity (Johnston *et al.* 2007) can decrease visibility and make

observational approaches difficult, and characteristics such as the presence of dangerous predators (e.g. the estuarine crocodile) in many estuaries can make certain sampling methods (e.g. underwater visual census) extremely dangerous. Past methods utilised in estuaries have included mostly netting techniques (e.g. cast nets (Sheaves and Johnston 2009), seine nets (Clark *et al.* 2003), trawls (Aglen *et al.* 1999)) and despite having to manage these difficulties, have provided a great deal of useful data. However, as with any technique, these gears have biases and limitations and have left some questions unanswered. While it is known that a variety of taxa utilise tropical estuarine edge environments (Sheaves 2005; Johnston and Sheaves 2008), the details of the patterns of spatial and temporal change in the distribution of taxa remain unclear.

The patterns of use of these estuarine edges are important to understand. Estuaries are crucial habitats as well as areas of economic importance (Costanza *et al.* 1997; Blaber *et al.* 2000), thus effectively managing and preserving these environments is essential. This is especially important due to the high level of outside influence on estuarine environments, particularly from anthropogenic sources, as estuaries receive some of the greatest direct impacts from human activity, which have the potential to directly or indirectly affect the fauna in these areas (Blaber *et al.* 2000). In order to most effectively protect these habitats, a thorough understanding of their use by estuarine fauna is necessary. As estuaries are highly variable (Sheaves 2006), organisms interact with their environment in complex ways and thus patterns of use can be expected to change both spatially and temporally, although little is known of the details of these patterns. Understanding the extent and use of connectivity between subtidal and higher intertidal areas, as well as changes in use along estuarine edges which are the most impacted parts of estuaries (Blaber *et al.* 2000), management plans can focus on areas of the most importance. By more fully understanding the movements of estuarine fauna and the way they utilise their environment, a more specific and

comprehensive management plan can be put in place that incorporates the variable nature and thus changing conditions found in tropical estuaries, as well as considering the range of interconnected habitats that are used by estuarine fauna (Johnston and Sheaves 2007).

In order to increase our understanding of these systems, a technique that can address both spatial and temporal questions more effectively is needed to provide information that has not been available with traditionally-used methods. Underwater videography fits these criteria because it provides information on nekton distribution over time that is not available from point sampling techniques. It is also a safe technique to use in these environments since they can be deployed from a boat without any need to enter the water. The overall aim of the study was to gain a better understanding of the spatial and temporal changes in the distribution of nekton along the margins of estuaries and thus to obtain clearer insight into the way these organisms utilise these habitats and respond to their varying conditions. Before this aim could be investigated, the protocols for using video techniques in this context had to first be established. The thesis thus commences by developing operational protocols for sampling along the margins of tropical estuaries with camera equipment (Chapter 2). To ensure that these camera protocols were a valid sampling technique for estuarine systems, Chapter 3 focuses on assessing the biases of this gear through direct comparison with cast netting, a previously-proven estuarine sampling technique. The remaining chapters focus on investigating the study's aim by breaking it down into two more manageable objectives:

- 1) To determine what temporal changes occur in the distribution of organisms in estuarine edge habitats over the tidal cycle;
- 2) To determine how site-specific habitat characteristics of estuarine edge environments (i.e. depth, rugosity, presence of structure, algal growth, and current flow) may be influencing the changes seen in faunal distribution.

These objectives are addressed in Chapters 4 and 5, respectively.

Chapter 2 An Evaluation of Camera Methods for Investigating the Identification, Distribution and Temporal Patterns of Estuarine Fishes

2.1 Introduction

Tropical estuaries are biologically rich areas, harbouring a diversity of organisms (Blaber and Blaber 1980; Sheaves and Johnston 2009) that they support by providing rich food resources, size-specific refuges and a vast array of available habitat types (Laegdsgaard and Johnson 2001; Sheaves 2009). However, these important estuarine environments are increasingly impacted by anthropogenic factors that have the potential to impair ecological functioning (Edgar *et al.* 2000). Understanding details of estuarine functioning is of the utmost importance in recognising and remediating the impacts of degradation, with a detailed knowledge of species compositions and distribution the most basic requirement.

Logistical difficulties due to the physical nature of tropical estuarine systems (i.e. turbid waters, large tidal movements, dense mangrove forests) and the presence of dangerous predators like estuarine crocodiles make investigating the distribution of fauna difficult. These conditions make techniques such as underwater visual census unviable. Given these limitations, it is often difficult to gather a comprehensive data set when sampling estuarine areas using traditional methods (Rozas and Minello 1997; Connolly 1999).

In the past, most sampling in estuaries has been conducted using a variety of netting techniques; however, every gear type has limitations and although netting methods have proven extremely useful, gaps in knowledge are still evident. Common netting methods for fish

collection, such as cast, gill, seine or trawl nets, are extractive techniques which are useful for providing identification and distribution data (Aglen *et al.* 1999; Clark *et al.* 2003; Sheaves and Johnston 2009). However, the effectiveness of each netting method differs due to characteristics such as species, mobility, behaviour, size class and preferred habitat (Rozas and Minello 1997; Baker and Minello 2011). For instance, gill nets are appropriate for larger mobile individuals, but often under-represent site-attached species (Ley 2005; Sheaves and Johnston 2009). Conversely, cast and seine nets are inefficient at capturing species sensitive to disturbance and possessing quick escape responses (Lugendo *et al.* 2005; Sheaves and Johnston 2009).

Other capture methods have similar issues. For instance, hook and line sampling is a targeted approach, only effective for species attracted to the specific bait being used (Hetrick and Bromaghin 2006). Technological advances continue to offer more sampling options, but these still have their own limitations. For example, while sonar is continually developing to provide more detailed information, it can only reliably distinguish between size classes, rarely between species (Romare *et al.* 2003), particularly in complex multi-species assemblages (Mueller *et al.* 2010). Consequently, by themselves, none of these methods provide a comprehensive view of community structure and so leave gaps in knowledge that are still to be filled.

Cameras have been used as an alternative sampling method that can fill some of the gaps in information left by other techniques. Still photographs have previously been used to identify fish species (Koslow *et al.* 1995; Bailey *et al.* 2007) and determine their distributions (Omori and Ohta 1981), as well as to identify and characterise habitats (Dyer *et al.* 1982). However, video cameras are often more useful because they capture more information, and can either be analysed as complete video files or in part as still frame-grabs, granting the researcher flexibility in analytical approach depending on the specific questions being addressed (Harvey *et al.* 2003;

Wisenden *et al.* 2004; Shucksmith *et al.* 2006). Videos have been used to study behaviour (Ang and Petrell 1997; Sheehan *et al.* 2010), species diversity and distribution (Burrows *et al.* 1994; Cappo *et al.* 2004; Stien *et al.* 2007), and size structure and abundance (Denny and Babcock 2004). Videos may be recorded using either baited (Cappo *et al.* 2004; Denny and Babcock 2004) or non-baited cameras (Burrows *et al.* 1994; Sheehan *et al.* 2010), and can be useful both in natural (Cappo *et al.* 2004; Shucksmith *et al.* 2006) and modified environments (Ang and Petrell 1997; Stien *et al.* 2007; Zion *et al.* 2007).

There are many advantages to using video methods for data collection. For instance, cameras are particularly effective for observing individuals in their natural habitat. These methods are also useful in conditions that limit the use of alternative methods, such as in extremely deep water (Bailey *et al.* 2007), studies in areas of uneven or rugged bathymetry, in which cameras can be used to monitor the bottom without having to come into contact with it (Shucksmith *et al.* 2006), or in areas of limited access, such as under layers of ice (Mueller *et al.* 2006). Video cameras can also be used to capture multiple types of data simultaneously such as behaviour, species composition, abundance and habitat associations. By analysing such data from recorded video footage, areas of uncertainty can be reviewed many times, a luxury not available with other methods. For instance, visual censuses can result in issues of diver presence scaring or attracting fish (Fernandez-Jover *et al.* 2008), diver bias that can make replication difficult, and uncertainty with transect size (Edgar *et al.* 2004)—all issues that can be eliminated using camera techniques. Videography also enables a large volume of data to be collected in a short amount of time, as many cameras can collect data simultaneously. Underwater cameras are now a low-cost method, and a non-extractive and non-destructive option for sampling (Bailey *et al.* 2007), providing additional benefit, especially for sampling in protected areas.

As with any sampling gear, video methods have a number of disadvantages. Camera effectiveness can be limited by water and environmental conditions (i.e. low light levels, high turbidity) which can severely decrease visibility, although camera settings may be adjusted to account for these factors in some instances (Willis and Babcock 2000; Mueller *et al.* 2006). Additionally, just as any technique is limited to a specific area or volume, cameras have a fixed maximum field of view which naturally restricts their ability to sample the entire water column, allowing only the portion that is in-frame to be represented. Deploying underwater cameras also generates a disturbance that may either attract or repel organisms, creating the possibility of sampling biases (Stoner *et al.* 2008). However, habituation may correct for this as cameras are left in one position for an extended period of time, and the disturbance of deploying cameras is relatively small compared to most other gears or sampling options. Structure and uneven bathymetry can also complicate the positioning and orientation of the camera. Electronic cameras are vulnerable to technological problems that do not affect simpler, alternative methods such as nets. Finally, evaluation of data from this technique can be difficult. Analysis of recorded data can be time-consuming, which must be included in any research plan. Cryptic species may also be overlooked, although this is a problem common to most sampling approaches.

To ensure robust data from video camera sampling, the potential issues with the gear need to be considered and addressed before using video methods for data collection in the field (Table 2.1). Specific settings, placement and water conditions must be determined for optimal collection of the most comprehensive and representative data. For example, many species occupy either the pelagic or benthic environment, or their preferred depth or distance from shore (Johnston and Sheaves 2007; Johnston *et al.* 2007), and so camera placement can have a substantial impact on the abundance and species diversity recorded in the area. Consideration of

these details in relation to the objectives of the study is essential to ensure non-confounding results.

Underwater videography may be a useful additional gear type for sampling in estuarine environments and for filling in the gaps in knowledge left by the limitations of more traditional methods. For example, the locations of entire life stages of many common estuarine species still remain unknown, which may be due to the fact that some habitats within estuaries have been subject to little investigation, such as within complex vegetated habitats (Connolly 1999; Guest *et al.* 2003) or in deep, structured habitats (Bradley 2013). Much of the knowledge of estuarine systems is thus limited to shallow, open-water habitats, although it is still apparent that the gears that are generally used to sample may still be missing important components of the assemblage in these areas as well (Baker and Sheaves 2006). The objective of this chapter is to develop operational protocols for video sampling of temporal and spatial distribution of fishes in tropical estuaries, as well as to evaluate the impact of biases and the approaches for minimising such impacts related to this video methodology.

2.2 Materials & Methods

Underwater cameras were utilised in two experiments to determine and evaluate the arrangement of equipment most suitable to this study in collecting data on the spatial and temporal distribution of fishes in a tropical estuarine environment. Investigations were conducted in tropical estuarine habitats of North Queensland, Australia. To ensure the most effective and comprehensive video testing method was established, many details of the technique were developed and evaluated during pilot fieldwork (Table 2.1).

Table 2.1 Summary of potential issues and concerns associated with underwater camera methodology, as well as the methods and solutions to address them. Issues must be addressed and validated before the successful use of cameras in tropical estuaries is possible.

General Issues	Specific Issues/Concerns	Methods Used to Address Issues	Solutions
Sampling Conditions	<p><i>Weather:</i></p> <p>Weather conditions (e.g. wind, precipitation) can affect the ability to properly deploy equipment, as well as other factors that can impact the quality of the data collected, such as visibility and current conditions.</p>	<p>Appropriate weather conditions were assessed via visual observations of the sampling sites as well as review of video data. Assessments were made by reviewing factors such as visibility and current, as well as logistical deployment of equipment, as they related to weather conditions.</p>	<p>Suspended sediment could be observed directly from the boat as well as in video footage after heavy winds or rains and after long periods of precipitation. Days with such weather conditions, as well as up to approximately two days following them, were subsequently avoided during sampling.</p>
	<p><i>Visibility:</i></p> <p>Low levels of visibility can negatively impact the ability to capture and then identify individuals on video footage.</p>	<p>Visibility was determined from video data by using points of reference as seen in video footage (e.g. structure or other features). Each video was then evaluated to determine if the ability to identify fauna was hindered due to too low visibility by analysing the correlation of observed species richness and visibility for each video.</p>	<p>Low visibility reduces the ability of cameras to capture details of the surrounding environment or its fauna. However, visibility of 0.25m can still provide useful data with approximately the same amount of positive identifications made as in higher visibility videos. Videos with visibility <0.25m had few positive identifications, and so were discarded and not used in analysis. Most video samples used had visibility between 0.5 and 0.75m. Light levels were not found to be affected by depth in any substantial way, with all daytime light levels found to be effective for sampling.</p>
	<p><i>Depth:</i></p> <p>Depth can play a role in the species that are observed, the extent of the water column captured by camera equipment (as deeper water emphasizes field of view limitations) and visibility conditions via changes to light levels.</p>	<p>The depth of each camera was measured during camera collection and was evaluated in relation to visibility and species richness.</p>	<p>Although depth was not found to substantially influence the species richness recorded by cameras, species richness was generally higher in cameras placed in shallower depths. This was especially true of floating cameras.</p>

	<p><i>Current:</i></p> <p>The issue of current can be broken down into two categories: speed and direction. High current speeds can create problems when setting cameras and can drag equipment out of place, and so current limits must be set. Current direction is also of concern, as it can affect the accuracy of identifications and influence what is observed by cameras. Cameras must also be placed in the same orientation in relation to current direction to create consistency between videos and avoid confoundment (due to videos placed in different directions and therefore in different orientations in relation to fish movement; fish generally swim upstream to maintain position).</p>	<p><i>Speed:</i> The range of usable current speeds was determined by observing the success of equipment deployment at various speeds of water movement (ranging from no current to approximately 0.25m sec⁻¹) as well as evaluating the quality of video gathered at those different speeds.</p> <p><i>Direction:</i> To determine the optimal orientation for cameras in relation to current, videos were evaluated from cameras placed in different directions to current movement.</p>	<p><i>Speed:</i> High current strength (>0.25 m sec⁻¹) was found to limit camera use. With strong currents, costly camera setups may be moved and/or lost. These conditions should be avoided (only sampling with moderate currents, with slow or nil current conditions ideal) or cameras should be placed in areas more sheltered from fast water movement.</p> <p><i>Direction:</i> To create consistency and avoid confoundment, cameras should be placed facing in a downcurrent orientation. This allows for individuals to be captured in frame for a longer amount of time while they showed minimal movement, as fishes move upcurrent relatively slowly (both advantageous for identification).</p>
	<p><i>Tide:</i></p> <p>The tidal ranges used for sampling can affect results, as they can impact other factors such as visibility and current. Although water movement created by tidal change is important while investigating temporal changes to faunal distribution, too much movement can create undesirable conditions. An additional concern is that camera batteries, file storage space and daylight hours restrict sampling throughout the tide, so only</p>	<p>The appropriate tidal range for collection of comprehensive data was determined by observing the current associated with various tidal “runs” (the change in water level between high and low tide) as well as the visibility associated with tidal heights. These observations were made using both visual observation and video data. The need for some tidal movement to observe the effects of tidal change on faunal distribution (as was the aim of this study) was also taken into consideration. Visual observation of the study site was used to determine the portion of the tidal cycle to sample; cameras were run</p>	<p>Large spring tides (>2m range, high tides >3m) result in strong current and poor visibility conditions (high turbidity), and so moderately-sized tides (between neap and spring) were found most suitable for sampling. Three or more days of large spring tides caused unsuitable visibility conditions to persist for approximately an additional three days of suitable tides. The smallest neap tides did not create enough tidal movement to sample tidal changes on faunal distribution (as was the aim). Thus, it was moderately-sized tides after the neap that were utilised for this study, in an effort to minimise suspended sediment, reduce current, and still allow for adequate tidal movement.</p>

	part of the tidal cycle can be examined on any given day.	during the bottom half of the tide (i.e. mid run out to mid run in) to keep sampling consistent between replicate days (and avoid confoundment). Using the bottom half of the tidal cycle ensured that fauna was confined to the main channel of the estuary and not dispersed throughout the fringing mangrove forests, allowing for a more comprehensive sampling of community structure.	
Camera Setup & Deployment	<p><i>Position in Water Column:</i></p> <p>Camera field-of-view limitations usually mean that only part of the water column can be recorded, so gear position in the water column and angle of the camera lens can greatly influence the specific area and species sampled.</p>	The optimal camera position in the water column for collection of the most comprehensive data was evaluated by utilising cameras at the surface and bottom of the water column. Species richness as well as specific species unique to each setup were assessed. Camera setups and deployment are described in detail in sections 2.2.1.1 and 2.2.2 of this chapter.	The weighted benthic cameras (angled horizontally to capture both the substrate and water column in the field of view) were found to record the most diverse range of species as well as the fewest observed fish that were unidentifiable, when compared to floating cameras. Bottom cameras were thus chosen as the most appropriate for addressing the aims of this study.
	<p><i>Distance from Shore:</i></p> <p>The lateral placement of cameras (and thus also relative depth) can have a large impact on the abundance and species diversity recorded and so must be considered carefully so as to accurately represent the target habitat.</p>	The most appropriate lateral positioning for camera equipment was determined by running cameras at intervals from the shoreline, outwards (as described in detail in section 2.2.3). Species richness recorded by bottom cameras was evaluated.	The greatest number of species was observed close to shore, with the peak approximately one to two meters from the shoreline.
	<p><i>Structure:</i></p> <p>Fauna have been found to congregate around underwater structure (Sheaves 1996; Laegdsgaard and</p>	The possibility of bias caused by underwater structure (e.g. roots and snags) was considered based on previous studies as well as using visual observation and video data. The impact	Presence of structure attracts fish and can potentially bias results (as seen via visual observation, video data, and past studies (Sheaves 1996; Laegdsgaard and Johnson 2001)), but the procedure in relation to the presence of structure while sampling is to be

	<p>Johnson 2001), creating the possibility of biased results while sampling. Although structure need not always be actively avoided, this possible prejudice of data collected around areas with structure must be considered.</p>	<p>of this possible bias on results was considered while forming video sampling guidelines.</p>	<p>determined on a case-by-case basis, based on what is appropriate for the collection of the target data. For instance, when sampling along a bank to determine distribution, structure is an important characteristic of the area and so must be neither targeted nor avoided; however, when targeting data on more general concepts and not on specific habitats, sampling should keep away from structure to avoid biased results.</p>
	<p><i>Length of Video:</i></p> <p>The ideal duration of each video must be determined, accounting for battery life, data storage space and time needed to collect the appropriate representative target data (also allowing for habituation of disturbed fauna).</p>	<p>The optimal video length was determined by evaluating footage for species richness and determining the point at which this number stopped steadily climbing (at which point it can be inferred that local fauna has been accounted for and any new species seen are representative of rare species for the area). Battery life of cameras, data storage space, the need for daily replicates, and the need for comprehensive data were also taken into consideration when determining video length.</p>	<p>It was determined that approximately 15 minutes was the ideal duration of a video sample. This allowed for multiple samples to be taken each sampling day (which was limited by battery life (approximately 3 hours) and file storage space (32GB)) while still collecting comprehensive data on the community. It can be inferred that local fauna was accounted for at this point in video samples, as species richness ceased growing in continuing observation of footage (Fig. 2.7).</p>
<p>Video Analysis</p>	<p><i>Analysis of Footage:</i></p> <p>The best approach for extracting information from the recorded video footage (i.e. what proportion of each video to analyse, how to assess abundances, best identification protocols, etc.) must be determined in order to maximise information extraction.</p>	<p>The most useful and efficient way to analyse video footage was determined by evaluating videos multiple times with various methods. The appropriate speed to view videos, methods of identification and taxonomic level of identification were determined this way.</p>	<p>The presence/absence of species (and species richness) was found to be the most effective and time-efficient means of analysis. Maximum number of individuals of a species observed together in a frame was also recorded to be used for abundance data (max. N). Footage was analysed at 1.5x fast forward, as this allowed for rapid review of samples with minimal likelihood of missing any fish passing quickly through the frame. Individuals were identified to the lowest possible taxonomic level, although many were put into less-specific taxonomic groupings to avoid misidentifications and create consistency.</p>

2.2.1 Initial Technique Development

Pilot fieldwork was conducted in the near-pristine estuarine systems of Deluge Inlet and Mendel Creek on Hinchinbrook Island in tropical North Queensland, Australia (Fig. 2.1) to determine the most suitable environmental conditions and camera setup for video sampling (detailed in Table 2.1). These areas receive freshwater drainage off the western side of Hinchinbrook Island and tidal influx from Hinchinbrook Channel, a narrow channel between the island and the North Queensland mainland. Both Deluge Inlet and Mendel Creek are lined with dense mangrove forests, composed primarily of *Rhizophora* spp., *Bruguiera* spp., *Ceriops* spp., *Avicennia marina* and *Sonneratia alba*. Sediments in the subtidal zone are primarily sand, with patches of macroalgae and seagrass in some areas.

