

Final Report

Determining the spatial distribution and abundance indices for Moreton Bay Bugs, *Thenus parindicus* and *Thenus australiensis* in Queensland to improve stock assessment and management



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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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| BRT | Boosted Regression Trees |
|-------|--|
| ACN | Authority Chain Number |
| CFISH | Commercial FISHeries logbook database of Queensland |
| CAP | Canonical Analysis of Principal coordinates |
| CI | Confidence Intervals |
| CL | Carapace Length |
| CW | Carapace Width |
| DAF | Queensland Department of Agriculture and Fisheries |
| ECOTF | East Coast Otter Trawl Fishery |
| GAM | Generalised Additive Model |
| GBR | Great Barrier Reef |
| GLM | Generalised Linear Model |
| IDW | Inverse Distance Weighting |
| JCU | James Cook University |
| LTMP | Long Term Monitoring Program of Queensland Fisheries |
| MARS | MARine Sediment database of Geoscience Australia |
| MPA | Marine Protected Area |
| RF | Random Forest |
| SD | Standard Deviation |
| SDM | Species Distribution Modelling |
| SRA | Scallop Replenishment Area |
| VEcv | Variance Explained by cross validation |
| | |

Abbreviations

Executive Summary

We report on the first comprehensive investigation into the spatial distribution of Moreton Bay Bugs within the Queensland East Coast Otter Trawl Fishery. This research was a collaboration between the Queensland Department of Agriculture and Fisheries and James Cook University, applying interdisciplinary approaches to successfully model habitat preferences of the two Moreton Bay Bug species and map their distributions along Queensland's east coast. Historic catch records were then split according to each species' spatial distribution. From these records, standardised catch rates were produced as indices of abundance for each species for use in future stock assessments of the Moreton Bay Bug fishery.

Background

Queensland's Moreton Bay Bug fishery has become increasingly important due to its rapid growth in value and the depletion of co-located Saucer Scallop stocks. Increasing demand has driven a shift in effort to the periodic targeting of Moreton Bay Bugs in the East Coast Otter Trawl Fishery (ECOTF), resulting in a need for greater management focus and assessment of bug stocks. However, the Moreton Bay Bug catch is comprised of two species, Reef Bugs (*Thenus australiensis*) and Mud Bugs (*T. parindicus*), each with different life histories and distributions. Both species have been recorded together without differentiation in logbook catch records since 1988, complicating interpretation of historical trends in the abundance of each species.

This project aimed to develop long-term indices of abundance for each Moreton Bay Bug species by allocating historic catch records between them based on each species' spatial distribution. The resulting species-specific abundance indices, in the form of standardised catch rates, will be used to inform stock assessment to better manage Moreton Bay Bug stocks.

Aims

This work had four objectives: 1) Implement a state-wide crew member observer program to obtain detailed photographic records of Moreton Bay Bug catches, to assist with determining the species composition and distribution of catches, 2) Undertake a stratified survey of Moreton Bay Bug catch rates, species composition, and seafloor properties in the main trawl fishing grounds off Townsville, 3) Use all available data sources to model, predict and map the spatial distribution of the two species of Moreton Bay Bugs along the Queensland coast, and 4) Produce

long-term standardised catch rates for each Moreton Bay Bug species that can be used as an index of abundance for stock assessment and management.

Methodology

Species distribution data were compiled from all available sources, including commercial catch records and fishery-independent sources. Around 80% of historic landings within the ECOTF occur in the Townsville and Gladstone regions, with the remainder distributed along Queensland's east coast from Cape York to Moreton Bay. While species distribution data from the Gladstone area had been collected periodically by a long-term monitoring survey, data were lacking for the remainder of the Queensland coast. As such, two surveys were conducted during the current study to inform the distribution of Moreton Bay Bug species: 1) A fishery-dependent crew observer program, whereby crew members recorded their Moreton Bay Bug catches with co-located photographs of the catch to allow species identification, and 2) A fishery-independent stratified random survey of Moreton Bay Bug species distributions on the fishing grounds offshore from Townsville.

Machine learning techniques were used to produce continuous rasters of seafloor sediment properties and other environmental variables. Species distributions were then modelled based on habitat preferences derived from locations with known species compositions. Historic catch records were then allocated between the two Moreton Bay Bug species based on each species' distribution and time series of standardised catch rates were produced for each species as indices of abundance to inform stock assessment.