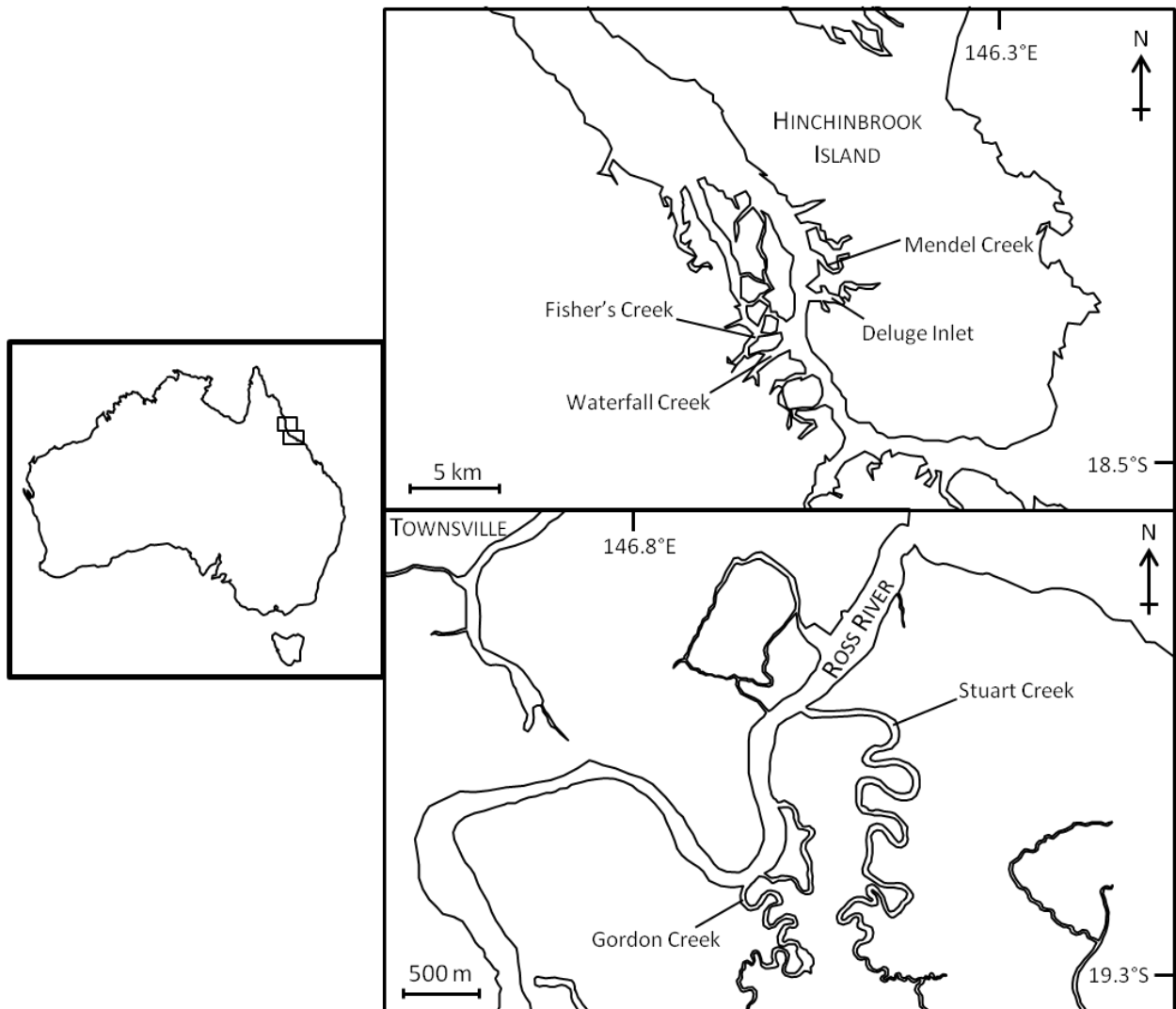


Figure 2.1 Map of sampling sites in North Queensland, Australia, showing locations of Mendel Creek, Deluge Inlet, Waterfall Creek and Fisher's Creek off Hinchinbrook Channel; Stuart Creek and Gordon Creeks are located off the Ross River in Townsville, Queensland, Australia.

All data were collected using ATC9K HD All Terrain Video Action Cameras by Oregon Scientific. Cameras were set to their highest resolution, capturing video at 30 frames per second with image quality set at "fine." This setting enabled the highest quality video to be captured, aiding in identification efforts, especially during times of low visibility. Videos were run for approximately 20 minutes each (approximately the amount of time needed to deploy all cameras within a site) throughout the bottom of the tide (Table 2.1). Due to the lack of artificial lighting with the camera equipment (as artificial lighting creates a type of

baited sampling which was not the aim for this study) as well as to address crocodile safety issues, all cameras were deployed during daylight hours.

2.2.1.1 Camera Systems

To investigate the most appropriate positioning of cameras for gathering comprehensive species distribution data, cameras were set up in two configurations: in floating housings at the surface (Fig. 2.2) and mounted on weighted bases and situated on bottom sediments (Fig. 2.3). The greatest depths in these particular sampling areas reached only approximately three meters, so the fields of view of the combined surface and bottom cameras captured the majority of the water column.

Floating cameras were positioned to record activity from the water surface, angled approximately 30° downwards, with the topmost part of the field of view just below and parallel to the water's surface. After testing a variety of setup options, cameras were housed in an elongated float made of dense Styrofoam and shaped to float on the surface without compromising camera orientation (i.e. preventing rolling)(Fig. 2.2a,b). A soft foam mat was secured over the float and camera to prevent the camera from overheating in the sun or from having moisture condense on the lens (Fig. 2.2c,d). The camera was anchored with a 2mm-diameter line with an intermediate small float to prevent excess line from drifting into the camera's field of view (Fig. 2.2a). Tethering the camera by its upstream end meant that it automatically pointed downcurrent (Table 2.1).

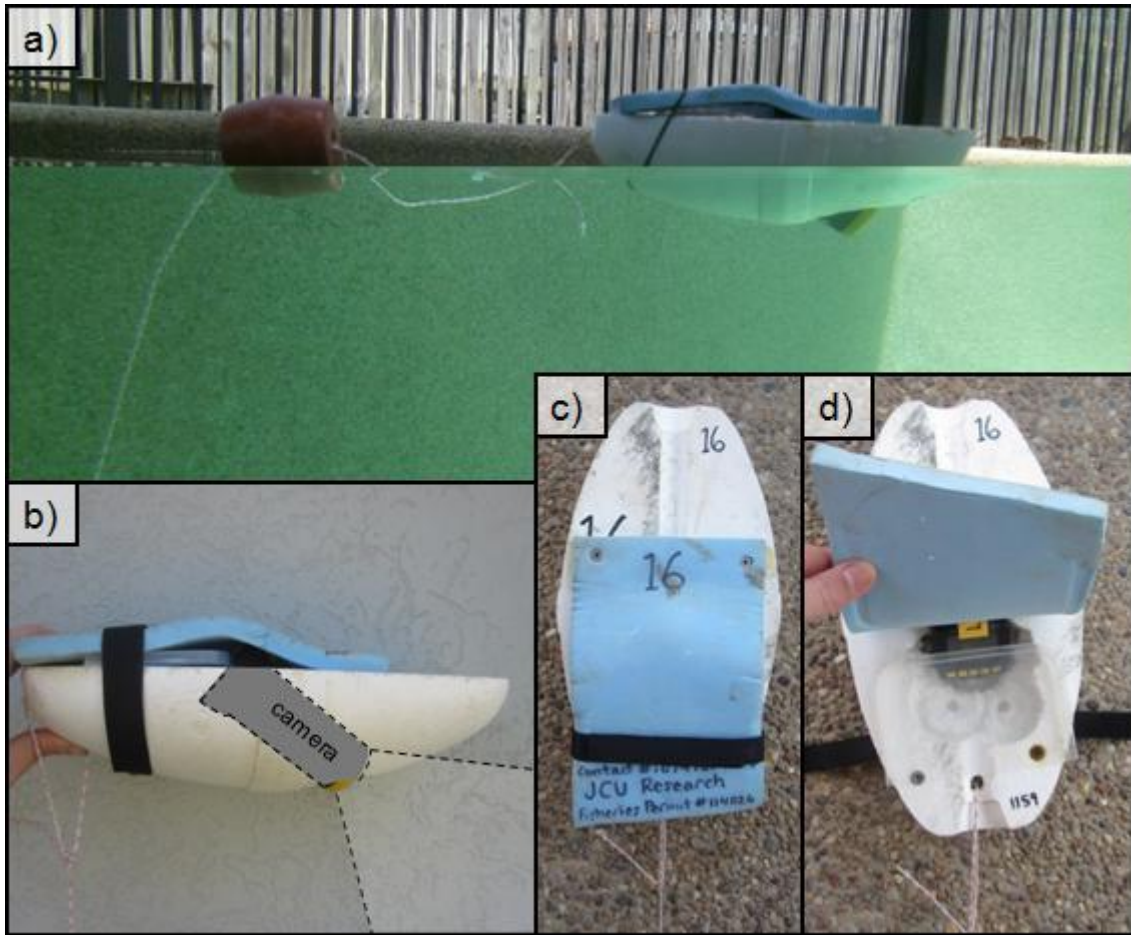


Figure 2.2 Floating camera setup: a) An example of how the floating camera setup is deployed in water; b) A demonstration of how cameras are housed within the floating setup; c) & d) A closer view of the floating housing with camera and protective foam mat.

Cameras mounted on weighted submersible bases were positioned to record activity from the substratum upwards (Fig. 2.3). This configuration enabled the benthic environment to be recorded, and in shallow sites included a majority of the water column. Cameras were mounted onto aluminium bases and oriented horizontally such that the lens was positioned approximately 5cm above the substrate (Fig. 2.3b). This provided a field of view in which both the substrate in front of the camera as well as a substantial amount of the water column was visible (Fig. 2.3c). During the study, substrate type and fish species could be identified for up to approximately two meters from the camera. A plastic fin above the camera caused it to orient downcurrent during deployment, the same as the surface cameras (Fig. 2.3). A 2mm-

diameter line attached the weighted setup to a buoy on the surface, and a small float between the base and surface buoy ensured that excess line did not drift into the camera's view (Fig. 2.3a).

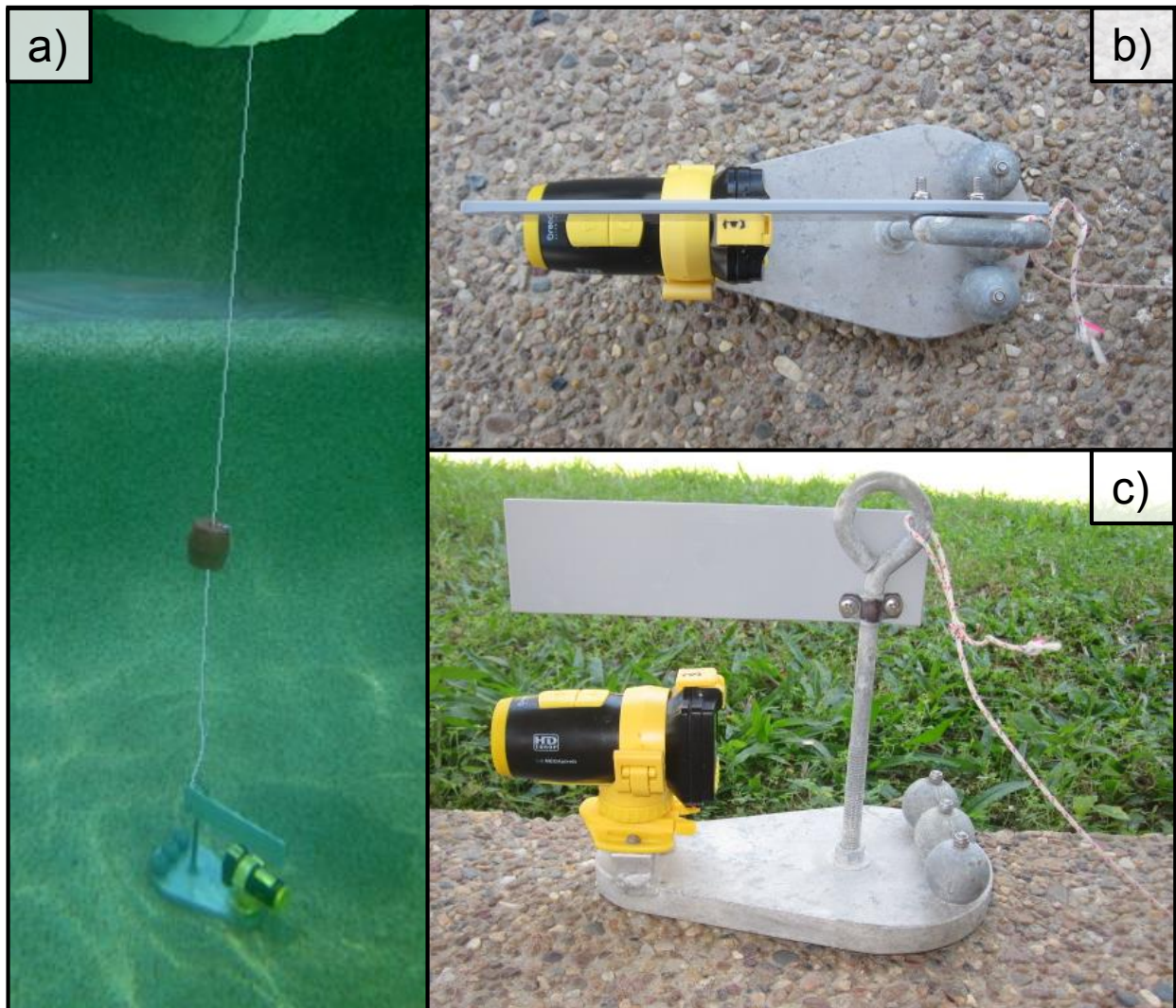


Figure 2.3 Bottom camera setup: a) An example of how the bottom camera setup is deployed in water; b) An above view of the weighted aluminium base and plastic fin with camera located at one end; c) A closer view of the weighted base and plastic fin with camera mounted on a small platform.

2.2.2 *Camera Position in the Water Column*

Surface and bottom camera systems were utilised to determine the most appropriate positioning of camera equipment within the water column (Table 2.1). The two setups were placed at increasing distances from the shoreline to evaluate both their differences in targeted species and data collection, as well as to evaluate their effectiveness for sampling at all depths and positions in the estuarine edge environment. For example, in shallow water, the field of view of either camera setup will encompass the majority of the water column, while deeper water results in only a small portion of the water column represented, highlighting the level of each system's capability of representing a diverse range of both benthic and pelagic species throughout the estuarine edge.

Cameras were deployed at two sites with known high concentrations of fish within Deluge Inlet and sampled on the 14th and 15th of May 2012 (Fig. 2.4). Two additional sites with unknown concentrations of fishes were sampled in Mendel Creek on the 13th of June 2012 (Fig. 2.4). Five floating cameras and four bottom cameras were set at each site (uneven numbers of camera gears were used due to limitations in the amount of camera equipment available during field work). Floating and bottom cameras were run in separate transects perpendicular to the shore and approximately 10m apart (Fig. 2.5), close enough to ensure that both were sampling the same local community. Floating cameras were placed at 2m, 6m, 10m, 14m, and 18m from shore, while bottom cameras were set at 4m, 8m, 12m, and 16m. This two-transect setup and alternating camera arrangement helped to ensure both independence of samples as well as that each camera type captured a range of depths and positions from shore.

Cameras recorded video for 20 minutes each at varying stages throughout the bottom half of the tide (Table 2.1). The number of replicate camera sets ranged from three to five per

day for each site, determined by camera battery life and data storage space. Final depth measurements were also taken for each sample by marking (and then measuring) water level on the attached line as cameras were retrieved. This design produced a total of 225 videos from the four sites.

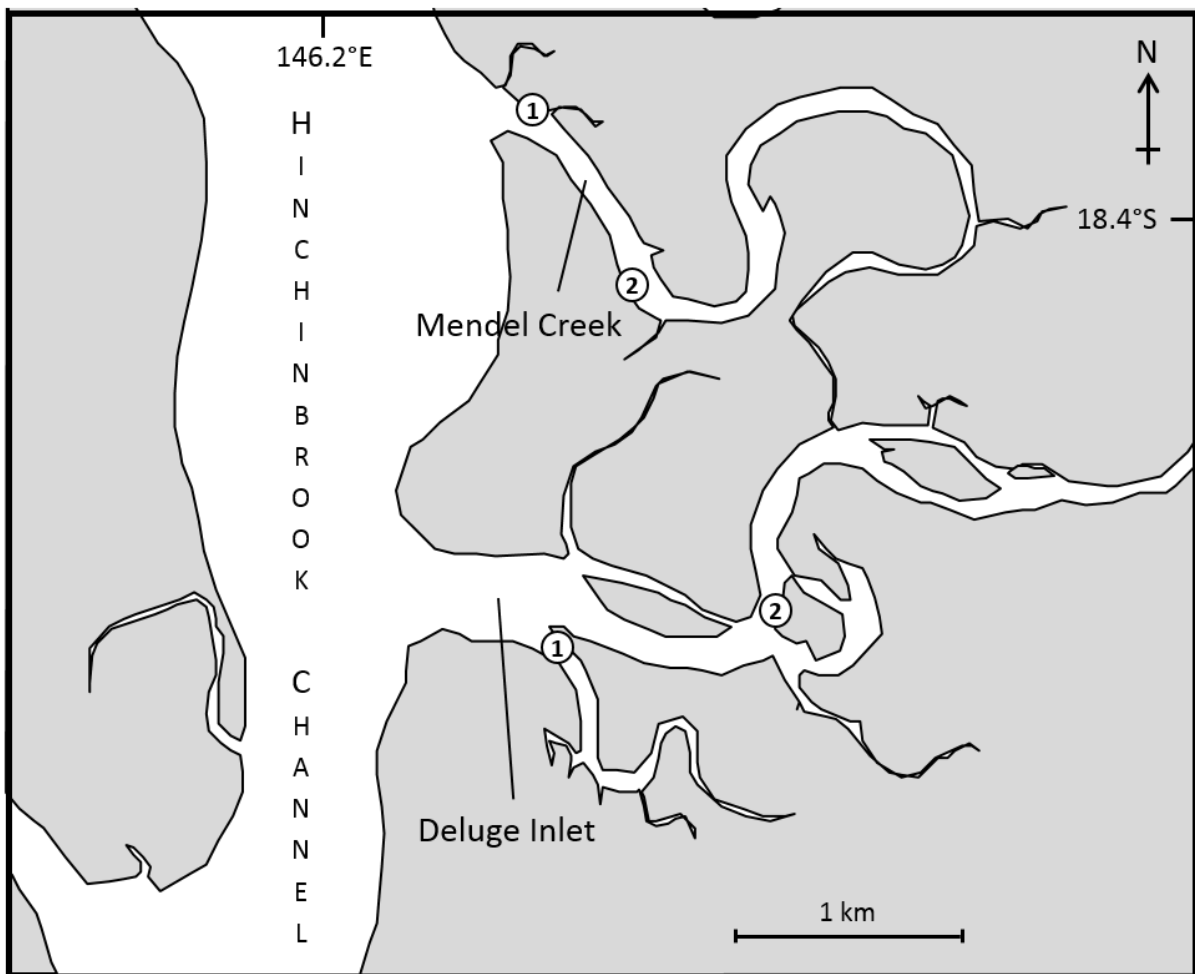


Figure 2.4 Map of study sites with approximate positions of the two sampling locations at Deluge Inlet and Mendel Creek.

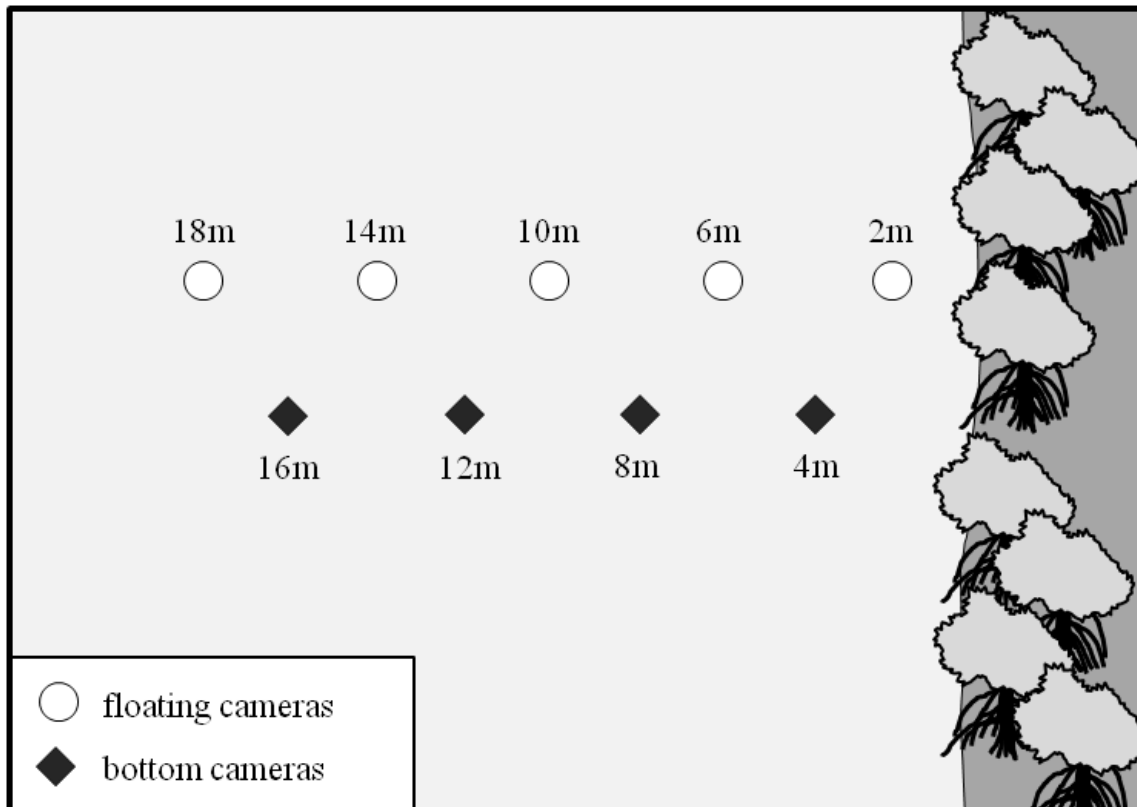


Figure 2.5 Diagram of the configuration of floating and bottom cameras used to assess camera setup and position in the water column.

2.2.3 Lateral Camera Placement

In the second phase of this study, weighted bottom cameras were used to investigate the lateral distribution of fish away from the bank (Table 2.1) to determine the ideal placement of camera equipment for sampling the community along the estuarine edge environment. To avoid any confoundment with habitat type, only areas with no apparent submerged structure were targeted (Table 2.1). A number of sites were utilised to ensure the results reflected general patterns relevant to tropical estuaries, without limiting results to specific patterns of a particular site.

Cameras were deployed at four sites off Hinchinbrook Channel (Paluma Creek, Mendel Creek, Waterfall Creek and Fisher's Creek), as well as two sites at the mouth of the Ross River near Townsville, Queensland (Stuart Creek and Gordon Creek) (Fig. 2.1). Sampling took place over two days, the 30th of July and the 15th of August 2012. Cameras were deployed in 20 transects spanning the six sites, with six cameras per transect resulting in 120 total video samples. Transects were deployed in independent pairs (with approximately 30m between transects) perpendicular to the shore, with cameras set at 1m, 3.5m, 6m, 8.5m, 11m and 13.5m from shore in each transect. Cameras were fitted with front-mounted, standard 50cm rods made of MIG welder cable (approximately 5mm diameter) directed forwards from the base of the camera and designed to rest on the bottom sediments to assist in determining relative visibility. Sites were sampled throughout the bottom of the tide during daylight hours (Table 2.1). Videos were run for 15 minutes (ideal video length)(Table 2.1). Final depth measurements were collected for each sample as cameras were retrieved.

2.2.4 Data Analysis

Videos were analysed in VLC Media Player on 1.5x fast forward (Table 2.1). The occurrence of each positively identified taxon was recorded from each video, as was max. N of each taxon, determined using the maximum number of individuals seen together in a frame. This removed the possibility of any individual being counted twice. Where necessary, videos were examined frame-by-frame to help properly identify individuals. Each individual seen in the video footage was identified to as low a taxonomic level as possible, with the majority classified to genus or species. However, only positive identifications (with no chance of misidentification) were recorded, causing some individuals to be classified into more general groupings rather than as individual species (Table 2.1). Twenty (*Camera*

Position in the Water Column, see section 2.2.2) or 15 minutes (*Lateral Camera Placement*, see section 2.2.3) of video were analysed (Table 2.1). For both experiments, analysis began after the initial sediment disturbance from the camera-setting process had settled (as stipulated in pilot work, with average time being approximately 30 seconds from the time the camera entered the water). If the video recorded for less than the 15 or 20 minutes (e.g. due to battery depletion), then analysis was done until the video file concluded. Visibility for each video was estimated to the nearest 10cm.

Max. N values, species composition, and species richness were used during analysis of video data. While max. N and species composition can provide direct and specific comparisons, species richness allows cameras to be assessed by how valuable they may be for sampling a large variety of species (the more species they sample, the more functional they may be for general uses), which is useful for developing sampling protocols. Position in the water column was assessed using a one-way ANOVA on $\log(x+1)$ transformed max. N data, using max. N values from each camera replicate to assess their relationship to camera type (floating or bottom cameras). Transformed data were used to normalise results. Lateral camera placement was assessed using a one-way ANOVA on $\log(x+1)$ transformed max. N data, using max. N values from each camera replicate and analysing their relationship to position from shore.

2.3 Results & Discussion

Protocols for the use of underwater video equipment to sample local fauna in tropical estuaries were determined and refined during initial field work in Deluge Inlet and Mendel Creek (Fig. 2.1), resulting in a robust methodology to be used for the remainder of the study (Table 2.1).

2.3.1 Initial Technique Development

Apart from defining ideal sampling conditions and deployment protocols for underwater cameras (Table 2.1), the details of video recording by camera equipment were refined during the initial stages of this study. The ideal length of each video sample was defined; around ten to fifteen minutes per video replicate was optimal, as by this time most videos had reached their maximum species richness (Fig. 2.6)(Table 2.1). Ten to fifteen minute samples captured the immediate local fauna, with new species after this point most likely originating from surrounding areas or being rare in that particular locale (Table 2.1). In this time, the boat sound and brief disturbance of sediment (30 seconds in most cases) caused from the setting of cameras could dissipate, and habituation of disturbed species was allowed for. However, fish were present and able to be identified almost immediately in many instances, which suggested that analysis of video data should begin immediately after settlement of initial disturbed sediment. For these reasons, and since 15 minute samples allowed for sampling at various periods throughout the bottom half of the tide while optimising battery life (Table 2.1), 15 minute videos were chosen for the rest of the study.

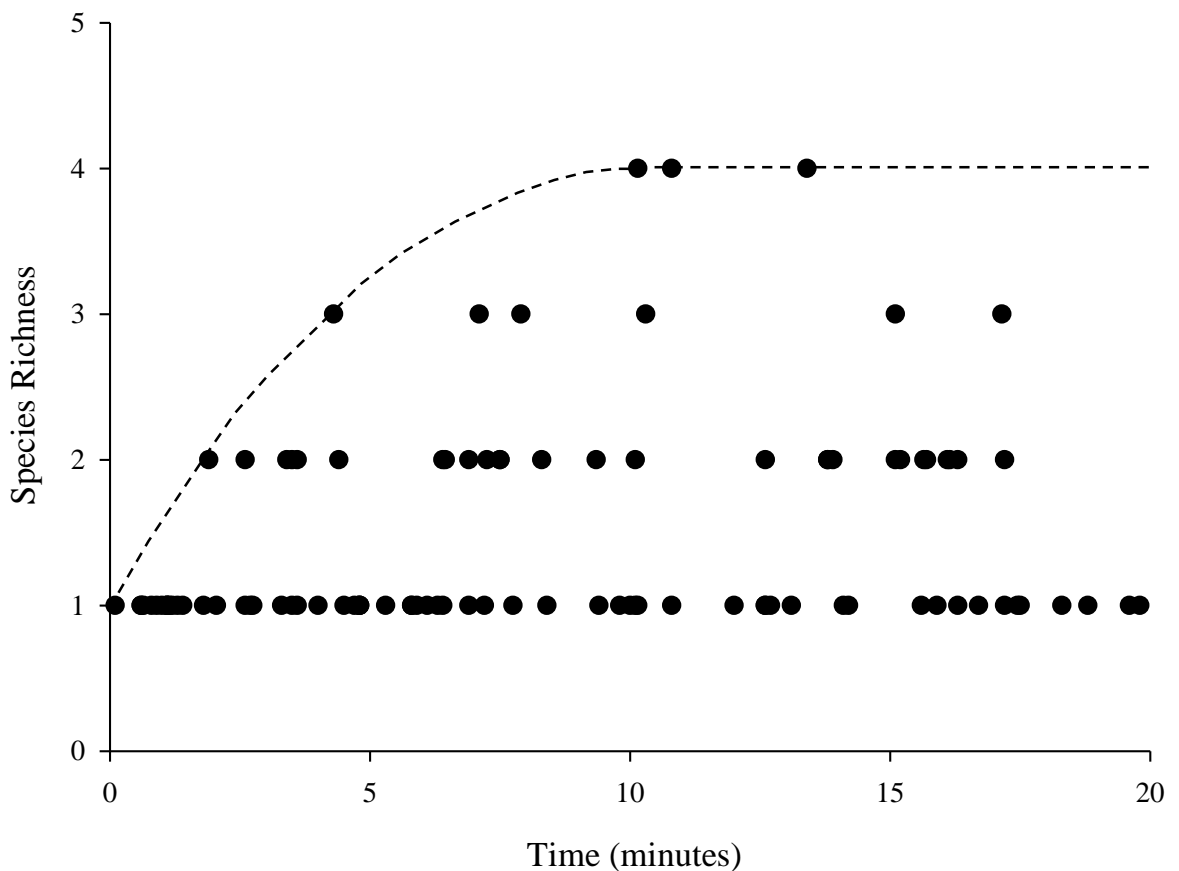


Figure 2.6 Species richness in relation to video analysis time, providing information as to appropriate video length. Data is from bottom cameras used in the first experiment in this study (section 2.2.2) and portrays species richness values at their appropriate times throughout each video (i.e. some videos may be represented by multiple points). Only videos with a species richness of ≥ 1 were used. Dashed line provides visual representation of the plateau in species richness at approximately 10 minutes into analysis.

Video samples were analysed at 1.5x fast forward, enabling rapid review of the video with minimal likelihood of missing any fish passing quickly through the frame, a possibility with faster video speeds which skip multiple recorded frames at a time (Table 2.1). Cameras recorded video at their highest resolution settings, as this provided the best quality images and thus allowed for more positive identifications, particularly during times of low visibility. Fish were identified to as low a taxonomic level as possible, usually to species or genus, and some only to family level (Table 2.2). Even where many individuals were identified to

species, some taxa were grouped more broadly to standardise results and allow for comparisons across the whole study. For instance, although there were a few occasions in which specific species of mullet were identified, the majority of mullet within the video data were unable to be classified more specifically than as “Mugilidae.” Thus, all mullet were classified as “Mugilidae” during analysis, regardless of the ability to identify them further. Another example is with the species *Lutjanus russelli* and *Lutjanus fulviflamma*— two species that can be easily confused. Sightings of either *L. russelli* or *L. fulviflamma* were recorded as part of the species group “*Lutjanus fulviflamma/russelli*.”

2.3.2 Camera Position in the Water Column

The use of both floating and bottom cameras allowed investigation throughout the water column, determining what camera setup was most useful for collection of comprehensive data (Table 2.1), or at providing the most complete picture of the surrounding community. By comparing the footage between the two setups, the extent to which video from benthic or surface cameras represented the entire assemblage at a location and what species were under-represented by either setup alone could be determined. Floating and bottom cameras showed distinct differences in abundances recorded. Cameras situated on bottom bases recorded significantly more individuals in their video footage than did the cameras in the floating housings ($df=1$, $F=44.008$, $p=0.000$)(Fig. 2.7). Videos from bottom cameras also included a greater number of species exclusive to this particular setup (Table 2.2). Bottom cameras recorded a total of 18 species, 13 of which were seen only by bottom cameras (Table 2.2). This is in contrast to the floating cameras, which recorded only 8 total species, 3 of which were exclusively observed by floating cameras (Table 2.2). However, *Lutjanus fulviflamma/russelli*, although only recorded by one floating camera in this

experiment (albeit in an extremely shallow depth of 0.7m, which would have allowed this individual to have been recorded by a bottom camera as well if it had been placed in the same location), was common in bottom cameras in pilot fieldwork performed in this system.

Bottom cameras also proved most reliable for positive identification, with 67% of fish seen during video analysis able to be positively identified. For floating cameras, only 34% were positively identified. Therefore, bottom cameras were found to be more reliable both for data collection and for positive identification.

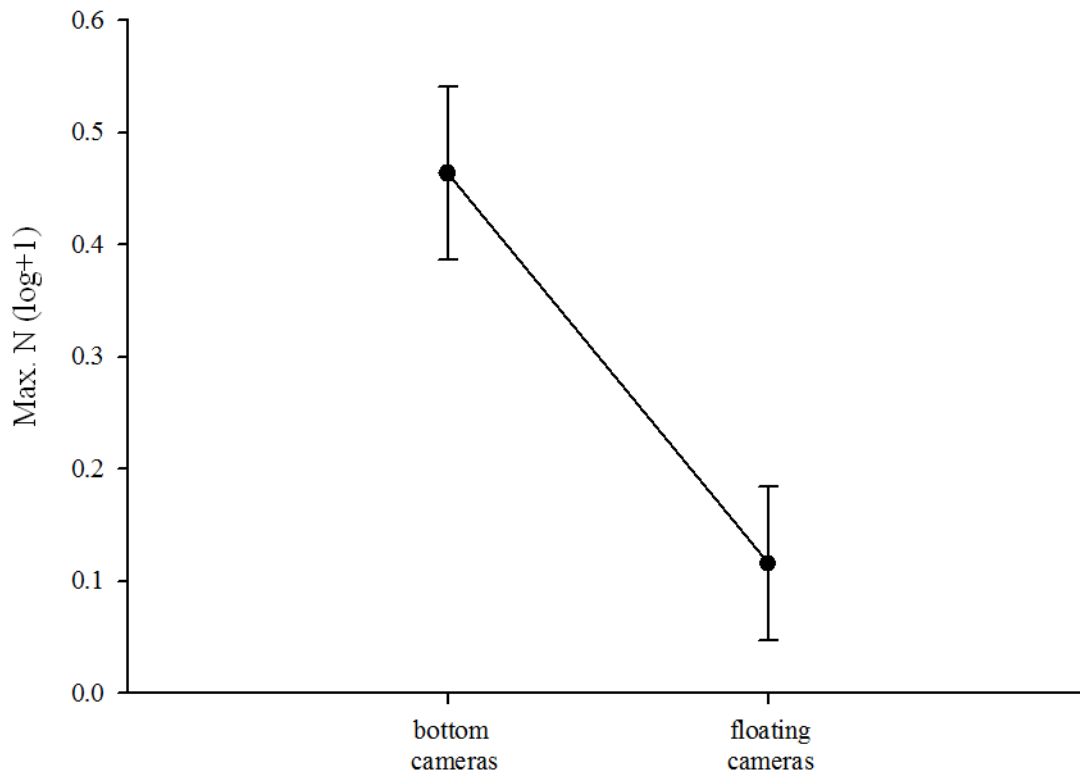


Figure 2.7 Max. N values by camera type (df=1, F=44.008, p=0.000). Vertical bars denote 0.95 confidence intervals.

Table 2.2 Species assemblage list from floating and bottom cameras (collected as described in section 2.2.2). Numbers indicate the proportion of samples (n) in which each species was recorded for each gear type.

	floating cameras (n=125)	bottom cameras (n=100)
<i>Acanthopagrus</i> spp.	0.02	0.04
Alpheidae	0	0.02
Ambassidae	0.03	0
<i>Caranx</i> spp.	0	0.03
<i>Chanos chanos</i>	0	0.03
Dasyatidae	0	0.02
<i>Drepane punctata</i>	0	0.05
<i>Gerres</i> spp.	0	0.18
Gobiidae	0	0.16
<i>Herklotsichthys castelnaui</i>	0.07	0.08
<i>Leiognathus equulus</i>	0	0.01
<i>Lutjanus fulviflamma/russelli</i>	0.01	0
Mugilidae	0.02	0.14
<i>Neoarius graeffei</i>	0	0.01
<i>Pomadasys</i> spp.	0	0.03
<i>Scomberoides</i> sp.	0	0.05
<i>Scylla serrata</i>	0	0.01
<i>Selenotoca multifasciata</i>	0.02	0.23
<i>Siganus</i> sp.	0	0.01
<i>Sphyraena</i> sp.	0.01	0.01
<i>Toxotes chatareus</i>	0.02	0

This difference in results from bottom and floating cameras, although providing useful insight into these estuarine systems by focusing on the bottom and surface communities, could also be due to a variety of factors and differences between the physical setups, suggesting that it may be both the bottom camera setup as well as position that was found more useful for gathering data in this study. For instance, bottom cameras may have produced more useable data partly due to their angle of view. Bottom cameras gave a lateral view of each individual in frame, making identifications much easier and producing more

positive identifications than the floating camera setups, which relied on an angled view from above to identify individuals. However, not only was identification difficult in floating footage, but fewer individuals were observed compared to bottom camera videos, suggesting that in addition to angle-of-view issues, the position in the water column impacted results as well. Cameras on weighted bottom bases were also more stable than floating cameras, as they were anchored to their placement location. In contrast, floating cameras moved according to both wind and current, sometimes orientating themselves in the wrong direction or changing direction due to strong gusts of wind. The constant movement of these floats may have caused bias by repelling fish. Additionally, floating cameras typically would not include the benthic habitat within their field of view, providing little information about any benthic-associated fishes present, the surrounding environment and no point of reference to assess visibility conditions. Bottom cameras provide valuable information such as bathymetry and habitat characteristics at each site (i.e. extent and type of vegetation, sediment type, underwater structure).

Depth was also investigated in relation to its influence on the results collected by floating and bottom cameras. Species richness in floating cameras had a significant negative relationship with depth ($r=-0.331989$, $p<0.05$). Floating cameras recorded few individuals in depths over 1.5m, and none at depths greater than 1.74m, despite cameras being deployed at depths of up to 3.8m (Fig. 2.8)(Table 2.1). In contrast, bottom cameras observed individuals across the entire range of depths sampled. Thus, floating cameras were only effective in shallow waters where most species were effectively sampled by bottom cameras anyway.

Bottom cameras successfully produced identifications throughout the range of depths sampled (Fig. 2.8). Although species richness values from bottom cameras were not significantly correlated with depth ($r=-0.087038$, $p>0.05$), the majority of identifications were

made at depths of one to three meters, which differed from the floating camera samples (Fig. 2.8). These observations may have also been affected by the limitations of the sampling environment (which only supported depths of little more than 4 meters), the characteristics of each sampling gear, and each camera's lateral position. This difference in results between bottom and floating cameras could also be because benthic habitats are most likely to house the most species (Omori and Ohta 1981; Aglen *et al.* 1999); densities of zooplankton and micronekton are often found to be concentrated near the bottom sediments during the day, creating an area of increased opportunity for food for many species (Omori and Ohta 1981).

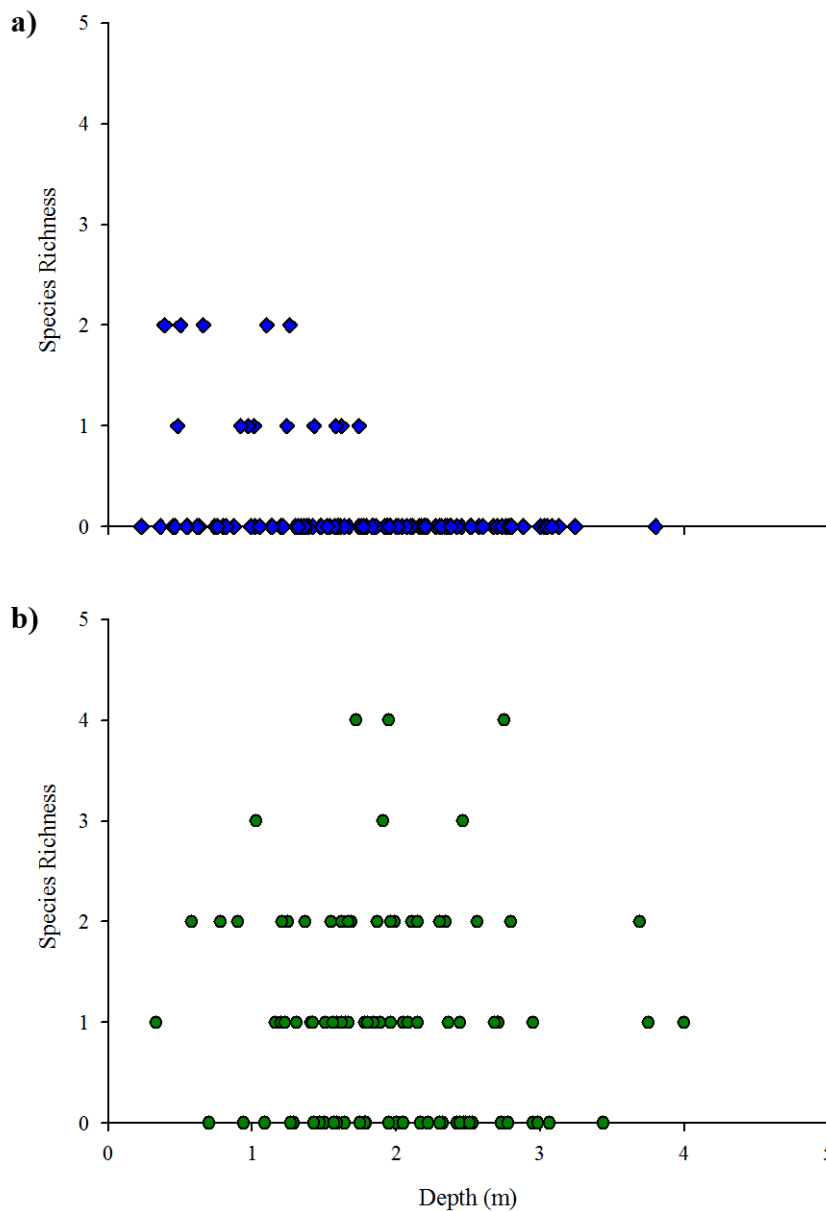


Figure 2.8 Species richness of floating (a) and bottom (b) cameras at various depths.