Results/key findings

Strong habitat partitioning was observed between the two Moreton Bay Bug species, resulting in highly successful species distribution modelling. The key drivers of species distributions were sediment mean grain size, depth, distributions of medium-, fine-, and very fine sand, and distance from the coast.

Using a Boosted Regression Tree model, these six variables explained 93% of the variance in species distributions and correctly predicted the dominant Moreton Bay Bug species at >99% of locations where dominant species was known. Overall, sediment properties, particularly mean grain size, were more important than depth or other geographic variables in explaining species distributions.

Allocation of historic logbook records between the two Moreton Bay Bug species using the outputs from the species distribution model indicated that the proportion of Reef Bugs comprising total annual landings has increased from ~67% in 1988 to ~93% in 2021. The change in catch composition over this period is likely due to increased targeting of Reef Bugs and reduced fishing effort in areas dominated by Mud Bugs (e.g., Far North Queensland).

Despite increased targeting of Reef Bugs, standardised catch rates (produced as indices of abundance for both species) remained relatively stable from 1988–2021. Targeting behaviour and vessel effects (i.e., improvements in fishing power through gear efficiencies and reductions in license numbers leading to consolidation of the most efficient fishers within the fleet) had the greatest influences on Reef Bug catch rates.

Implications for relevant stakeholders

This study provides Fisheries Queensland with robust data with which to assess the stock of Moreton Bay Bugs in Queensland, an increasingly important catch component of the ECOTF. The provision of standardised catch rates and species distribution data allow Fisheries Queensland to make evidence-based management decisions to ensure the long-term sustainability of both Moreton Bay Bug species in Queensland.

The strong support provided by industry, particularly in relation to the Crew Observer Program, resulted in a robust combination of data from fishery-dependent and fishery-independent sources covering much of the ECOTF. This revealed strong habitat partitioning between Moreton Bay Bug species throughout the fishery. This habitat partitioning, based on each species' preference for different types of habitats, allowed the development of a highly credible species distribution model.

Using the species distribution model to split historic logbook records between both Moreton Bay Bug species revealed a marked increase in the proportion of Reef Bugs comprising total landings from 1988 to 2021. Over the same period, indices of abundance for both species remained relatively stable, despite increased targeting of Reef Bugs in recent years.

The stability of Reef Bug abundance since 1988 despite increased targeting may be due to management measures offsetting the effects of increased fishing on the Reef Bug population. These measures include a nominal reduction in fishing effort since 1988 (e.g., via a trawl management plan and licence buy backs) and extensive area closures (e.g., in the Great Barrier

Reef Marine Park), where source populations with no fishing mortality may contribute to the fished stock via spill over or larval dispersal.

Recommendations

Fisheries Queensland should use the outcomes of this study to assess the status of Moreton Bay Bug stocks in Queensland. Specifically, the standardised catch rates derived herein represent robust indices of abundance for use in future stock assessments.

Quantifying the contribution made by Moreton Bay Bug populations in protected areas (e.g., in areas of the Great Barrier Reef Marine Park closed to trawling) to the fished stock, in terms of spill over and/or larval dispersal, would provide a more comprehensive understanding of stock dynamics.

Future sampling of Moreton Bay Bug populations in data poor areas (e.g., off Cape York or Mackay) would help validate model predictions of species compositions in those areas.

Keywords

Moreton Bay Bugs, Bay Lobster, Shovel-Nosed Lobster, Reef Bug, *Thenus australiensis*, Mud Bug, *Thenus parindicus*, Scyllaridae, species distribution modelling, machine learning, Random Forest, Boosted Regression Trees, sediment modelling, Great Barrier Reef, Crew Observer Program, fishery-independent survey, standardised catch rates, index of abundance.

1 Introduction

1.1 Background

Moreton Bay Bugs, also known as Bay Lobsters, are Scyllarid lobsters distributed around the northern coastline of Australia. The Scyllaridae (slipper lobsters) diverged from closely related Palinuridae (spiny lobsters) approximately 250 million years ago and are distinguished by their lack of whip-like antennae in favour of compressed, plate-like antennal flagella. This characteristic adaptation, as well as a dorso-ventrally compressed morphology, allows the Scyllaridae to exploit open habitats with loose sedimentary substrates where they find refuge by burying in the sediment instead of seeking structural refugia in the reef or rocky habitats preferred by spiny lobsters. It is in such sandy, open habitats that Scyllaridae are principally caught by trawl fisheries.