The information gathered by both bottom and floating camera systems is reliant on a diverse range of factors and characteristics. Structure, current, surrounding and submerged vegetation, the presence of other species, bathymetry and sediment type, wind and weather will all influence the data collected by cameras, as well as any gear type sampling in estuaries. The presence of these influencing factors can make analysis of data from these

areas challenging when their values are unknown (e.g. unknown visibility conditions or sediment type that may influence what fauna are observed), as it can be difficult to discern which variables may be influencing results. In this way, cameras provide a good insight into these issues, as they are able to record information on many of these factors when recording data. Bottom cameras especially benefit from having a point of reference in these instances.

Bottom cameras collected the most comprehensive data while sampling a tropical estuarine edge environment in this study. Therefore, the most useful setup for further data collection would be to utilise all available cameras on weighted bottom bases and to forgo the use of floating camera setups, as little additional data are provided by this method (Table 2.1).

2.3.3 *Lateral Camera Placement*

During the first experiment (*Camera Position in the Water Column*, see section 2.2.2), the number of species recorded by both bottom and floating cameras was found to decrease as cameras were placed farther from the shoreline (Fig. 2.9a)(Table 2.1). Both camera setups documented their highest number of identified species at their closest-to-shore position (Fig. 2.9a), although species richness values from bottom cameras were not found to be significantly correlated to position ($r=-0.126614$, $p>0.05$). Floating cameras showed a significant decrease in species richness with increasing distance from shore (Fig. 2.9a)($r = -0.378419$, $p<0.05$). However, due to the lack of data collected by floating camera setups at deeper sites, it is possible that these methods may not represent a comprehensive sampling of species at those distances farther from shore and thus at those positions of greater depths; distance from shore was significantly correlated with depth ($r=0.604630$, $p<0.05$).

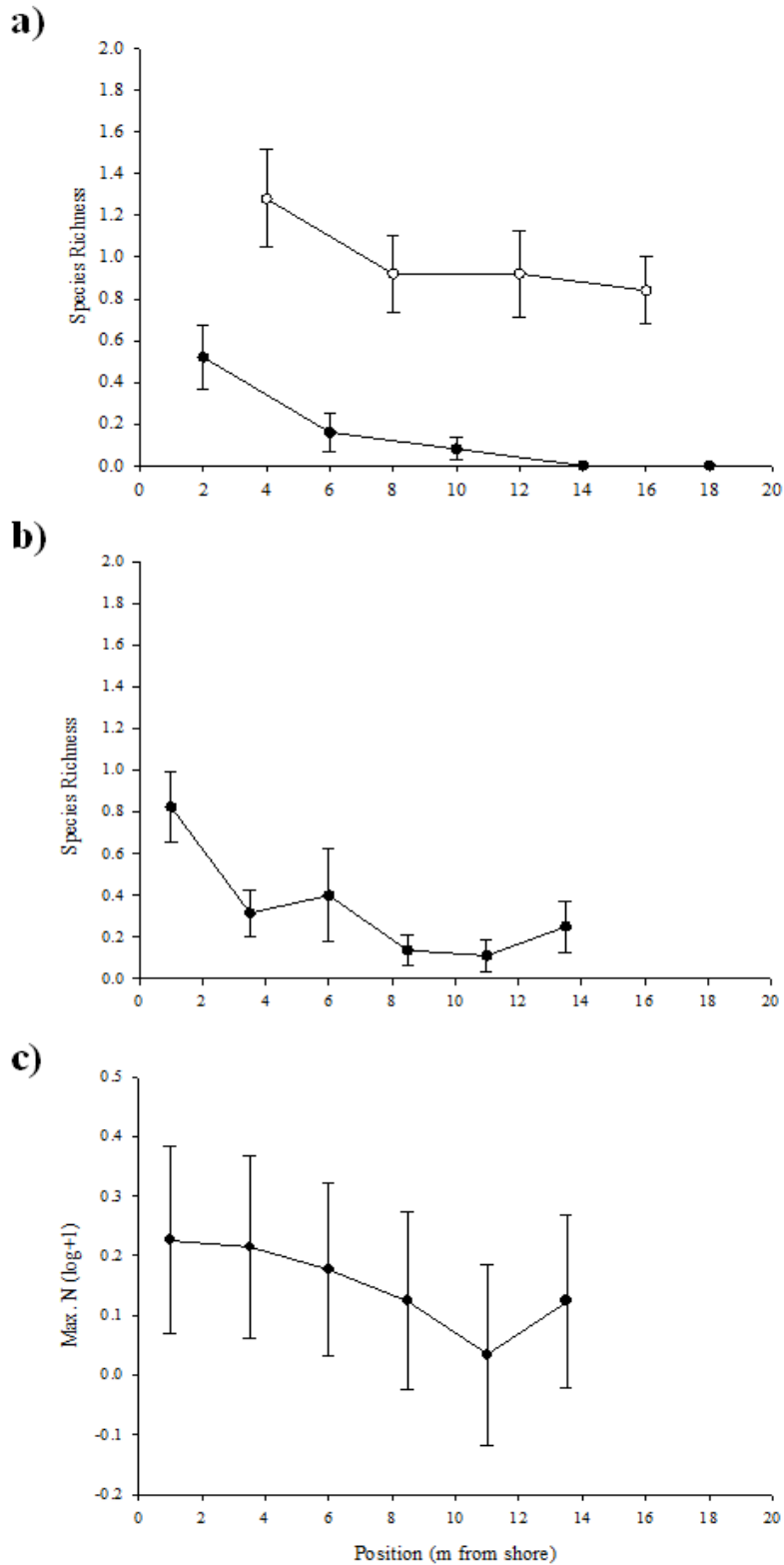


Figure 2.9 Presence of organisms at varying positions from shore; **a)** Mean species richness data from the first experiment (see section 2.2.2). Unfilled points represent bottom camera data, filled points represent floating cameras. Vertical bars denote standard error; **b)** Mean species richness of bottom cameras from the second experiment (see section 2.2.3). Vertical bars denote standard error; **c)** One-way ANOVA plot of mean max. N ($df=5$, $F=0.86331$, $p=0.508$). Vertical bars denote 0.95 confidence intervals.

The bottom cameras used in the second experiment (*Lateral Camera Placement*, see section 2.2.3) produced similar results. Species richness significantly decreased with increasing distance from shore ($r=-0.308203$, $p<0.05$)(Fig. 2.9b, Table 2.1). Although the cameras closest to the shoreline recorded higher numbers of species more consistently than any other position (Fig. 2.9b), a large proportion of samples from all positions observed no species, resulting in the mean species richness for all positions to be less than one (Fig. 2.9b). However, to isolate the effect of depth/distance from shore from other confounding habitat factors, these cameras were specifically placed in areas with no structural complexity. Many species prefer areas with structure (Sheaves 1996; Gratwicke and Speight 2005), which may explain the low number of individuals that were observed. This could also partly explain why larger numbers of species were seen at closer-to-shore positions throughout the study, where a greater amount of proximal structure can be found (i.e. the fringing mangrove forest), and which can be utilised as the tide rises. With no structure available in these areas during lower tidal levels, closer-to-shore positions may also be more favourable for prey species that use shallower water to avoid larger predators (Paterson and Whitfield 2000; Baker and Sheaves 2007).

Although significantly more species were seen closer to shore, there was no significant relationship between position and max. N ($df=5$, $F=0.86331$, $p=0.508$)(Fig. 2.9c). However, the highest max. N was recorded at the closest-to-shore position. It is possible that the large numbers of empty replicates may have had a weighted effect on these analyses.

Overall, cameras at closer-to-shore positions provided the greatest species richness and max. N values. Additionally, 9 out of 12 species observed by cameras during this portion of the study were detected at the 1m and 3.5m positions (those closest to shore). This suggests that a fairly comprehensive sample of the community was consistently observed at these distances and that these positions may be the most useful for further sampling. These

closer positions also support shallower depths, allowing for bottom cameras to record the majority of the water column and limit the number of species missed due to field-of-view limitations.

Although it is possible that the pattern observed in this study was driven by the characteristics of bottom cameras, which may not be highly efficient at sampling mobile and pelagic species that utilise habitats farther from shore, these data are supported by trends found in past studies, in which organisms in these systems were found close to the shoreline (Gibson 2003; Romare *et al.* 2003; Johnston and Sheaves 2007). Fish residing in edge zone waters typically utilise the intertidal zone as it is flooded to access food, for protection from potential predators, and to increase reproductive success in some cases (Gibson 2003). Many species take advantage of nearby edge structure, of which accessibility is tidally-dependant, and remain in edge zones for this reason (Romare *et al.* 2003). The presence of such organisms may then attract piscivores. Many fish also prefer to maintain a specific depth and so will move with the tide in order to stay as constant as possible (Gibson 1973). These patterns may account for the high max. Ns and species richness observed in closest-to-shore positions and are supported by the data found via underwater cameras in this study, further validating their use in estuarine edge habitats.

2.3.4 *Conclusions*

The use of underwater camera methods for field-based research has been closely evaluated and validated, addressing potential issues and limitations. If these video methods are to be utilised, they should be used in the manner summarised in this chapter. After close analysis of the logistical use of these underwater cameras as well as the quality of data

extracted from them, bottom cameras set as described were found to be most effective and useful for addressing the aims of this study.

Although bottom cameras have their own limitations, they have been found to be an extremely useful sampling gear for tropical estuarine environments. This novel technique can help to fill the knowledge gaps left by alternative methods. Together, comprehensive answers can be obtained, as each technique utilised alone in these environments has its own deficiencies. Bottom camera limitations, such as the inability to sample organisms higher in the water column, the inability to confidently distinguish between similar species, or the inability to utilise this gear outside of daylight hours, suggest that they may not be as useful as the sole method used in certain studies. It is the nature of the target data that will determine the appropriate methodology, although it is highly probable that the most useful and comprehensive information for many investigations will be gathered via a combination of camera use and other methods. For the collection of data on the identification and distribution patterns of estuarine fishes along a small, prescribed area, bottom cameras can be extremely effective and useful.

Chapter 3 Assessing the Validity of Videography Techniques for Sampling Nekton in Tropical Estuaries through Comparison with Cast Netting Procedures

3.1 Introduction

Underwater videography is a useful new technique for sampling the local fauna in areas such as tropical estuaries (see Chapter 2). This video method allows observational information to be gathered with a non-extractive and non-destructive approach, recording samples for multiple reviews if necessary and providing information on the presence of nekton and habitat characteristics. Videos can also offer behavioural observations, data that are unavailable from traditional netting methods. However, underwater videography has limitations, as does any gear type. With equipment set on the benthos and restrictions to cameras' fields of view, it is possible that some local fauna may be missed or underrepresented. For more extensive information on underwater videography techniques, refer to Chapter 2.

Although videography techniques were developed and refined during the procedures described in Chapter 2, the biases of this method must be assessed before they can be utilised for estuarine research. To ensure that the use of underwater cameras is a valid sampling technique for these systems, it must be directly compared to a recognized effective sampling approach. As netting techniques are the traditional methods used for sampling tropical estuaries (refer to Chapter 2), a netting approach such as cast netting makes for a suitable comparison.

Cast nets are ideal for appraising the validity of camera techniques. Cast nets have been used extensively for sampling estuarine environments (e.g. Layman *et al.* 2005; Sheaves and Johnston 2009; Sheaves *et al.* 2010) and have provided valuable information on nekton population parameters. Their extensive use is due to the advantages of this method. Although cast nets sample small areas, they provide high replication (thus allowing for large areas to be investigated) (Baker and Minello 2011) while providing extensive and specific species assemblage data (e.g. Sheaves *et al.* 2007; Sheaves and Johnston 2009; Nyanti *et al.* 2012). Cast nets enable the researcher to capture fish with little chance of injury and so allow close examination of individuals to aid in identification, while still returning them to their environment unharmed (Sheaves *et al.* 2007), allowing cast nets to be both a detailed and non-destructive technique. The success of this netting method is useful for comparison against video techniques, to assess any taxa that may be missing from camera samples and highlight any limitations of this new gear.

Limitations associated with cast netting can also be useful for comparison, as they can highlight the advantages that underwater videography may provide. Just like any sampling method, cast nets have limitations (Baker and Minello 2011). For instance, cast nets create disturbance as they are deployed and then sink through the water column, which has the potential to scare fish and possibly bias results (Johnston and Sheaves 2007; Johnston *et al.* 2007). This can cause cast nets to underrepresent certain species, such as those more sensitive to disturbances or surface species that distance themselves from the approaching boat (Johnston *et al.* 2007). This netting technique is also incapable of gathering certain types of data, such as information on behaviour and relationships, which can really only be assessed by observational approaches. Although some behaviours and relationships may be inferred, such as schooling behaviour

deduced from the repeated capture of many individuals of the same species together (Brehmer *et al.* 2006), cast nets (or any netting method) do not provide definitive behavioural information.

Cast nets and underwater cameras are vastly different techniques. Cast nets take a vertical sample of the water column from one moment in time and provide many replicates, while cameras record many minutes (20 minutes in this study) of footage from the benthos, where they can record anything that swims by or is located in front of the lens, up to approximately 1 meter away, and provide fewer replicates in the same time frame (see Chapter 2). These different techniques can be used together to assess the biases associated with underwater videography, and can highlight this method's weaknesses and strengths. The report of this information is important, as the limitations of a technique must be fully understood before analysing the data that it collects. The aim of the present study was to assess any biases and limitations associated with underwater videography in tropical estuarine environments by directly comparing this new technique to the previously-proven sampling technique of cast netting. This direct comparison highlights the advantages and disadvantages of camera methods and can determine if videography is a viable sampling option for gathering data from tropical estuaries.

3.2 Materials & Methods

3.2.1 In-Field Sampling

Sampling was conducted in the near-pristine estuarine systems of Deluge Inlet and Mendel Creek on Hinchinbrook Island (Fig. 2.1), in tropical North Queensland, Australia (for site descriptions, see Chapter 2). Cast nets were used in conjunction with cameras to directly

compare catch data with video data. Weighted bottom cameras (see Chapter 2 for a full explanation of camera equipment, deployment, and validation of technique) were deployed in two sites in each creek (Fig. 2.4), exactly as described in section 2.2.2 of Chapter 2. Nets were used in the same four sites while cameras were recording, but were not deployed within 25m of cameras, to avoid disturbance. A monofilament drawstring cast net with a 2.4m radius and 5mm mesh was used to sample the nekton across three zones corresponding to video placements: from 0 to 5 meters from the shore, from 5 to 10 meters from shore, and 10 to 15 meters offshore. Nets were cast at these distances ten times each within a stretch of approximately 500m of shoreline. This system was then replicated for each site on each sampling day. This resulted in a total of 60 net samples and approximately 16 twenty-minute bottom camera replicates per site for each sampling day, totalling 360 nets and 98 video samples overall. The order in which samples from each zone were collected was randomised and nets were spaced far enough apart (approximately $\geq 10\text{m}$) to ensure independence. All samples were collected in daylight during the bottom half of the tide (i.e. mid run out to mid run in). Individuals captured in the nets were identified, counted, and released unharmed.

3.2.2 Data Analysis

Although 15 minute videos were identified as the ideal length for analysis (Chapter 2), it took at least 20 minutes to deploy all cameras and return to collect the first under the design of the study (for a full explanation of video length validation and analysis protocols, refer to Chapter 2). Hence, twenty minutes of each video sample was reviewed. Fish in videos were identified to as low a taxonomic level as possible, however, some individuals could only be

classified into higher taxonomic groupings (refer to Chapter 2). Although most individuals were identified to species in the cast net samples, species were combined into the same taxonomic groups for both gears to facilitate comparisons between the two methods.

Although comparing results from two disparate gear types can complicate interpretation due to differences in effort, the goal of this study was to gain a different look at faunal composition than that provided by cameras, so as to assess any biases and limitations associated with this new videography technique; cast nets provided this different perspective from camera methods. The differences in assemblage composition sampled by each gear were evaluated by directly comparing the frequency of occurrence (the proportion of samples that each taxa was recorded in) of taxonomic groups in cast net and video samples. One-way ANOVA was used to compare taxonomic richness of individual samples (log transformed data were used to normalise the distribution of variables) between cast nets and cameras. As all other variables (e.g. study site, day, time) were the same between the gear types, comparison simply highlights the differences between the two gears.

3.3 Results

Taxonomic composition varied markedly between gear types. Of the 48 combined taxa recorded, 39 were sampled by cast nets, 21 by cameras, and only 12 were recorded in both cast nets and cameras (Fig. 3.1a,b). Of these shared taxa, only *Herklotsichthys castelnaui* was included in the top five most frequently-occurring taxa for both methods. The other four most frequently-occurring taxa captured with cameras were benthic-associated taxa (*Selenotoca multifasciata*, Gobiidae, *Gerres filamentosus* and Mugilidae)(Fig. 3.1a), whereas those most

frequently-occurring in cast nets were a mixture of benthic and more pelagic taxa (*Penaeus merguensis*, *Leiognathus equulus*, *Acanthopagrus pacificus* and Ambassidae)(Fig. 3.1b). The majority of taxa most frequently-occurring for either method were also sampled by the other gear type, while showing that the main differences in taxonomic occurrence between methods are mostly driven by taxa not commonly captured by either method (Fig. 3.1a,b). The trend toward capture of benthic taxa by cameras and pelagic taxa by cast nets was reflected in the less abundant taxa as well. Of the 9 taxa recorded solely by cameras, 8 were either benthic or demersal. In contrast, 22 of the 27 taxa exclusive to cast nets were either pelagic or partly pelagic. Additionally, of the five benthic taxa that were sampled solely by cast nets, four were various species of prawn, which are often unable to escape a descending net due to the nature of their escape response (Watson *et al.* 1992; Xiao and Greenwood 1993).

Additionally, mean taxonomic richness per individual cast net (mean=1.09) and video sample (mean=1.01) were not significantly different (df=1, F=0.98285, p=0.32202). This indicates that although individual samples were composed of fairly different taxa, they evaluated a similar proportion of the community.

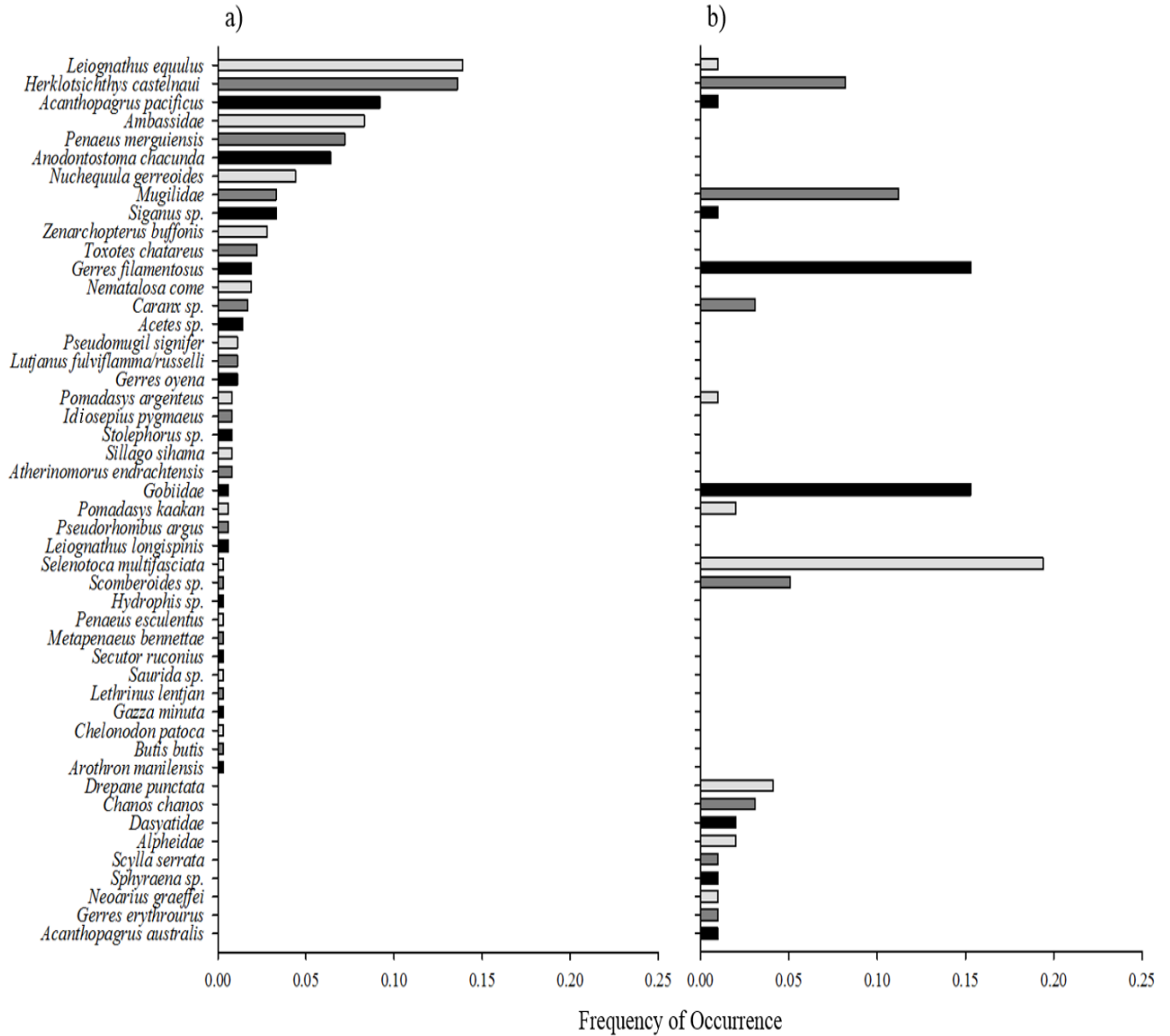


Figure 3.1 Frequency of occurrence of taxa found in (a) cast net and (b) camera data. Frequency of occurrence is represented by the proportion of the number of samples in which each taxon is present out of the total number of samples (n) for cast nets (n=360) and cameras (n=98).