Two genera of Scyllaridae are important in fisheries catches in Australia: *Thenus* (Moreton Bay Bugs) and *Ibacus* (Balmain Bugs), while two less important genera of slipper lobsters (*Scyllarides* and *Parribacus*) are caught incidentally in lesser quantities. Australian landings of Moreton Bay Bugs amount to ~400–700 tonnes annually, of which over 80% is landed in Queensland's East Coast Otter Trawl Fishery (ECOTF), with the remainder distributed from Torres Strait to Shark Bay in Western Australia (Roelofs et al. 2020). In comparison, around 200 tonnes of Balmain Bugs are landed annually, mostly from northern New South Wales and Southeast Queensland, although *Ibacus* landings also occur around the southern coast of Australia in smaller quantities (Haddy et al. 2007). In addition to their greater landings by weight, Moreton Bay Bugs are also considerably more valuable than Balmain Bugs due to their greater size, yield per recruit, and market demand (Courtney 2002).

From 1988, when commercial logbooks were introduced in Queensland, until 2000, both Moreton Bay Bugs and Balmain Bugs were recorded together as 'Bugs' in logbooks, leading to complexity in calculating *Thenus* landings during this period (see Section 7). From 2000, logbooks were updated to include separate boxes for recording Moreton Bay Bugs and Balmain Bugs. Landing records of Moreton Bay Bugs indicate that within the ECOTF, two main grounds off Gladstone and Townsville contribute ~80% of landings (Figure 1).



Figure 1. Landings of Moreton Bay Bugs recorded in 30' logbook reporting grids comprising the top 95% of total landings in the East Coast Otter Trawl Fishery from 2000–2021. The main grounds off Townsville and Gladstone each contribute ~40% of total landings in Queensland. Trawl Management Regions are numbered 1–5.

Moreton Bay Bugs comprise two species: Reef Bugs (*Thenus australiensis*) and Mud Bugs (*T. parindicus*) (Figure 2). Reef Bugs (also known as Sand Bugs) are typically found in deeper areas (30–60 m) with coarser substrates and were historically landed as bycatch mainly in association with Redspot King Prawns (*Melicertus longistylus*) and Saucer Scallops (*Ylistrum*)

balloti). Mud Bugs (also known as Tiger Bugs) are typically found in shallower areas (10–30 m) with finer sediments and caught in association with Tiger Prawns (*Penaeus esculentus* and *P. semisulcatus*), Endeavour Prawns (*Metapenaeus endeavouri* and *M. ensis*), and Banana Prawns (*P. indicus*). Minimum legal size for both species is 75 mm carapace width (CW). While both species were originally caught incidentally when targeting penaeids or scallops, there has been a shift in recent years to periodic targeting of the larger Reef Bugs. This shift has likely been driven by several factors, including their increasing market value and depletion of the co-located Saucer Scallop stock.



Figure 2. Distinguishing features of the two Moreton Bay Bug species: Reef Bugs (Thenus australiensis, left) and Mud Bugs (T. parindicus, right). Source: DAF (2022).

1.2 Project need

According to anecdotal advice from fishers, the targeting of Reef Bugs began after 2000 and became common from ~2012 onwards. This is supported by an observed increase in Moreton Bay Bug landings around the same period despite reduced fishing effort compared to historical levels (Figure 3). The shift in fishing behaviour to periodic targeting of Reef Bugs as they became more profitable has created a need for the first assessment of Moreton Bay Bug stocks to evaluate how they are responding to targeted fishing pressure. However, producing standardised catch rates to assess long-term trends in abundance is complicated by

the fact that both *Thenus* species have been recorded together as a multi-species complex in logbook records. It is therefore necessary to adopt an approach to identify species in historical catch records, based on location, to produce reliable indices of abundance for each species.



Figure 3. Annual landings of Moreton Bay Bugs (top panel) vs. annual nominal fishing effort (bottom panel) in the East Coast Otter Trawl Fishery from the beginning of logbook records in 1988. Red dashed lines denote the mean for each panel. Licence numbers are approximate numbers of otter trawl fishery symbols held. The Trawl Management Plan 1999 legislated annual closed seasons for the northern and southern sectors of the fishery, the introduction of a Vessel Monitoring System, and efforts to reduce bycatch and environmental impacts. The Great Barrier Reef Marine Park rezoning in 2004 resulted in the exclusion of trawling from large areas previously open to the fishery and extensive compensation to affected fishers.

Here we apply a multi-step species distribution modelling (SDM) approach to allocate logbook records between Thenus species. As a first step, existing information on Thenus species distributions, biology, and behaviour relevant to the SDM process were collated. Historical data on species distributions were compiled from several sources: 1) a formative thesis on Thenus populations in Queensland (Jones 1988); 2) a mark-recapture tagging study (Courtney 1997); and 3) a long-running fishery-independent survey off Gladstone that coincides with one of the two main Moreton Bay Bug fishing grounds (Dichmont et al. 2000). To add to the existing information on species distributions we conduct two new surveys: 1) a broad-scale fishery-dependent crew observer program along the entire east coast of Queensland; and 2) a targeted fishery-independent survey of Thenus populations off Townsville; the second of two main Moreton Bay Bug fishing grounds. Continuous habitat data were then produced by collating hydrologic and geologic data from a diverse range of sources, and modelling seafloor properties for the entire extent of the ECOTF to a depth of 80 m (beyond which Thenus records are rare). Species and habitat data were then brought together to identify species habitat preferences and use them to model species distributions throughout the fishery. Based on these distributions, logbook records were then split between both Thenus species and long-term standardised catch rates were produced for each species as indices of abundance for stock assessment.

2 Objectives

This project had four key objectives:

- Implement a state-wide crew member observer program to obtain detailed photographic records of Moreton Bay Bug catches, to assist with determining the species composition and distribution of catches.
- (2) Undertake a stratified survey of Moreton Bay Bug catch rates, species composition and seafloor properties in the main trawl fishing grounds off Townsville.
- (3) Use all available data sources to model, predict and map the spatial distribution of the two species of Moreton Bay Bugs along the Queensland coast.
- (4) Produce long-term standardised catch rates for each Moreton Bay Bug species that can be used as an index of abundance for stock assessment and management.

3 Analysis of existing data sources

Available sources were interrogated for data on the spatial distributions of the two *Thenus* species. Data were concentrated in the area off Gladstone, where a fishery-independent long-term monitoring program (LTMP) has been undertaken periodically from 1997 to monitor the Saucer Scallop (*Ylistrum balloti*) stock (Dichmont et al. 2000; O'Sullivan et al. 2005). During these surveys, data on *Thenus* species composition, sex, and length have been recorded since 1998 (Figure 4). Such data, however, were sparse throughout the rest of the fishery. Jones (1988) provided useful biological information and findings on movement and behaviour, but suitable species-specific distribution data were limited to a single location (Figure 4). Finally, a mark-recapture tagging study (Courtney 1997) investigated mortality and growth estimates and provided raw data that is analysed herein to assess movement and species distributions.



Figure 4. Availability of Thenus species distribution data at the beginning of this project.

3.1 Species biological information

This section summarises biological information on *Thenus* species compiled from extant sources including Jones (1988), Courtney (1997), and the LTMP survey off Gladstone and compares it with information collected during the current study (Townsville *Thenus* spp. survey 2021; Section 5).

Both *Thenus* species follow similar spawning and recruitment patterns. Low level year-round spawning is punctuated by spawning peaks in austral spring and summer followed by recruitment pulses in summer and autumn of young-of-the-year into the population (Jones 1993, Jones 2007). Growth is relatively rapid, with growth curves indicating both species attain the minimum legal size of 75 mm CW (~54 mm carapace length (CL); Milton et al. 2010) between 1–2 years of age, though females grow faster than males and attain greater maximum sizes (Jones 1993, Courtney 1997).

Mean sizes of Mud Bugs were smaller than Reef Bugs in all data sources examined and were below the minimum legal size in all sources except the LTMP survey off Gladstone (Table 1, Figure 5). The larger mean size of Mud Bugs in the LTMP survey likely reflects the fact that this survey is fishery-independent and samples some areas with low bug densities exposed to low fishing pressure. In all other data sources, measurements were derived from commercial fishery-dependent sources or sampled in heavily fished areas (Townsville *Thenus* spp. survey). Mud Bugs in these surveys are exposed to high fishing pressure on Tiger Prawn fishing grounds (limited in spatial extent thus concentrating fishing effort) and many larger adult Mud Bugs are likely removed from these populations. This leaves larger numbers of juveniles that bring down mean sizes of Mud Bugs in these regions. Mean sizes were similar for both sexes in all surveyed populations, although females tended to dominate the largest size classes for both species (see Appendix 14.3).