3.4 Discussion

Comparing the composition of taxa sampled by cameras to that of a different technique such as cast nets highlights the types of organisms that are vulnerable and underrepresented by each gear type, and so can evaluate the sampling efficiency of videography methods. As sampling was conducted at the same sites and times with both cameras and cast nets, all taxa present at the time were available to be sampled by either gear. However, the assemblage sampled by each method shared relatively few taxa, with only 20% of the total combined assemblage list in common. Only one of the five most common taxa sampled by cast nets and those by cameras was shared between the two methods: a highly abundant schooling fish (*Herklotsichthys castelnaui*) (Robertson and Duke 1990a). The remaining four most frequently-occurring organisms sampled by cameras were benthic-associated or demersal taxa, while those from cast nets were a mixture of benthic and more pelagic taxa. This correlates with previous work, which found cast nets to represent pelagic taxa fairly thoroughly (Baker and Minello 2011). Cast nets, as nets descending from the surface, are most likely to capture individuals in the pelagic zone which have less time to react and flee (Baker and Minello 2011). Cameras have a fixed maximum field of view which naturally restricts their ability to sample the entire water column, allowing only the portion that is in-frame to be represented, in this case, the benthic area (see Chapter 2). The assemblages sampled by each gear reflect this bias. The exception was the high number of *Penaeus merguensis* sampled by cast nets, which are highly effective for sampling penaeid prawns (Johnston *et al.* 2007), probably due to the tactile (rather than visual) and random-direction escape response of prawns (Watson *et al.* 1992; Xiao and Greenwood 1993); video methods often miss these taxa due to their cryptic nature (Primavera 1997). In fact, four of the five benthic taxa sampled solely by cast nets in the present study were prawns (most

likely due to the aforementioned reasons), leaving only one other benthic taxon sampled exclusively by cast nets, further highlighting the emphasis of cast nets on pelagic taxa. The majority of the most frequently-occurring taxa in both gears were also sampled to some degree by the other gear type, suggesting that common taxa (although more effectively represented by one gear type) are sampled by both methods, with the major differences in assemblage composition between sampling gears resting in less common taxa. These less common taxa also reflected the bias toward benthic taxa for cameras and pelagic taxa for cast nets. Thus, while both capable of sampling common estuarine fauna, cameras and cast nets provide a different representation of the assemblage.

Cameras focussed mostly on demersal species with moderate mobility, and captured taxa with an array of site-attachment, from those that remained in a particular area (i.e. Gobiidae) to those with high levels of movement (ie. *Sphyræna* sp.), and focussing primarily on those taxa characterised by moderate movement (Table 3.1). The taxa unique to camera methods displayed similar characteristics as well (Table 3.1). This reveals the diverse range of species susceptible to camera methods, although few pelagic species were observed by this gear (not surprising when the cameras were sited on the sediment surface). In contrast, cast nets sampled 27 taxa not represented in any video samples and the majority of these taxa were pelagic and characterised by a moderate amount of movement (Table 3.2). This emphasised the increased sampling efficiency of cameras toward benthic species and of cast nets toward pelagic.

Table 3.1 Characteristics of taxa sampled by camera methods. Taxa only sampled by cameras are in the upper portion of the table; those also sampled by cast netting are located in the lower portion and are indicated by (*). References were used to guide ecological zone, mobility and site-attachment classifications. (Hyland *et al.* 1984; Davis 1988; Bade 1989; Robertson and Duke 1990a; Sheaves 1993; Bagarinao 1994; Stewart and Jones 2001; Meyer *et al.* 2007; Froese and Pauly 2011; O'Toole *et al.* 2011; Jardine *et al.* 2012; Corcoran *et al.* 2013)

Taxon	Ecological Zone	Mobility	Site-attachment	Frequency of occurrence (cameras)	Frequency of occurrence (cast nets)
<i>Drepane punctata</i>	demersal	mobile	moderate movement	0.041	0
<i>Chanos chanos</i>	pelagic, demersal feeding	mobile	moderate movement	0.031	0
Alpheidae	benthic	limited mobility	site-attached	0.02	0
Dasyatidae	benthic	mobile	moderate movement	0.02	0
<i>Acanthopagrus australis</i>	demersal	mobile	limited movement	0.01	0
<i>Gerres erythrourus</i>	demersal	mobile	moderate movement	0.01	0
<i>Neoarius graeffei</i>	demersal	mobile	moderate movement	0.01	0
<i>Scylla serrata</i>	benthic	moderately mobile	limited movement	0.01	0
<i>Sphyraena</i> sp.	pelagic	mobile	extensive movement	0.01	0
<i>Selenotoca multifasciata</i> *	demersal	mobile	moderate movement	0.194	0.003
<i>Gerres filamentosus</i> *	demersal	mobile	moderate movement	0.153	0.019
Gobiidae*	benthic	moderately mobile	site-attached	0.153	0.006
Mugilidae*	demersal	mobile	moderate movement	0.112	0.033
<i>Herklotsichthys castelnaui</i> *	pelagic	mobile	moderate movement	0.082	0.136
<i>Scomberoides</i> sp.*	pelagic	mobile	moderate movement	0.051	0.003
<i>Caranx</i> sp.*	pelagic	highly mobile	extensive movement	0.031	0.017
<i>Pomadasys kaakan</i> *	demersal	mobile	limited movement	0.02	0.006
<i>Acanthopagrus pacificus</i> *	demersal	mobile	limited movement	0.01	0.092
<i>Leiognathus equulus</i> *	demersal	mobile	moderate movement	0.01	0.139
<i>Pomadasys argenteus</i> *	demersal	mobile	limited movement	0.01	0.008
<i>Siganus</i> sp.*	demersal	mobile	moderate movement	0.01	0.033

Table 3.2 Characteristics of taxa sampled only by cast nets. Only taxa with a frequency of occurrence >0.003 (sampled more than once) were included. References were used to guide ecological zone, mobility and site-attachment classifications. (Moynihan 1983; Robertson and Duke 1990b; Salini *et al.* 1990; Minami and Tanaka 1992; Xiao and Greenwood 1992; Hyndes *et al.* 1997; Newman *et al.* 1997; von Byern and Marwoto 2009; Froese and Pauly 2011)

Taxon	Ecological Zone	Mobility	Site-attachment	Frequency of occurrence
<i>Ambassidae</i>	pelagic	mobile	moderate movement	0.083
<i>Penaeus merguensis</i>	benthic	limited mobility	site-attached	0.072
<i>Anodontostoma chacunda</i>	pelagic	mobile	moderate movement	0.064
<i>Nuchequula gerreoides</i>	demersal	mobile	moderate movement	0.044
<i>Zenarchopterus buffonis</i>	pelagic	mobile	moderate movement	0.028
<i>Toxotes chatareus</i>	pelagic	mobile	moderate movement	0.022
<i>Nematalosa come</i>	pelagic	mobile	moderate movement	0.019
<i>Acetes</i> sp.	pelagic	moderately mobile	moderate movement	0.014
<i>Gerres oyena</i>	demersal	mobile	moderate movement	0.011
<i>Lutjanus fulviflamma/russelli</i>	demersal	mobile	moderate movement	0.011
<i>Pseudomugil signifer</i>	benthopelagic	mobile	moderate movement	0.011
<i>Atherinomorus endrachtensis</i>	pelagic	mobile	moderate movement	0.008
<i>Sillago sihama</i>	pelagic	mobile	moderate movement	0.008
<i>Stolephorus</i> sp.	pelagic	mobile	moderate movement	0.008
<i>Idiosepius pygmaeus</i>	pelagic	moderately mobile	limited movement?	0.008
<i>Leiognathus longispinis</i>	demersal	mobile	moderate movement	0.006
<i>Pseudorhombus argus</i>	demersal	mobile	limited to moderate movement	0.006

Overall, camera methods provided a different perspective of the estuarine community, recording information on taxa not sampled by cast nets, although apparently underrepresenting some taxa as well. These results are most likely attributed to a multitude of characteristics that set camera gears apart from netting methods. As mentioned previously, the positioning of cameras on the benthos can most likely account for the pelagic species that were underrepresented in camera samples, with pelagic taxa in the water column above and beyond the cameras' field of view missed in deeper water. However, the edge environment is most often characterised by shallow water, so the impact of this limitation may be diminished. Apart from limitations to field of view, cameras can theoretically provide a perfect sample of the local community, capturing everything that passes by. However, this depends on the level of water clarity, which can be a limitation (Chapter 2), as well as any bias the presence of camera equipment may cause. However, based on basic observations throughout this study, the presence of camera equipment was not found to substantially repel nekton, and any attraction that occurred was a minimal local phenomenon and thus probably had little effect. In addition, minimal disturbance is created from setting this gear (see Chapter 2), allowing taxa to be recorded that may normally be deterred by disturbances created from other techniques, providing an advantage over cast netting methods. Cameras are also more highly capable of sampling in and around the complex structure that often characterises the estuarine edge. However, probably the most profound difference between camera and cast net methodologies is time. Whereas cast nets provide a sample of the assemblage in an area from one moment in time, videography captures not one moment, but in this case, twenty minutes. This allows for a more comprehensive and dynamic view of the community in a specific location at a specific time, and as a result, allows for thorough investigation of spatial and temporal patterns.

Cameras also provide the advantage of being able to capture relationship and behavioural data—information unavailable from netting techniques. For example, an apparently symbiotic relationship between a burrowing alpheid shrimp (family Alpheidae) and individual gobiid fish (family Gobiidae) was observed in multiple video samples. This relationship has not previously been reported for tropical estuaries, and has been almost exclusively recorded in purely marine environments (Karplus 1987; Thacker *et al.* 2011; Jaafar and Hou 2012). This novel observation would be impossible with any netting method and highlights the advantages and opportunities that camera methods present.

Camera techniques provided a new perspective of the estuarine community and observed species missed by cast nets; this not only highlights some of the advantages of camera use, but some of the limitations of cast netting procedures as well. Cast nets create disturbance when entering the water, influencing surrounding fauna to a much higher degree than camera methods. Cast netting techniques are also inefficient at sampling burrowing organisms, such as the alpheid shrimp that were sampled by camera methods and mentioned previously. However, cast nets do still provide advantages, depending on the target data. For instance, cast net catches are brought to the surface where they can be closely examined before release. This enables researchers to make much more taxonomically-specific identifications, as well as providing the opportunity to collect biological samples. Cast nets are also able to sample a large area within one day of sampling, which allowed for a sample size of 360 nets in the present study, compared to only 96 camera samples.

In conclusion, camera methods provided a new perspective of the estuarine community and were found to be a valid sampling gear for this area. Although less efficient at capturing data on pelagic species that are found in cast netting samples, cameras have advantages over netting methods and did provide information on demersal taxa missed by cast nets, as well as on behaviours that are impossible to observe with netting techniques. In addition,

videography methods may prove to be beneficial not only on their own, but in addition to netting techniques, dependent on target data. Cameras and cast nets may be useful together to create the most comprehensive view of the community, or may pair well in gathering separate information. For instance, while cameras are beneficial for observing benthic and more site-attached species, cast nets can use this information to locate target taxa for gathering biological samples. Whether used as the sole sampling procedure or in conjunction with other techniques, camera methods can add crucial insight into the ecology of estuarine systems.

Chapter 4 Temporal Changes in Species Distribution Along Shallow Edge Habitats in a Near-Pristine Tropical Estuary

4.1 Introduction

Intertidal areas, such as mangrove-lined tropical estuaries, are particularly influenced by tidal fluctuations, with intertidal areas regularly flooded and drained with the ebb and flow of the tides. These estuarine systems are often turbid (due to sediment stirred up by moving water) (Uncles *et al.* 2002), are mixing zones for fresh and salt water (Pritchard 1967; Staunton-Smith *et al.* 2004), and experience high currents due to large volumes of water moving through restricted channels (Pugh 1996). Water depth also varies greatly with the ebb and flow of the tide. These changes in water level periodically create an in-forest aquatic environment within the fringing mangroves that forms and diffuses with the tidal cycle (Sheaves 2005).

Water movement from changing tides affects estuarine fauna in a variety of ways, particularly those species that occupy near-bank habitats where tidal changes have substantial influence. Distributions of organisms are affected by rising and falling tides, as many mobile fauna migrate into intertidal areas during high tide (Marine 1989; Henderson and Bird 2010) to make use of the periodically available in-forest aquatic habitat provided by the fringing mangrove forest for shelter and refuge, or for foraging (Ellis and Bell 2004; Sheaves 2005). For these reasons, the edge and in-forest environments of estuaries are vitally important to many species, as is the influence of the tide that makes edge habitats available. However, the organisms that utilise these complex intertidal areas are forced out of them when the tide retreats, so must then survive without the protection provided by mangrove forests during low

tides (Johnston and Sheaves 2007). It is during these low tidal levels that many nekton disperse to alternate foraging or refuge habitats (Johnston and Sheaves 2007). Smaller prey species are often among the most common taxa to utilise in-forest habitats, as many larger predatory organisms are unable to follow them into the intricate roots of the mangrove forest (Sheaves 2005). However, some predators wait for retreating tides in order to ambush their prey when they are forced to leave the mangroves (Sheaves 2005). Thus, tidally-available habitats provide a complex of interacting benefits and dangers for the fauna that utilise them.

Estuaries provide valuable habitats for many species, and understanding the way these habitats function is important for maintaining and preserving these environments. Although it is well-established that some estuarine fauna utilise the fringing mangrove forest when it is available at high tides (Ellis and Bell 2004; Sheaves 2005; Johnston and Sheaves 2007), our understanding of the use of edge habitats during times when the complex refuge of the mangrove forest is unavailable remains deficient (Johnston and Sheaves 2007). The aim of this study was to investigate the temporal changes to faunal distribution associated with tidal movement along the margins of a tropical estuary, and thus to consider how the use of the estuarine edge by nekton changes over the lower portion of the tidal cycle when the mangrove forest is inaccessible. Underwater videography was used to address this aim, gaining new insight into faunal distribution patterns by providing a different perspective from past methods (see Chapter 3), as well as granting advantages over older techniques such as the ability to record samples over time to best investigate temporal patterns (see Chapter 2).

4.2 Materials & Methods

4.2.1 *In-Field Sampling*

Bottom-set video cameras were used to evaluate the temporal patterns of faunal distribution along the margins of a tropical estuary. For a detailed explanation of camera equipment and deployment protocols, as well as site descriptions, refer to Chapter 2. Investigations took place in the near-pristine estuarine system of Deluge Inlet on Hinchinbrook Island, Queensland, Australia, along approximately 1.5km of mangrove shoreline. As most nekton were observed close to shore during previous fieldwork (Chapter 2), cameras were set approximately one to two meters from the water's edge, one in each of 18 "zones" along the bank (Fig. 4.1). Each zone was marked via GPS coordinates, ensuring consistency throughout sampling, and far enough apart to be independent. Cameras were placed within approximately four meters to either side of the GPS zone markers. The bank was sampled on three separate days over five months (early, mid, and late dry season), on June 14th, August 27th, and October 12th 2012, with cameras placed repeatedly within the same zones. Cameras were placed along the bank in twenty-minute sets throughout the bottom of the tidal cycle (early run out, mid run out, late run out, early run in, mid run in), although a mismatch between appropriate tides and available light on August 27th and October 12th prevented the early run out of the tidal cycle to be sampled.

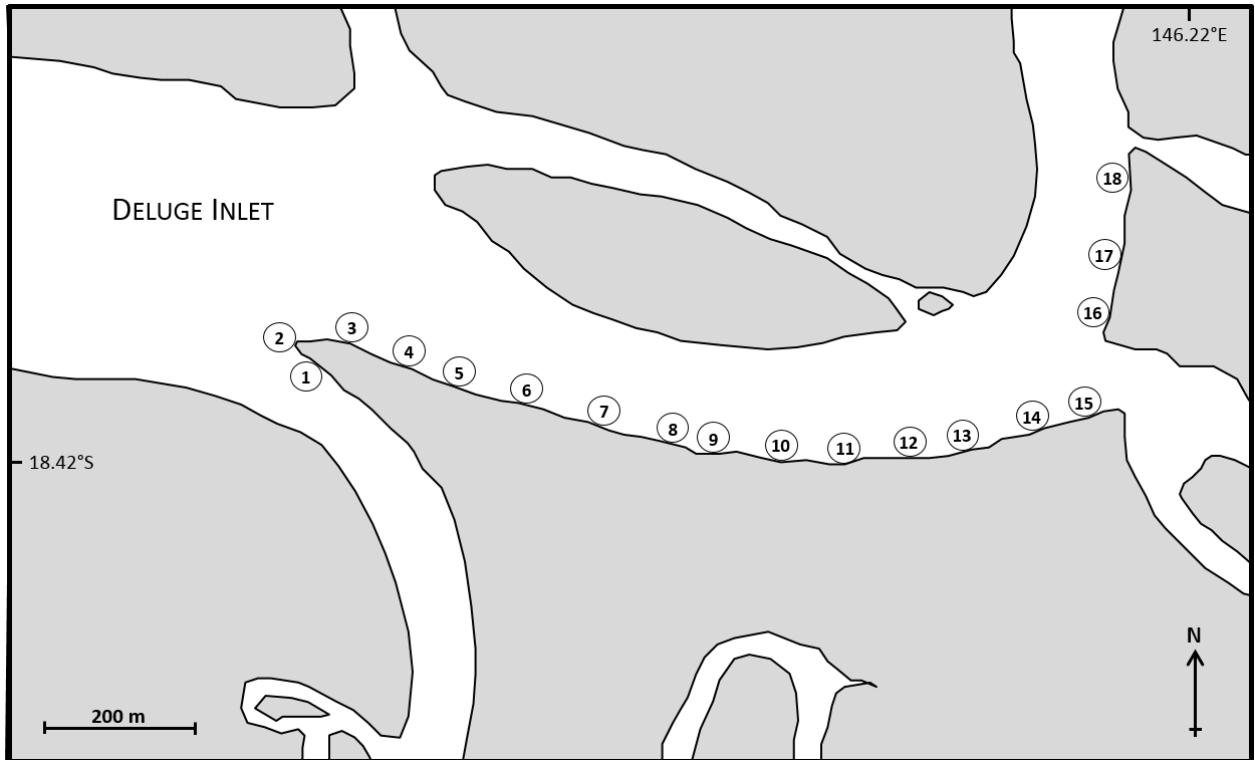


Figure 4.1 Location of eighteen zones for camera placement along a bank in Deluge Inlet, Queensland, Australia.

To evaluate if any observed patterns were consistent among locations, cameras were run in a similar configuration at two additional locations. Thirteen zones (due to a shorter, although similar, bank) were sampled along Mangrove Point, a bank approximately five kilometres north and across Hinchinbrook Channel from Deluge Inlet, on September 24th 2012 (Fig. 4.2). Seventeen zones were sampled along Stuart Creek, located near Townsville, Australia, on October 10th 2012 (Fig. 4.2).