Table 1. Mean carapace length $(mm) \pm standard$ deviation measured for both Thenus species from all available sources.

| Source | Mud Bug | Reef Bug |
|------------------------------------|-----------------|-----------------|
| Jones (1988) | 43.5 ± 11.2 | 61.4 ± 12.5 |
| Courtney (1997) | 49 ± 8.4 | 68.6 ± 10.5 |
| LTMP Scallop Survey 1998–2021 | 55.2 ± 13.2 | 68 ± 13.1 |
| Townsville Thenus spp. Survey 2021 | 46.4 ± 9.3 | 65.5 ± 11.6 |



Figure 5. Size frequencies of Mud Bugs (top) and Reef Bugs (bottom) from Courtney (1997) mark-recapture project (left), the LTMP survey off Gladstone 1998–2021 (centre), and the 2021 Townsville survey (right). Dashed lines are the observed mean carapace lengths and red lines are the minimum legal sizes in carapace length (54 mm converted from 75 mm carapace width). See Appendix 14.3 for sex-specific size frequencies.

Sex ratios differed among data sources but generally revealed greater numbers of males than females (Table 2). Sex ratios were similar for both species in Jones (1988) and Courtney (1997), but Mud Bugs in the LTMP survey off Gladstone and Reef Bugs in the Townsville Survey were more skewed towards males. Mud Bug sex ratios (M:F) ranged from 1.1:1 to 2:1, while Reef Bug sex ratios ranged from 1:1 to 1.6:1.

Table 2. Sex ratios (*M*:*F*) recorded for both Thenus species from all extant data sources and the current work.

| Source | Mud Bug | Reef Bug |
|------------------------------------|---------|----------|
| Jones (1988) | 1.2:1 | 1.2:1 |
| Courtney (1997) | 1.1:1 | 1:1 |
| LTMP Scallop Survey 1998–2021 | 2:1 | 1.4:1 |
| Townsville Thenus spp. Survey 2021 | 1.2:1 | 1.6:1 |

Sex ratios in favour of males within similar ranges as described here have been reported for congeneric *Thenus* species over a large geographic area, ranging from 1.1:1 for *T. unimaculatus* in India (Radhakrishnan et al. 2013) to 2:1 for *T. orientalis* in the South China Sea (Shirota and Ratanachote 1977). The observed male sex bias in *Thenus* species is likely not attributable to sex-based differences in catchability caused by reproductive behaviours because male sex bias is observed even in immature size classes. In the LTMP survey, where large numbers of juveniles have been recorded for both species, sex ratios in immature young-of-the-year <40 mm CL were dominated by males in both Mud Bugs (N = 62, M:F = 1.7:1) and Reef Bugs (N = 768, M:F = 1.6:1).

In a review of sex bias in crustaceans, Ewers-Saucedo (2019) reported that local drivers of sex bias, e.g., competition for mates or resources, are unlikely to be expressed in species with broad-scale larval dispersal, to which *Thenus* species belong (Jeena et al. 2015, McMillan et al. In Review). Males are slower growing than females in both Mud Bugs and Reef Bugs (Courtney 1997). Therefore, a possible driver of male sex bias in Moreton Bay Bugs may be sex-based differences in growth, which can cause the slower growing sex to dominate smaller size classes, while the largest size classes are dominated by the faster growing sex (Ewers-Saucedo 2019), i.e., females in Moreton Bay Bugs (see Appendix 14.3).

In addition to biological information, raw data from Courtney (1997) and the LTMP survey off Gladstone informed movement and species distributions in the current study.

3.2 Movement of *Thenus* species from a mark-recapture study

Courtney (1997) used a large mark-recapture study of both *Thenus* species to investigate growth and mortality rates, and assess the effects of tagging on growth, moulting, and survival (see also: Courtney 2001). In the context of the present work, raw data from that study were interrogated to investigate: 1) information on catch composition useful for modelling species distributions; and 2) information on movement of *Thenus* species. The latter is particularly important, since an assumption of our species distribution modelling approach is that species proportions at sampled locations are not likely to change due to large-scale movements of either species that could alter species compositions.

3.2.1 Methods

The tagging methods used are outlined in detail in Courtney (1997). Here we focus on our analyses of movement in *Thenus* spp. using the raw data from that work. A feature of mark-

recapture studies based on recaptures made by commercial trawlers is that the distance of each trawl may confound the distance moved by an animal, since it is impossible to know where exactly along the trawl the animal was picked up by the trawl gear. Assessments of movement should therefore be interpreted with caution. In this dataset, a small number of very large movements between release and recapture points were observed that likely resulted from delayed discovery of tags and transport on vessels to new locations between recapture and reporting. For this reason, animals that moved more than the mean daily distance + 3 standard deviations were deemed likely invalid and excluded from analyses. This resulted in the omission of 5 out of 205 recaptured Mud Bugs and 12 out of 827 recaptured Reef Bugs.

3.2.2 Results

Mean daily distance moved (\pm SD) was 178.8 \pm 233.1 m for Mud Bugs and 146.7 \pm 245.6 m for Reef Bugs (Figure 6). Mean overall displacement was 2.9 \pm 5.6 nm for Mud Bugs and 3.5 \pm 4.7 nm for Reef Bugs. Both species displayed weak relationships between time at liberty and distance moved with R² = 0.19 for Mud Bugs and R² = 0.11 for Reef Bugs (Figure 7). Maximum observed displacements were 63 nm for Mud Bugs and 47 nm for Reef Bugs (Figure 7). Maximum time at liberty prior to recapture was 388 days (mean \pm SD: 53 \pm 62 days) for Mud Bugs and 517 days (mean \pm SD: 99 \pm 98 days) for Reef Bugs.

There were no discernible patterns of directional movement, instead movements appeared to be stochastic and possibly related to foraging behaviour. Neither species appeared to move from the broad habitat types they were originally caught and released in, i.e., inshore habitats for Mud Bugs and deeper areas for Reef Bugs (Figure 8).



Figure 6. Histograms of daily displacement for Mud Bugs (left) and Reef Bugs (right).



Figure 7. Distance between release and recapture locations as a function of time at liberty for Mud Bugs (N = 200) and Reef Bugs (N = 815). Linear trends with standard errors are fitted for both species.



Figure 8. Release locations and movement vectors selected for presence of both Thenus species tagged in 1993–1995 by Courtney (1997). Where no vector is visible, individuals were recaptured very close to the release location. Maximum time at liberty in days is given for each species.

3.2.3 Discussion and Conclusions

Analysis of the movement of *Thenus* spp. using the mark-recapture tag data from Courtney (1997) indicated that Moreton Bay Bugs do not move large distances, with most animals moving short distances of <3.5 nm. For reference, the distance trawled by most vessels in the ECOTF on a single trawl is >3.5 nm and the fishery reporting sites used by fishers are 6 x 6 nm. Moreton Bay Bugs therefore displaced on average less than the distance covered by a trawl shot. Similar small-scale movements have been reported in other lobsters. For example, 95% of tagged European Lobster (*Homarus gammarus*) moved <2.1 nm (Smith et al. 2001) and 85% of Southern Rock Lobster (*Jasus edwardsii*) moved <2.7 nm (Linnane et al. 2005).

Movements of Moreton Bay Bugs appeared stochastic rather than directional with weak relationships between distance moved and time at liberty. Importantly, neither species appeared to make large scale movements from habitats with which they have known habitat associations into habitats preferred by the other species. These movement patterns were similar to those reported by Jones (1988), who also found non-directional movement and concluded that *Thenus* do not undertake migrations. These findings provide confidence that the movements of Moreton Bay Bugs generally cover small distances with no broad species shifts between habitats. These limited movements are therefore conducive to species distribution modelling based on habitat preferences.

3.3 Species distributions from a long-term monitoring survey

Fishery-independent surveys are widely employed in fisheries management because they help monitor population parameters and ecological relationships that can be masked by fishing behaviours in fishery-dependent data. Fishery-independent surveys are expensive and logistically complex, but often benefit from the scientific design of the sampling process. Long-term fishery-independent surveys can help build time series that are useful in evaluating trends in fished populations over time without relying on commercial catch and effort records.