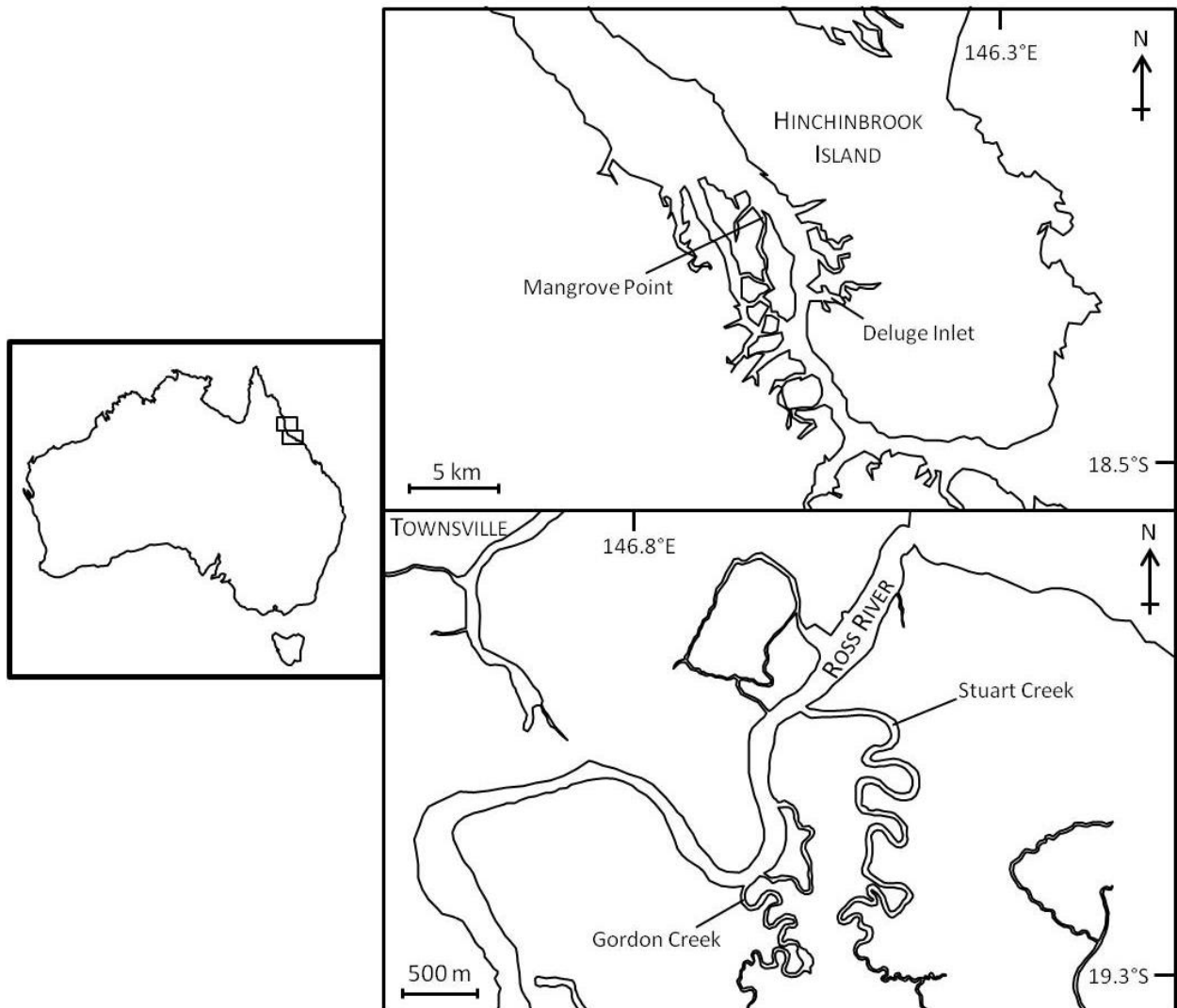


Figure 4.2 Map of replicate sampling sites: Mangrove Point near Hinchinbrook Island, Australia, and Stuart Creek, near Townsville, Australia.

4.2.2 Data Analysis

Videos were analysed following the procedure outlined in Chapter 2, noting taxa occurrences as well as recording the max. N of each taxon (the maximum number of individuals of each taxon observed together in a frame). Species occurrence data were converted to proportions to determine the frequency of occurrence along the edge (for each taxon, the number of zones observed out of the total number of zones) at each tidal stage

(early run out to mid run in) for each day. Non-metric multidimensional scaling (nMDS) was performed on frequency of occurrence data for taxa from Deluge Inlet observed in greater than 5% of samples. Using frequency of occurrence data focussed the nMDS results on temporal differences in distribution between tidal stages, rather than on any spatial changes due to differences in zone. A Bray-Curtis distance matrix was used for the ordination of data. Vectors were projected on the biplot, indicating the direction of greatest increase in occurrence of taxa most highly correlated with the space. The direction of these vectors was determined by regression of each taxon on the nMDS space. The length of each vector reflects the R^2 value for each regression, thus signifying the strength of the correlation with the space. Only taxa with R^2 values of greater than 0.4 were included as vectors on the nMDS configuration. This included all taxa except *Drepane punctata*, which had a low representation on the two dimensions (R^2 value of 0.006).

A multivariate classification and regression tree (mCART) was used via TreesPlus software (De'Ath 2002) to evaluate the drivers of tidal distribution changes in the estuarine edge habitat. Classification and regression trees are a useful statistical tool for investigating patterns and relationships between variables, and can be employed on data that may prove problematic with other techniques (i.e. data with missing values, that are unbalanced, have high-order interactions, or that contain non-linear relationships between variables)(McLean 2012). Groups are formed from data sets by repeated splitting, each split selected based on maximising the within-sample homogeneity of variance (each split results in two groups that are as homogenous as possible and mutually exclusive). All trees were created as described by De'Ath (2002) and Sheaves (2006). Fourth-root transformed max. N data from all three sampling locations were used (to moderate the influence of samples with extreme values) with tidal stage (early run out, mid run out, late run out, early run in, mid run in) and location (Deluge Inlet, Mangrove Point and Stuart Creek) as explanatory variables. This was to assess

the impact changing tides had on nekton max. N values and to assess the generality of the observed patterns and ensure that they are not unique to Deluge Inlet. To select the tree size with the best overall fit, 100 ten-fold cross validations were run, and the smallest tree with a cross-validation error within one standard error of the best tree was selected as the final model (the 1-SE rule as described by Breiman et al. (1984)), thus producing a biologically interpretable and valid tree (Sheaves and Johnston 2009). Only taxa seen in greater than 5% of all samples were used for analysis.

Mean max. N Deluge data for the five most prevalent taxa (*Acanthopagrus* spp., Clupeidae, Gobiidae, *Lutjanus fulviflamma/russelli*, and *Gerres* spp.) were analysed graphically to assess taxon-specific temporal patterns in edge habitats. Data from all zones for the three sampling days were combined to determine mean max. N for each tidal stage, creating a bar plot and providing a simple and effective means of displaying the temporal patterns of prevalent edge taxa.

4.3 Results

The occurrence of most taxa in edge habitats in Deluge Inlet changed over the tidal cycle (Fig. 4.3). Most taxa occurred more often in samples from the early run out and mid run in tidal stages (times of high water levels on the ebb and flood tides, at which the water begins to drain and flood the mangrove forest, respectively)(Fig. 4.3). Gobiidae was an exception to this general trend, and was in fact negatively correlated with some species (i.e. the vector representing Gobiidae was at a right angle to taxa such as Clupeidae)(Fig. 4.3). Gobiidae did not show a strong correlation to any tidal stage (Fig. 4.3). The bottom portion of the tide (mid run out to early run in) supported lower occurrences of most taxa, although there was some variation in these results over the three sampling days (Fig. 4.3).

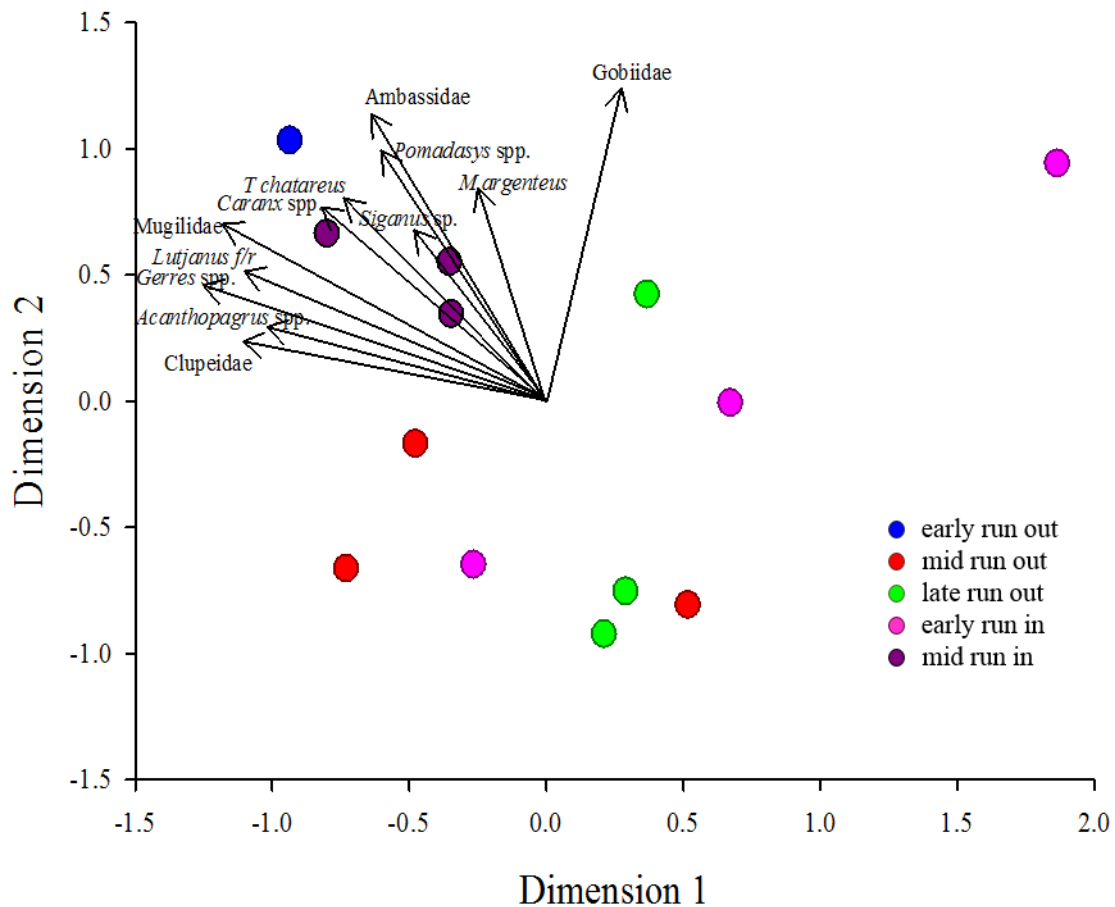


Figure 4.3 Non-metric multidimensional scaling analysis (nMDS) of Deluge species occurrence data. Species observed in >5% of samples and with R^2 values of >0.4 are represented in the figure. Stress is approximately 0.1. Twelve points represent data from five tidal stages (early run out, mid run out, late run out, early run in, mid run in) over three sampling days.

The mCART analysis showed higher max. N for most nekton in edge habitats during the higher tidal levels (early run out, mid run in)(Fig. 4.4), consistent with the trends observed in the nMDS (Fig. 4.3). As expected, location was an important factor in explaining the variation between samples, although tidal stage was approximately equal in importance. Tide caused the first tree split, breaking between higher water levels (early run out, mid run in) and lower (early run in, late run out, mid run out)(Fig. 4.4). As tide continued to split throughout the tree, branches were consistently determined by water level (Fig. 4.4). Thus,

tidal level drove patterns of max. N in a similar way across all three sampling sites. No pattern was seen in location branching (Fig. 4.4).

The max. N values of the most prevalent taxa in Deluge Inlet display similar patterns, both to the nMDS and mCART results, and to each other (Fig. 4.5). All five taxa had highest max. N values at higher tidal levels, which then decreased as the tide retreated and once again increased upon the rise of the tide (Fig. 4.5). Similar to the nMDS analysis, Gobiidae was the taxon with the weakest trend, although they still showed this general pattern (Fig. 4.5).

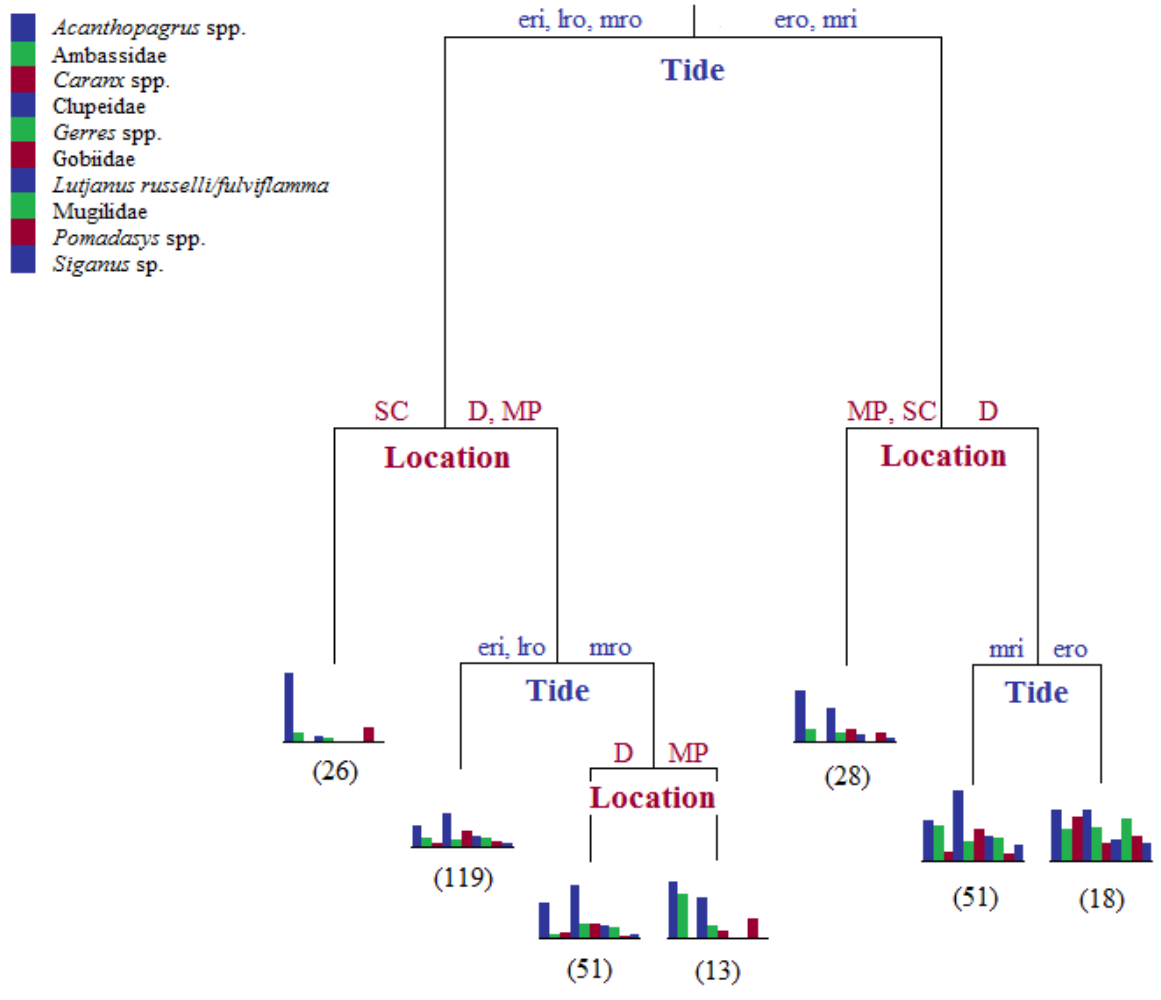


Figure 4.4 Multivariate regression tree (mCART) investigating species max. N over tidal stage (ero: early run out; mro: mid run out; lro: late run out; eri: early run in; mri: mid run in) and location (D: Deluge Inlet; MP: Mangrove Point; SC: Stuart Creek). Only the 10 species that were present in >5% of all samples were used in analysis. Max. N data were fourth root transformed; barplots show the multivariate species mean at each node, and the numbers in parentheses are the number of replicates.

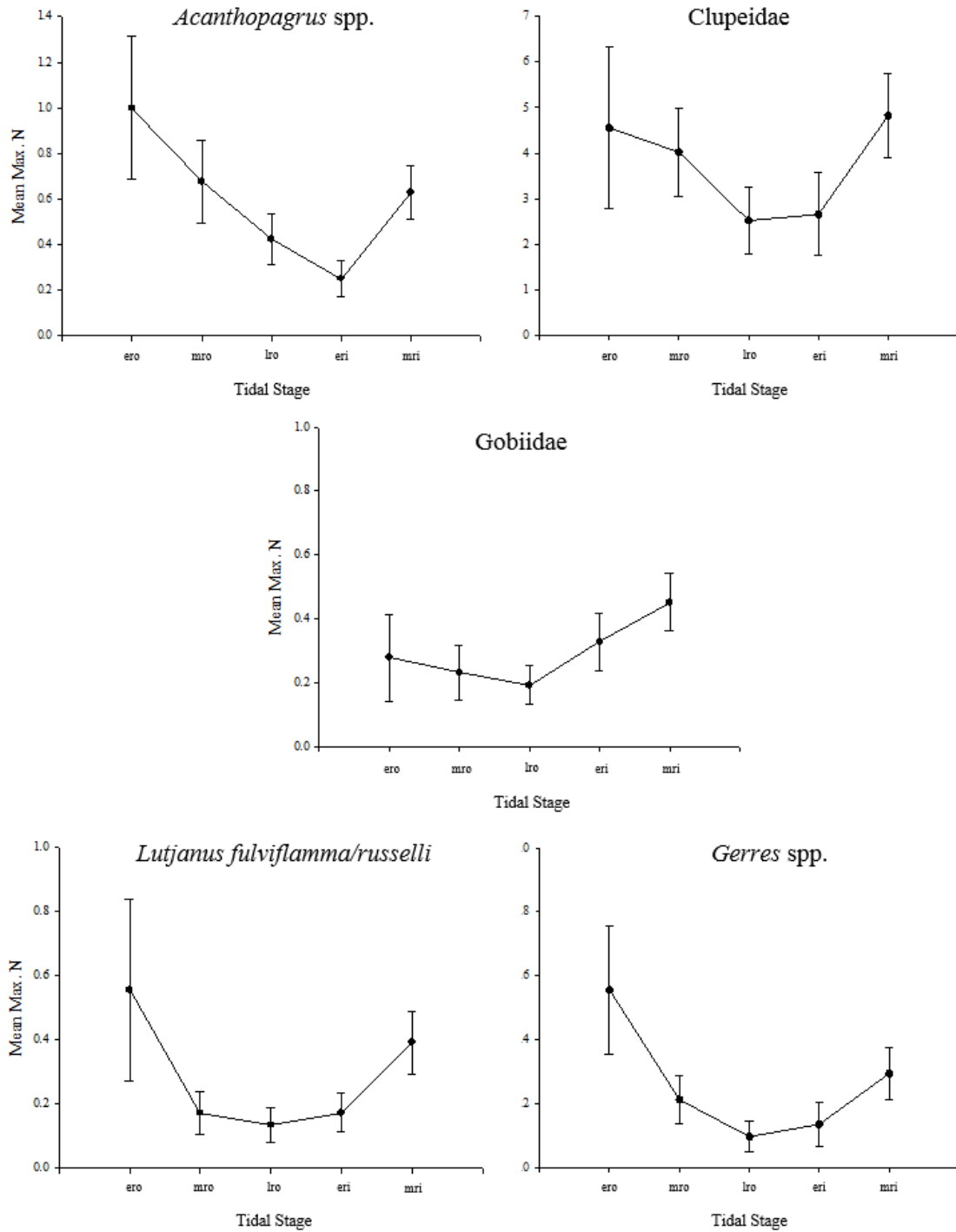


Figure 4.5 Mean max. Ns of the five most prevalent species observed in video samples in Deluge Inlet through the tidal cycle (ero: early run out; mro: mid run out; lro: late run out; eri: early run in; mri: mid run in). Note that y-axis scales vary among species. Error bars denote 1 standard error.

4.4 Discussion

The distribution of fauna in an estuarine edge habitat changed with tidal movement throughout the present study. Consistent between taxa and locations, nekton decreased in max. N with the retreating tide, and increased as the tidal level rose, with the highest max. Ns occurring at the highest tidal levels tested. The highest tidal levels tested in the present study were the mid run in and early run out (about mid-tide), at the approximate tidal heights water first inundated then drained from the mangrove forest. The fringing mangrove forest is a resource extensively utilised by estuarine fauna when it becomes accessible during high tides (Beck *et al.* 2001; Ellis and Bell 2004; Sheaves 2005). The high max. Ns of nekton at higher water levels as observed in the present study are thus likely to be attributable to fauna entering and leaving the mangrove forest as tidal height allows. However, as the use of the mangrove forest is intrinsically linked to tidal fluctuations, it is not clear which factor, tides or mangroves, is the primary driving force of changes in abundance.

Although past studies agree that the use of vegetated intertidal habitats is an important component of estuarine edge ecology (Beck *et al.* 2001; Ellis and Bell 2004; Sheaves 2005), the movements of nekton after forced evacuation from the complex forest habitat during low tide are less clear. With decreased max. Ns at lower tidal levels throughout the present study, it is evident that not all nekton that aggregate along the edges for the utilisation of the mangrove forest at high tide remain in the shallow edge habitat when the tide retreats (Johnston and Sheaves 2008). Although higher turbidity at low tide could potentially explain this pattern of lower max. Ns at this time (simply due to less taxa observed by cameras due to decreased visibility, not because they are not present), low visibility videos were discarded (see Chapter 2) and no substantial increase in the occurrence of high turbidity videos was observed for low tide samples; thus, turbidity most likely had minor effects on observed

distribution patterns. It is possible that this decrease in max. N of nekton at low tide is due to movement of taxa away from the edge in response to the lack of resources, such as refuge or food, provided by the edge habitat when the complex root system of the mangrove forest is unavailable. Some past studies have argued that the shallow water in these edge environments provides refuge from predators that inhabit greater depths (Blaber and Blaber 1980; Boesch and Turner 1984; Paterson and Whitfield 2000), however, other studies argue that the situation is more complex (Sheaves 2001; Baker and Sheaves 2007). It is probable that the edge environment does offer some level of refuge potential at low tidal heights, although this potential is substantially less than that provided by similar-depth water in the mangrove forest during high tides. These nekton that disperse at low tide may move in order to utilise alternate subtidal structured habitat for refuge or shelter or to locate foraging grounds; these alternate habitats may simply be the more extensive shallow waters available at low tide in the main channel (Johnston and Sheaves 2008) or entirely different systems (e.g. other estuaries, non-coastal areas)(Simpfendorfer *et al.* 2010). However, these low tide movements are not well known.