Fisheries Queensland has periodically run a fishery-independent survey of the Saucer Scallop fishery off Gladstone – Hervey Bay since 1997 (Dichmont et al. 2000), encompassing one of the two main Moreton Bay Bug fishing grounds. Useful data on bug populations has also been collected in this survey since 1998. The survey ran annually, with extensive sampling between 1997–2000 and 2017–2022. Surveys between 2001–2006 were limited to the Scallop Replenishment Areas (SRAs) and a single stratum (T30, Figure 9). No surveys were undertaken between 2007-2016. The survey occurs primarily in October each year, prior to

the opening of the Saucer Scallop season. Between 2001-2006, the survey was redesigned twice to account for changes in management over this period, including the implementation of a rotational harvest strategy in the SRAs and changes to the Great Barrier Reef Marine Park zoning in 2004. From 2017 onwards, the survey was extended to include strata off Fraser Island and the Sunshine Coast to capture possible range shifts in Saucer Scallops (Figure 10).



Figure 9. Design of the fishery-independent LTMP survey from 2017–2021. SRAs = Scallop Replenishment Areas permanently closed to fishing in 2017 (north to south: Yeppoon A & B, Bustard Head A & B, and Hervey Bay A & B).

Although information on bug species has been recorded incidentally since 1998, changes to the survey design, sampling effort and distribution, area closures (marine protected areas), and gear used (e.g., introduction of bycatch reduction devices and turtle excluder devices) during the first phase of the survey to 2006 result in more comparable data being available for the second phase of the survey from 2017 onwards when all these aspects were standardised. Here we investigate the data from the LTMP survey to assess Moreton Bay Bug population trends and species distributions.

3.3.1 Methods

The survey design is described in detail in Dichmont et al. (2000) and O'Neill et al. (2019). Since 2017, three vessels have taken part in the survey each year, each towing configurations of commercial otter trawl gear. Short transects ~1 nm in length were sampled at randomly distributed sites throughout a stratified survey area. Sampling effort is allocated among survey strata proportional to strata area and the distribution of commercial CPUE. Sampling stations are randomly distributed throughout the survey area each year (Figure 10).

To standardise the effects of fishing power (e.g., differences in gear or experience), a series of adjacent calibration trawls (up to 30) were undertaken by participating vessels at the beginning of each survey. A reference vessel that has taken part in all survey years is attributed a fishing power value of 1. Raw catches (count data) during calibration trawls were used as the response variable in generalised linear models (GLMs) for each year with vessel and site as explanatory variables and swept area (trawl distance x net swathe) as an offset using a Quasi-Poisson distribution. The reference vessel's calibration traves was then divided by the exponentiated coefficients of other vessels to derive calibration factors for those vessels. Calibration factors could then be used as offsets in subsequent models (which has the effect of multiplying raw catches by calibration factors) to model standardised catch rates of bugs per hectare.

Standardised catch rates were produced to account for differences in sampling strata, year, time of night, and lunar phase using a Quasi-Poisson GLM. Due to the patchy distribution of Mud Bugs limited to a small number of strata in the LTMP dataset (Fig. 11), catch rate modelling was conducted on Reef Bugs only, which dominated the dataset and were widely distributed among strata. The model used catch as the response variable (count data) with strata, year, time of night and lunar phase as explanatory variables. The natural logs of swept area and calibration factor were used as offsets. ANOVA tests were used to determine if

explanatory variables made significant contributions to the model. Predicted mean catch rates were calculated using mean values for explanatory variables. Modelling was performed in the R statistical environment (R Core Team, 2022). Distributions of *Thenus* species within the survey boundaries were modelled using the Kriging spatial analyst tool in ESRI ArcGIS.



Figure 10. Distribution of sampling effort by year in the LTMP survey from 2017–2021. SRAs = Scallop Replenishment Areas closed to fishing since 2017.

3.3.2 Results

Reef Bugs dominated the species composition of the LTMP survey with 18,449 Reef Bugs recorded compared to 275 Mud Bugs from 2017–2021. Kriged distributions of each species indicated Mud Bugs were limited to small pockets of the survey area where suitable habitat likely occurs, while Reef Bugs were much more widely distributed, though typically clustered (Figure 11). Reef Bug densities, when standardised for the effects of vessel, year, and lunar phase, were greatest in several Scallop Replenishment Areas (SRAs: Yeppoon B, Hervey Bay A, and Hervey Bay B) (Figure 12). Time of night had no effect on bug catches. Lunar phase, when standardised for vessel, year, and strata showed increased catchability of bugs around the full moon (Figure 13). Standardised catch rates followed a general increasing trend from 2017–2021 (Figure 14). A similar increasing trend, though with lower densities of bugs, was observed when only areas open to fishing were analysed by excluding the Scallop Replenishment Areas (i.e., fishing closures) that had some of the highest catch rates (Figure 15).