Although the patterns of change in max. N of estuarine edge nekton in the present study are clear, they are further supported by observations of specific taxa. For instance, pelagic species are more likely to be observed by benthic camera equipment during periods of low tides (and thus lower water levels) rather than high, as deeper water constitutes a larger space above camera equipment in which species may be present, but not be seen. Benthic organisms, such as Gobiidae (Keith 2003; Hernaman and Munday 2005), have the same likelihood of being recorded by cameras at all water levels. Although distributional patterns of Gobiidae were most unlike those of other taxa, possibly due to the grouping of gobiid species into a general taxonomic classification, Gobiidae still supported the same general distribution patterns as seen in other species (including such pelagic taxa as Clupeidae), with

numbers increasing with higher tidal levels (Fig. 4.5). The reduced expression of this general trend by Gobiidae can probably best be explained by the greater site-attached nature of this family due to their utilisation of burrows (Reavis 1997). Individuals probably locate burrows farther from high intertidal areas as to ensure they are submerged throughout the tidal cycle (thus they are missed by cameras placed 1 to 2m from shore at high tides, often within the flooded intertidal zone). However, as Gobiidae still displayed higher max. Ns at higher tidal levels and lower max. Ns at low tide, this indicates that the patterns observed in this study are robust and not an artefact of changing efficiency of the gear in varying depths, thus supporting the conclusion that numbers of organisms increase as tides rise and decrease as they fall.

Based on the results from the present study, tidal changes have a direct impact on the distribution patterns along an estuarine edge; however, there is still unexplained variation suggesting the possibility that other factors may contribute to these patterns. As estuaries are extremely connected environments, many characteristics of estuarine habitats (e.g. tidal movements, water quality) are intricately linked (Sheaves 2009). This interconnectedness of estuarine properties may explain why no one variable was found to have a dominant influence over max. N patterns. It is most likely a combination of interlinked factors that drive faunal distributions along estuarine edges and lead to the patterns observed in this study. Further research would be needed to more thoroughly understand these temporal patterns of nekton movement and their driving forces, specifically the movements of nekton that disperse from the edge at low tide.

Chapter 5 The Impact of Site-Specific Habitat Characteristics on Nekton Distribution Along Shallow Edge Habitats of a Near-Pristine Tropical Estuary

5.1 Introduction

Many estuarine nekton, particularly juvenile fish, aggregate around the shallow edges of tropical estuaries (Blaber and Blaber 1980; Baker and Sheaves 2007). At high tide, many species enter fringing mangrove forests for feeding and refuge (Sheaves 2005). However, with the retreating tide, these complex intertidal habitats are no longer available, forcing organisms to utilise subtidal areas (Johnston and Sheaves 2007). Habitats immediately adjacent to mangroves often provide little complex structure and thus have lower refuge value than the proximal forest, meaning fauna exiting must manage risks associated with this change or relocate to sub-tidal complex habitats (Johnston and Sheaves 2007) that may be located some distance from the mangrove edge (Sheaves 1996). Estuarine nekton utilising the edge are thus often forced to move to various habitats throughout the tidal cycle. However, despite the reduced refuge potential brought about by a drastic decrease in available complex structure, large abundances of fishes often occur along shallow estuarine edge habitats (Baker and Sheaves 2007; Johnston and Sheaves 2007).

The “shallow-water refuge paradigm” may explain the use of these shallow environments (Baker and Sheaves 2007). It states that small and juvenile fishes face lower predation risk and thus increased refuge value in shallow-water environments because of the inability of larger predators to forage in shallow water. This paradigm has been widely accepted for estuarine environments (e.g. (Blaber and Blaber 1980; Boesch and Turner

1984; Paterson and Whitfield 2000)) but recent work (Sheaves 2001; Baker and Sheaves 2007) has questioned its generality. For instance, predatory activity in shallow water is often underestimated (Baker and Sheaves 2006) and there is a lack of research directly comparing shallow- and deep-water predator abundances and predation pressure (Baker and Sheaves 2007). While these studies that question the validity of the shallow-water refuge paradigm do not unequivocally refute it, they do highlight that the drivers of estuarine fauna distribution in these habitats are largely unknown or at least poorly understood (Sheaves 2001; Johnston and Sheaves 2008).

Estuarine faunal distributions are spatially heterogeneous (Rozas and Zimmerman 2000; Rönnbäck *et al.* 2002; Ellis and Bell 2004), and are influenced by a diversity of factors. For instance, fish are often at highest densities along shallow edges (McIvor and Odum 1988; Collares-Pereira *et al.* 1995; Williams and Zedler 1999; Ellis and Bell 2004; Johnston and Sheaves 2007), while the presence of submerged structure is also important in determining faunal distribution (Sheaves 1996). Submerged structure can provide refuge from predation, as it limits the movement of predators and obstructs visual foraging (Savino and Stein 1982; Bartholomew *et al.* 2000; Ellis and Bell 2004). Vegetation also influences distribution in a similar way, creating refuge for many fauna (Savino and Stein 1982), as well as serving as a food source (Laegdsgaard and Johnson 2001). Rugosity, or the small-scale topography and characteristics of the substrate, can also impact distribution. The characteristics and type of sediment may determine the ability of burrowing species to construct burrows (Alexander *et al.* 1993; Schratzberger *et al.* 2004), determine the occurrence of vegetation (Bornette and Puijalon 2011), and regulate the presence of particular food sources (such as burrowing infauna (Alexander *et al.* 1993; Schratzberger *et al.* 2004)), all of which can determine the presence and abundance of different species. Finally, current flow can impact distribution by influencing energy expenditure of fauna

(Hoffman *et al.* 2006) or creating rich foraging grounds by aggregating food sources such as zooplankton via water movements such as upwelling (Alldredge and Hamner 1980).

Habitat characteristics including depth, structure, vegetation, rugosity, and current flow can influence the distribution of fauna along the margins of tropical estuaries. The uneven distribution of these characteristics along the edge creates a heterogeneous distribution of nekton, with some areas of substantially higher abundance than others. These “hot spots” of abundance highlight the areas that are characterised by conditions that create the most suitable habitat. However, determining the combination of factors that create this ideal locale can be challenging due to the difficult nature of sampling in these dynamic habitats (Rozas and Minello 1997). Additionally, it is often difficult to examine the impact of any one variable due to the complex and interacting way that variables influence estuarine fauna (Ellis and Bell 2004; Johnston and Sheaves 2007). However, it is important to understand the influence these habitat characteristics have on nekton in these areas and the distribution patterns that result.

Although edges are among the most valuable estuarine habitats (Rozas and Minello 1997), they are also among the most vulnerable because they are most directly subject to anthropogenic impacts such as development and bank modification (Blaber 2002; Bond and Lake 2005; Johnston and Sheaves 2008). Consequently, understanding the spatial distribution of nekton along bank edges and the factors that drive patterns of distribution is crucial for effective management and conservation of these important habitats (Cross and McInerney 2005; Isaak and Thurow 2006; Johnston and Sheaves 2008). The aim of this study is to determine how site-specific habitat characteristics along the margins of a tropical estuary influence the distribution patterns of nekton.

5.2 Materials & Methods

5.2.1 In-Field Sampling

Distribution of estuarine fauna was investigated along the margins of a tropical estuary using bottom-set cameras. For a detailed explanation of camera equipment and deployment protocols, refer to Chapter 2. Sampling was conducted along a mangrove shoreline spanning approximately 1.5 kilometres in Deluge Inlet (for complete site description, see Chapter 2). Camera equipment was used for sampling along the edge in 18 “zones” along the bank (Fig. 5.1), exactly as detailed in section 4.2.1 of Chapter 4. To investigate the bank on a broader spatial scale, zones were also grouped in sets of three for some of the analyses. As part of this broader scale, the impact of two ‘pressure points’, or areas at which flow is disrupted by the junction of a side tributary into the main creek, was considered. Based on field observations, the influence of these areas covered approximately the first three and last three zones along the bank. Thus, zones were grouped into threes to incorporate these pressure point areas. These six groups of three along the bank thus reflected their associated bank type (i.e. pressure point or linear bank) and were simply labelled ‘A’, ‘B’, ‘C’, ‘D’, ‘E’ and ‘F’ (Fig. 5.1). As described in Chapter 4, data were collected during the early, mid and late dry season, on June 14th, August 27th and October 12th 2012. Cameras sampled along the bank throughout the bottom of the tidal cycle (early run out, mid run out, late run out, early run in, mid run in), although a lack of alignment between daylight hours and necessary tidal stage on August 27th and October 12th prevented the early run out from being sampled. Cameras were run for twenty minutes for each replicate, the minimum amount of time logistically necessary for placement and the collection of all 18 cameras, as well as to allow for adequate sampling of the nekton community (see Chapter 2). Depth measurements were taken when cameras were collected (as described in Chapter 2).

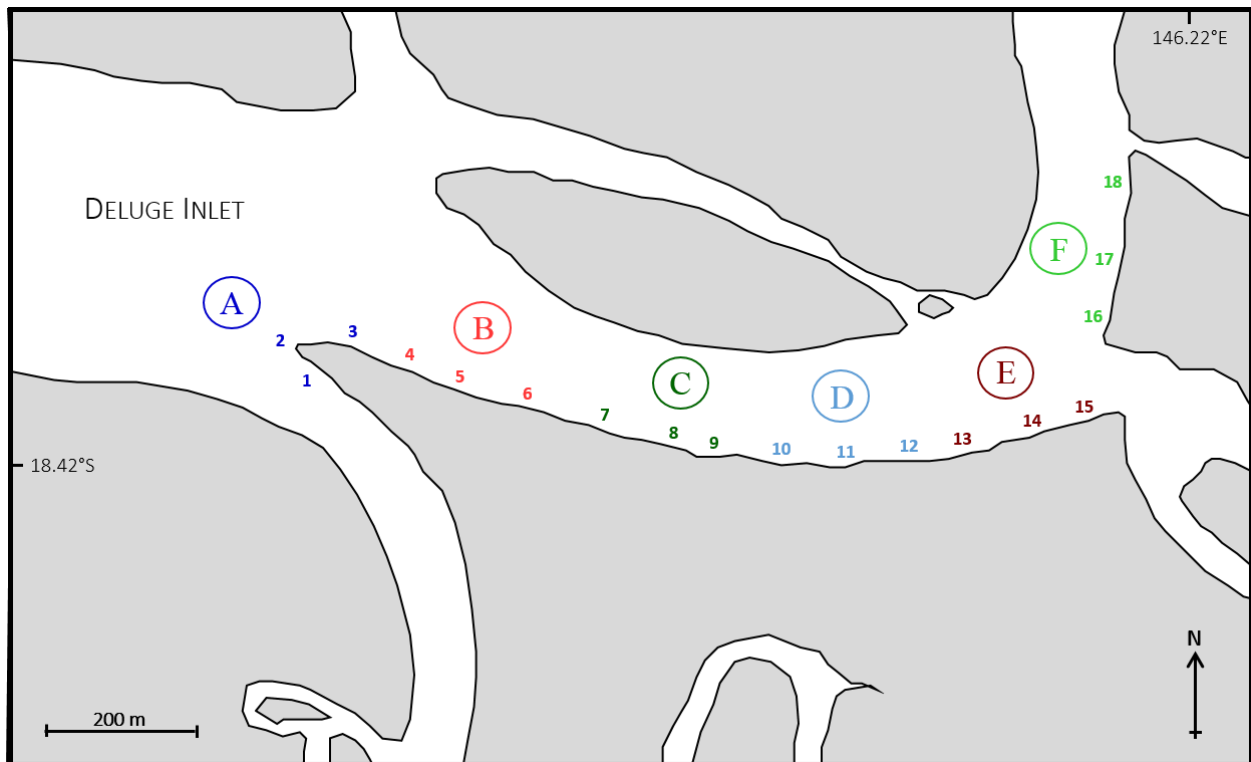


Figure 5.1 Map of Deluge Inlet, indicating the placement of the 18 zones used for sampling. Zones were also grouped for analyses to reflect bank type: 'A', zones 1-3; B, 4-6; C, 7-9; D, 10-12; E, 13-15; F, 16-18.

5.2.2 Data Analysis

Video samples were assessed in the same way as outlined in Chapter 2. Footage, paired with in-field observations, was also used to categorise each zone based on habitat characteristics: presence of algae, presence of structure and rugosity. Presence of algae and structure were both estimated based on observations from video samples that determined if structure and/or algae were apparent in each zone (i.e. present or absent). Rugosity was determined from video samples, classifying the benthos as either uneven or flat. The general current flow pattern for each zone (i.e. linear or turbulent) was also assessed via visual

observations while in the field. The depth measurements taken during pickup of each camera were also used during analysis of the effect of habitat characteristics.

Mean max. N data (see Chapter 2) for the five most prevalent taxa (*Acanthopagrus* spp., Clupeidae, Gobiidae, *Lutjanus fulviflamma/russelli* and *Gerres* spp.) were analysed graphically to assess patterns in distribution in edge habitats. Data for the three sampling days were combined to determine mean max. N for each tidal stage, creating a bar plot for each zone and bank type, and providing a simple and effective means of displaying the pattern of distribution data along the bank.

A multivariate classification and regression tree (mCART) was used to evaluate if any habitat characteristics correlated with distributional patterns observed along the margins of Deluge Inlet, using TreesPlus software (De'Ath 2002). For further details on the use of regression trees, see Chapter 4 or refer to De'Ath (2002) or Sheaves (2006). Fourth-root transformed max. N data were used (to moderate the influence of samples with extreme values) and analysed using bank type categories (i.e. A (zones 1-3), B (4-6), C (7-9), D (10-12), E (13-15) and F (16-18)) and habitat features (presence of algae, presence of structure, rugosity (uneven or flat), depth, and current flow (linear or turbulent)) as explanatory variables. The tree size with the best overall fit was selected using the 1-SE rule (Breiman *et al.* (1984), see Chapter 4). Only species seen in greater than 5% of all samples were used for analysis.

5.3 Results

The mean max. Ns of the five most prevalent taxa (*Acanthopagrus* spp., Clupeidae, Gobiidae, *Lutjanus fulviflamma/russelli* and *Gerres* spp.) varied substantially among edge

habitats (Fig. 5.2). However, four of the five taxa (with the exception of Gobiidae) showed elevated max. N values at both the beginning and end of the bank that was sampled, in the 'A' and 'F' areas, or the two 'pressure points' along the bank (Fig. 5.2).

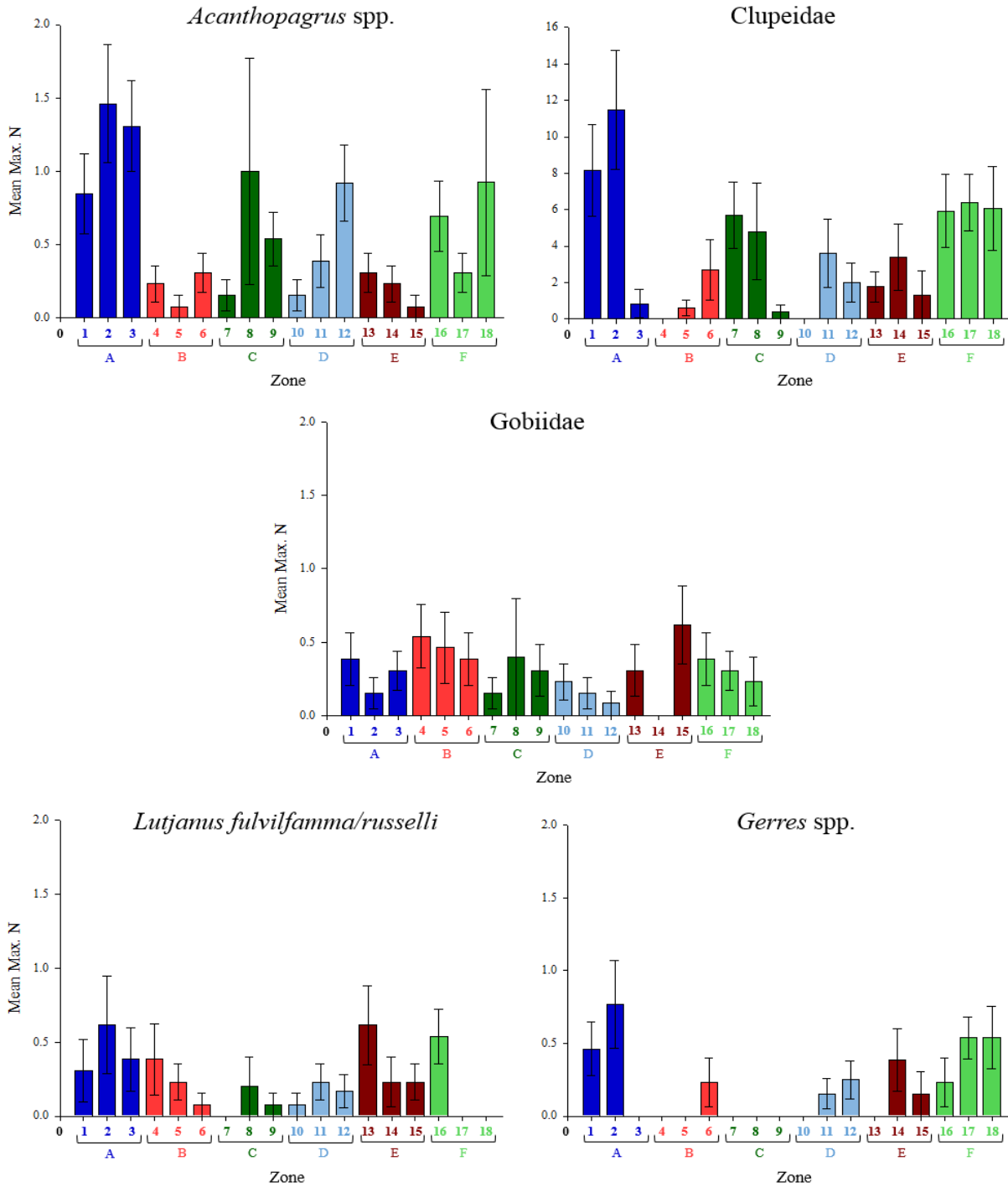


Figure 5.2 Mean max. N of the five most prevalent taxa across 18 zones sampled in Deluge Inlet. Zone numbers and bars are coloured to reflect type of bank: blue: 'A', zones 1-3, light red: B, 4-6, green: C, 7-9, light blue: D, 10-12, dark red: E, 13-15 and light green: F, 16-18. Bars depict mean max. N over three sampling days (14/06/12, 27/08/12, & 12/10/12). Error bars denote standard error.

The mCART showed 'type of bank' was the most influential in determining distributional patterns along the bank, with depth also showing substantial effects (Fig. 5.3). The split caused by type of bank (the first on the tree) separated between the two pressure point groups and those along the linear bank, with greatest max. Ns at the pressure point areas (i.e. groups A and F)(Fig. 5.3). The taxa that most reflected this trend were Clupeidae and *Acanthopagrus* spp. (Fig. 5.3). Depth further split the pressure point groups, with taxa such as Clupeidae and Ambassidae showing preference for shallow water and others such as *Acanthopagrus* spp., *Pomadasys* spp. and *Siganus* spp. showing a preference for deeper water (Fig. 5.3). Current flow was moderately important as per an analysis of variable importance for the mCART, although did not cause any splits on the tree (Fig. 5.3). Rugosity, the presence of structure, and algal growth had minimal impact on distribution patterns and also caused no splits in the mCART (Fig. 5.3).

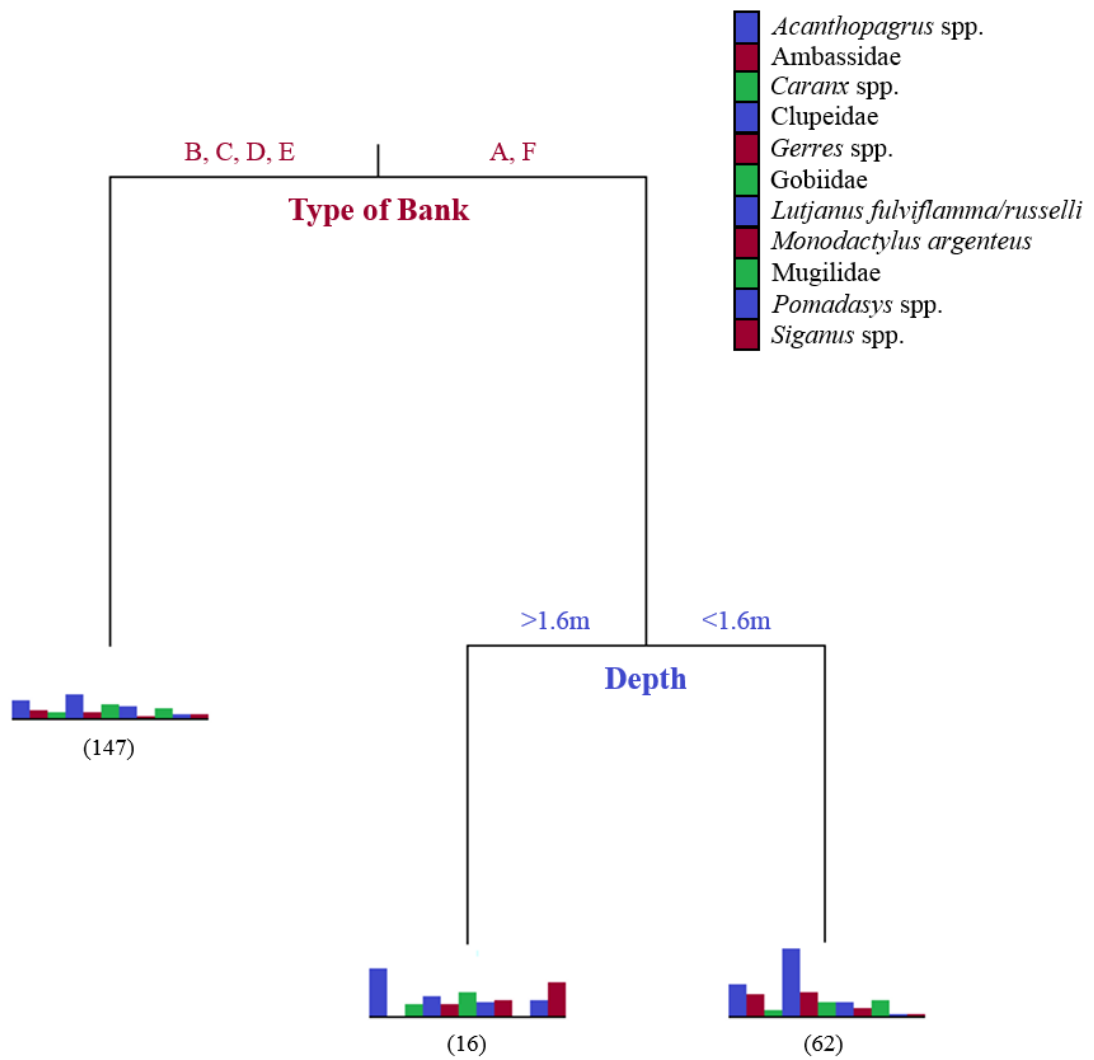


Figure 5.3 Multivariate classification and regression tree (mCART) investigating max. N in Deluge Inlet as it relates to habitat type, classified by: **type of bank** (A, B, C, D, E, F), **depth**, current flow (linear or turbulent), structure (present or absent), algal growth (present or absent) and rugosity (flat or uneven). Only the 11 taxa that were present in >5% of samples were used in analysis. Max. N data were fourth-root transformed; barplots show the multivariate taxa mean at each node, and the numbers in parentheses are the number of replicates.

5.4 Discussion

High nekton max. N values were consistently observed in specific areas throughout this study. In both the general graphical representations of mean max. N of the most prevalent species (Fig. 5.2) and the mCART analysis (Fig. 5.3), the greatest concentration of nekton were in the 'A' and 'F' areas of the bank: 'hot spots,' with higher concentrations of nekton than surrounding areas (similar to the use of the term 'hot spot' in Malakoff (2004); and Reese and Brodeur (2006)). These increased max. N values were observed in these locations for all of the taxa included in the mCART analysis (Fig. 5.3) and particularly reflected in observations of *Acanthopagrus* spp. and Clupeidae, which were also the two most prevalent taxa in this study (Fig. 5.2).

As the type of bank had the greatest impact on nekton distributions, it is clear that broader-scale patterns had the most influence in this system. The broad-scale pressure point areas of the bank created hot spots, probably due to the turbulent current flow in those areas. At these locations (A and F), current encountered a point of land between the main channel and a side tributary that interrupted linear current flow (Fig. 5.1), causing turbulent flow conditions; this contrasts with all other bank types (B, C, D and E), where the flow was mainly linear and parallel to the bank. The higher max. Ns observed in these disrupted current areas may be due to an aggregation of food sources such as zooplankton, creating ideal foraging conditions in these areas with turbulent or upwelling water (Alldredge and Hamner 1980). However, as current flow did not create any splits on the mCART as an independent variable, it is probable that disrupted current influenced distribution on a broad scale, but not at finer zone-level. The absence of current-driven splits could also be explained by a radiating effect of turbulent current into neighbouring linear-current zones, creating broad-scale current effects, but no substantial fine-scale impact of current between zones. It is

also probable that other factors at the pressure point areas influenced the high max. Ns observed there. This could be submerged snags nearby, or access to easy connections that lead to other useful habitats.

Depth was also an important influential factor in determining nekton distribution patterns, particularly within the pressure point areas (Fig. 5.3). Past research has been divided on the influence of depth on distribution in estuarine edge habitats, with some suggesting that it has little influence (Halliday and Young 1996; Meager *et al.* 2003), and others contending that it has substantial impact (Vance *et al.* 1996; Ley *et al.* 1999). The shallow water refuge paradigm that was largely accepted in the past contends that organisms utilise shallow water because it provides increased refuge from predation (Blaber and Blaber 1980; Boesch and Turner 1984; Paterson and Whitfield 2000). The results of the present study suggest that the influence of depth on taxa distribution was substantial, although preference for certain depths varied between taxa. Although the 'A' and 'F' areas were fairly shallow in nature, the majority of taxa in these locations preferred greater depths (Fig. 5.3), suggesting that increased refuge value of shallow water may not be as important as other factors for determining hot spots and that there are probably other factors contributing to nekton distribution. Of course, it is also possible that a combination of increased foraging in slightly deeper waters at these sites, paired with the availability of shallow water nearby, together create an ideal area for hot spot formation. It is also possible that depth preferences may also be due to differences between taxa and their associated refuge requirements. For instance, smaller-bodied taxa (i.e. Clupeidae and Ambassidae) may find refuge in the shallower depths that they generally preferred, while deeper-bodied taxa (i.e. *Acanthopagrus* spp., *Pomadasys* spp. and *Siganus* spp.) may find refuge in slightly deeper water.

The other habitat characteristics that were investigated in this study (presence of structure, rugosity and algal growth) may contribute to hot spot formation to some degree, although the low importance that was given these variables in the mCART analysis (Fig. 5.3) suggests that any influence was most likely minor. In some instances, this contrasted with past work; the minimal influence of structure on nekton distribution in the present study differs from past research, which has found structure to play a key role in determining distribution of organisms (Sheaves 1992). However, the species found to be most influenced by the presence of structure by Sheaves (1992) (i.e. *Epinephelus malabaricus* and *E. coioides*) were not strongly sampled by the present study, making the comparison weak. Nonetheless, the results from this study suggest that the influence of the factors determining distribution is complex, and variables such as structure may not be highly important for certain taxa in edge habitats. It is also possible that other factors that were not investigated in this study may also play a role.

In conclusion, the results of the present study suggest that site-specific habitat characteristics influence the distribution of fauna along the estuarine bank. Some of these variables have more impact than others and a certain combination of factors can cause hot spots of nekton abundance to form in specific areas. These conclusions do not accord with a random walk model (Bartumeus *et al.* 2005) that could be seen as a null hypothesis of random assortment along estuarine margins, and so further support the claim that distribution is heavily influenced by habitat characteristics.

Although these conclusions are supported by the strong trends observed within the results of the present study, there was a substantial amount of variation that remains unexplained. The complex interaction of site-specific habitat characteristics and the distribution of estuarine nekton may explain some of the variation that was found throughout

the present study. It is also possible that this variation was due to other factors contributing to the patterns that were observed. For instance, areas of prolonged access to the mangrove forest may have influenced distributions, or other variables (e.g. salinity, current strength, etc.) may have impacted results (McIvor and Odum 1988; Johnston and Sheaves 2007). In addition, all the variables that were investigated in the present study were not evenly distributed amongst 'zones' (i.e. there was an uneven number of 'zones' with uneven and flat rugosity), possibly skewing results and adding to the variation that was observed. Still, the variability throughout the study may simply be attributed to the complex and dynamic nature of estuarine systems and the interconnected way in which these habitat variables relate (Blaber 1980; Morin *et al.* 1992; Sheaves 2009). This variation and the complex interactions of habitat characteristics leave questions to investigate in the future, such as the impact of other untested variables on the formation of abundance hot spots.

The patterns in nekton distribution that were observed in the present study suggest that there are certain areas that are more suitable for nekton habitation than others. Although the present study has given important insight into distributional patterns and their driving forces along the estuarine bank, further research is necessary to investigate these relationships. Increasing the understanding of the role these site-specific habitat characteristics play in impacting estuarine fauna and the many complex factors that influence these environments is essential to increasing the ability to protect and manage these important habitats.

Chapter 6 General Discussion

Prior to the present study, the understanding of faunal distribution within a tropical estuarine environment was often limited to studies focussing on general information about where taxa were located throughout the estuary and potential reasons why these areas were utilised over others (Blaber and Blaber 1980; Sheaves 2001; Ellis and Bell 2004; Johnston and Sheaves 2008). However, how these distributional patterns change both spatially and temporally drew little focus. Consequently, the complex way in which estuarine taxa utilise these variable and dynamic environments is not fully understood (Sheaves *et al.* 2010). The goal of the present study was to gain a better understanding of the distributional changes and patterns of movement associated with estuarine fauna at both a spatial and temporal scale, and thus to obtain clearer insight into the way these organisms utilise edge habitats and respond to their varying conditions. While supporting the claims made by previous studies regarding such faunal movements as the periodic use of mangrove forests (Sheaves 2005), this study has also provided new insight into the patterns of change associated with the movement of estuarine taxa and shown that edge habitats, although they may be used briefly by nekton, are important habitats for estuarine taxa.

6.1 Key findings

Abundances of estuarine edge fauna increased at higher tidal levels, just before the fringing mangrove forest was flooded with water and after it was drained, and decreased as the tide retreated (Chapter 4). This pattern suggests that the distribution of estuarine nekton is driven by habitat connectivity and the utilisation of higher intertidal habitats (Ellis and Bell

2004; Johnston and Sheaves 2007). The increased abundance of nekton just before tides flood the mangroves suggests that the use of mangrove forests is important for many estuarine taxa, with many nekton utilising these complex habitats when they were available at high tides (Chapter 4)(Sheaves 2005). However, unlike the hypothesis in Chapter 1 that predicted fauna would remain in the edge habitat throughout low tide, the distinct decrease in abundance along the edge at low tide shows that many organisms do not stay in edge habitats at this time (Chapter 4). This brings forth questions of where these taxa move to, which will be briefly discussed in section 6.3.

Site-specific features of the estuarine edge influenced nekton distributions as well, with many variables related to distribution in a complex way (Chapter 5). Depth and current were probably particularly important in determining patterns of faunal distribution along the edge (Chapter 5). Although past studies have disagreed on the importance of depth influencing habitat selection (Halliday and Young 1996; Vance *et al.* 1996; Ley *et al.* 1999; Meager *et al.* 2003)(see Chapter 5), the substantial effect of depth in comparison with the other habitat characteristics measured in this study (particularly structure, which has been found to have a substantial influence in the past (Sheaves 1996)) suggests an important link between depth and distribution (Chapter 5)(more on depth in section 6.2). The high abundances in two ‘hot spot’ areas along the bank further validate that site-specific characteristics influence distribution and suggest that current is probably a highly influencing factor, due to the turbulent current that characterised these areas (Chapter 5). Taxa thus demonstrated a particular preference for turbulent flow, probably due to increased foraging opportunities (Aldredge and Hamner 1980)(Chapter 5)(more on current in section 6.2). However, the results of this study concluded that while depth and current were probably highly influential on the distributional patterns of estuarine fauna, it is probably a complex interaction of many

factors that determine distribution, and as these characteristics change with tidal fluctuations, distribution patterns emerge (Chapters 4, 5).

6.2 Potential drivers of habitat-use patterns

The patterns of movement and distribution of estuarine nekton are driven by the needs of the taxa involved. Although habitat use can be driven by many factors (Rountree and Able 2007), there are two basic requirements: feeding and refuge (Boesch and Turner 1984; Sheaves *et al.* 2006). Understanding these needs can elucidate the reasons for the utilisation of certain habitats at specific times as well as the changes that are observed in patterns of movement and distribution. As the characteristics of estuarine banks change over time mostly due to the fluctuating tide, the refuge and foraging potential at these edge sites change. The need for refuge and feeding are thus met unequally between sites and between times of day.

The changing balance of feeding and refuge opportunities in edge and adjacent intertidal and subtidal habitats are the probable drivers responsible for the patterns of distribution of estuarine nekton observed throughout the present study (Sheaves 2005), facilitated by the temporal changes to connectivity that can regulate movements between habitats. While mangroves can provide refuge and foraging opportunities at high tide (Laegdsgaard and Johnson 2001; Sheaves 2005), the competing need for refuge and foraging must also be taken into consideration when evaluating the movement of taxa away from the bank at low tides. This movement may be explained by the utilisation of the main channel, which may provide refuge and foraging opportunities at low tide. However, as tides begin to rise again, taxa return to the edge environment, likely awaiting the flooding of the mangrove forest (Sheaves 2005). This suggests that this edge environment provides an important, if

brief, purpose for these taxa, as they utilise this area before and after the mangroves are available rather than moving directly from their low-tide habitat into the mangroves.

The movements between habitats within the estuary (e.g. from the edge into the mangrove forest) are driven by both the level of connectivity between them and the resources each habitat can offer. Organisms move to the most favourable available habitat at any given time. For instance, refuge is of great importance for many estuarine taxa and is probably provided by the mangroves at high tide (Sheaves 2005) and potentially by the main channel at low tide, which may provide refuge in the form of shallow water once the tide has sufficiently fallen (Blaber and Blaber 1980; Boesch and Turner 1984; Paterson and Whitfield 2000; Johnston and Sheaves 2008) or via submerged structure (Savino and Stein 1982; Bartholomew *et al.* 2000; Ellis and Bell 2004). Although the validity of the shallow-water refuge paradigm has been questioned (Sheaves 2001), the increased refuge potential of shallow water has not been disproved (Sheaves 2001; Baker and Sheaves 2007)(for more discussion of the shallow-water refuge paradigm, see Chapter 5). When refuge is unavailable or the need for food is greater, the level of foraging opportunities offered by each habitat may drive distribution, and is probably the reason why areas of turbulent current flow saw increased nekton abundance, as turbulent current flow can often lead to upwelling of zooplankton and other food sources, creating ideal foraging conditions (Alldredge and Hamner 1980).

The differences between habitat use for different taxa can probably also be explained by the balance of refuge and foraging needs, which differs between taxa (Sheaves 2005). For instance, some taxa (e.g. Clupeidae, Ambassidae) showed preference for shallow water, whereas others preferred deeper areas (e.g. *Acanthopagrus* spp., *Pomadasys* spp., *Siganus* spp.). These patterns may be driven by differences in refuge requirements, with smaller-

bodied fish (e.g. Clupeidae, Ambassidae) favouring shallow water and deeper-bodied fish (e.g. *Acanthopagrus* spp., *Pomadasy* spp., *Siganus* spp.) favouring deep water.

6.3 Implications for connectivity

Estuaries are dynamic, complex systems especially due to the high levels of connectivity among habitats, and between these systems and others (Sheaves 2009). The movements of nekton observed in the present study highlight connectivity between subtidal and intertidal habitats within the estuary. As abundances substantially decreased along the edge at low tides (Chapter 4), the taxa observed during higher water levels must have moved on to other areas, taking advantage of physical connections between areas of the estuary or between systems (depending on the scale of movement), as well as adding to the transfer of resources. The movement of estuarine nekton has implications for the transfer of energy and nutrients (Quevedo *et al.* 2009; Sheaves 2009). The level of connectivity and energy transfer is dependent on the trophic level of the moving taxa as well as the extent of their movement (Quevedo *et al.* 2009). For instance, a fish that feeds within the protection of the mangroves is forced out of the forest at low tide, moving the forest production to other parts of the estuary. Without the refuge of the mangrove forest, this individual may also be more susceptible to predation at this time, and if it is eaten, it would then facilitate the transfer of forest production to higher trophic levels that may further move it to other systems offshore (Sheaves and Molony 2000). However, the degree of movement is unclear in the present study, as it was simply the absence of taxa that was observed. These missing individuals may have simply moved to shallow areas in other parts of the channel (Johnston and Sheaves 2008), moved to utilise more productive foraging areas or submerged structure, or, like the sawfish studied by Simpfendorfer *et al.* (2010), it is possible that they may have moved to an

entirely different system (i.e. out of the estuary), creating a much more extensive connection. Thus, connectivity between habitats can occur at a range of scales, with smaller daily movements through edge habitats and between low and high tide habitats facilitating larger-scale movement of mangrove production to other systems.

6.4 Implications for conservation and management

The patterns of movement and distribution of estuarine nekton observed in the present study can aid in building more effective conservation and management plans for these areas. Estuarine edge environments are not only crucial habitats for many estuarine fauna and important economic areas for society (Costanza *et al.* 1997; Blaber *et al.* 2000), but they are also the most influenced by anthropogenic impacts (Blaber *et al.* 2000). As demonstrated in the present study, edge habitats play an important role in estuarine ecology and are used in dynamic ways. This may simply refer to their use as pathways between habitats, as a transition zone in which taxa reside before utilising adjacent habitats, or the use of edge habitats as feeding grounds for predators that utilise the forced evacuation of the mangrove forest as the tide retreats. Understanding the dynamic uses of estuarine edges is essential to ensure these valuable habitats are not overlooked in the development of conservation and management plans. As edges serve as transition zones for movement between connected subtidal and higher intertidal habitats, access to important refuge and foraging areas (e.g. the mangrove forest) may be interrupted if these habitats are altered. Edges also provide some form of valuable resource for estuarine fauna, which is evident from taxa that reside in these areas while awaiting access to the fringing mangrove forest, rather than staying in alternate habitats and quickly moving through these transition zones when the forest is available. These edge environments are thus important habitats for many estuarine taxa, which could be

greatly affected if these areas were not properly managed. Understanding the movement of taxa and the way they utilise these areas thus allows for more site-specific conservation and management plans to be put in place, and suggests that management plans consider the range of interconnected habitats that are used and the pathways that join them (Nagelkerken *et al.* 2013).

6.5 Challenges & directions for future research

Estuaries have challenging sampling conditions and can be difficult environments in which to conduct research (Rozas and Minello 1997). To overcome some of these difficulties and to provide a different perspective from the methods traditionally used, as well as to provide a technique capable of making observations over time rather than as point samples, underwater videography protocols were developed and utilised to sample these systems (Chapter 2, 3). However, as with any gear, it was not without its limitations. With cameras situated on benthic sediments and despite working primarily in sites <2m deep, pelagic fauna higher in the water column may have been underrepresented. Cameras were also dependent on water clarity. Although protocol development determined that visibility $\geq 0.25\text{m}$ was suitable for data collection with this method (Chapter 2), turbidity was still a constraint. As a result, some closely-related species were unable to be distinguished during identification and were thus grouped together, causing fine-scale ecological data between such species to be lost. Despite these challenges, underwater videography proved to be a useful technique that allowed for the examination of fine-scale spatial and temporal patterns in distribution and edge use and set the present study apart from past research. Using non-destructive videography techniques that were highly effective for sampling edge habitats (Chapter 2, 3),

observations of distribution could be made in real-time and patterns of change could be observed, providing an advantage for collecting this data over other methods.

Underwater videography will thus provide a valuable tool for future research, especially in areas of difficult access with traditional equipment, such as the mangrove forest. Although the use of the mangrove forest is widely accepted (Sheaves 2005), there is little direct evidence of the movement into and utilisation of the mangrove forest by estuarine nekton (Robertson and Duke 1990a; Vance *et al.* 2002). Videography would be very useful for investigating where estuarine taxa go at high tide and how they utilise those habitats. Further exploration of the movement of taxa at low tide would be additionally beneficial to locate the areas to which edge taxa move at these times as well as to identify how they utilise those areas. Additionally, further work would be useful to identify the source of the large amount of unexplained variation that remained in the data collected in the present study, although this is most likely due to the highly dynamic nature of estuaries (Sheaves 2006). However, this variation highlights the complexities of estuarine systems that must be thoroughly investigated to become clear, and thus deserve more attention in the future, such as the complex relationship observed between site-specific characteristics and distribution of taxa in this study (Chapter 5). To fully understand the effect of site-specific characteristics on edge distributions, investigation of the seascape configuration of adjacent connected habitats is necessary, as the characteristics of areas in close proximity to those investigated may affect the distributions that were observed (Pittman *et al.* 2010; Boström *et al.* 2011). In addition, the relationship of estuarine taxa with other site-specific variables not tested in this study (e.g. current strength, salinity, etc.) may also be of interest (Thresher 1983; Cyrus and Blaber 1992). Although these additional investigative topics may prove useful in the future to build on the results found in this study, the present investigation strongly demonstrates the importance of estuarine edge habitats and habitat connectivity for estuarine fauna.

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