Figure 11. Kriged distributions of Thenus species densities from the LTMP survey off Gladstone 2017–2021. Left panel: Mud Bugs, right panel: Reef Bugs. SRAs = Scallop Replenishment Areas closed to fishing since 2017. Note density scales differ between species.



Figure 12. Reef Bug catch rates (bugs per hectare) in each stratum of the LTMP survey from 2017–2021 standardised for effects of year, lunar phase, and vessel. Strata are arranged left to right as they occur from north to south. Error bars = 95% CI. See Figure 10 for strata key.



Figure 13. Modelled effects of lunar phase on Reef Bug catch rates in the LTMP survey from 2017–2021 standardised for effects of strata, year, and vessel. Error band = 95% CI.



Figure 14. Reef Bug catch rates for each year from 2017–2021 in the LTMP Survey standardised for effects of strata, lunar phase, and vessel.



Figure 15. Reef Bug catch rates for each year from 2017–2021 in the LTMP Survey including only areas open to fishing (i.e., excluding Scallop Replenishment Areas), standardised for effects of strata, lunar phase, and vessel.

3.3.3 Discussion and Conclusions

The long-term monitoring survey off Gladstone was dominated by Reef Bugs, suggesting the habitat in this area is better suited to Reef Bugs than Mud Bugs. Mud Bugs were found in low densities in isolated patches, particularly in the 'Yeppoon A' SRA. Both species displayed clustered distributions (Figure 11). Benthic species often have clustered distributions that likely result from the distribution and availability of preferred habitat (Elliot 1977). Clustered distributions have previously been observed in Thenus species (Jones 2007) as well as other Scyllarid (Spanier and Lavalli 1998) and Palinurid lobsters (Goni et al. 2001, Butler 2003). Here we observed dense Reef Bug population clusters particularly in and around areas closed to fishing (SRAs). The locations of SRAs were selected due to the role these areas were believed to play in sustaining Saucer Scallop recruitment. It is unclear whether these dense clusters result from the provision of suitable habitat in these areas alone or whether the absence of fishing mortality in SRAs drives population increases within the SRAs and in adjacent areas. Saucer Scallops are a known preferred prey item for Thenus spp., which are proficient at opening bivalves (Jones 1988), but the low densities of scallops recorded in SRAs in recent years (Courtney et al. 2021) do not seem likely to support the large Thenus population clusters in and around SRAs.

Standardised catch rates of Reef Bugs showed a general increasing trend from 2017–2021. This was the case both when the entire survey area was analysed (Figure 14) and when only strata open to fishing were included in the analyses (Figure 15), though catch rates were consistently lower in areas open to fishing. Catch rates were highest in the Hervey Bay SRAs and adjacent strata as well as the 'Yeppoon B' SRA. The Maheno and Sunshine Coast strata yielded low catch rates, suggesting they may provide lower quality habitat for *Thenus* species.

It is unclear why catch rates increased from 2017–2021. The reduced fishing mortality in SRAs (permanently closed to fishing in 2017 following a previous regime of rotational closures) may play a role in increasing the biomass of bugs over time both within SRAs and in adjacent areas where some spill over may occur. Anecdotal evidence supplied by fishers suggests that bug catch rates near the boundaries of SRAs and other closed areas (e.g., 'green zones') are higher and attribute this to spill over of bugs from large, protected source populations. Estimates of *Thenus* biomass contained in 'closed' areas within the Great Barrier Reef Marine Park range from 45% for Mud Bugs to 54% for Reef Bugs (Pitcher et al. 2007).

Time of night had no effect on bug catch rates, which is consistent with previous observations that bugs can even be caught during the day when they are buried with just their eyes exposed (Jones 1988, Jones 2007). Lunar phase tended to influence catchability of bugs with greater catches around the full moon. Anecdotal advice from fishers suggests that the full moon is a preferred period to target Reef Bugs, although opinions vary on whether this is because catchability is greater then, or because prawn catchability is reduced in luminated conditions, so fishing effort is simply shifted to bugs.

Similar catch rate increases around the full moon have been observed in other lobsters, including Southern Rock Lobster (*Jasus edwardsii*) (Linnane et al. 2013, Feenstra et al. 2014) and Norwegian Lobster (*Nephrops norvegicus*) (Moller and Naylor 1980). Prescott (1988) reported spatial variation in effects of lunar phase on activity levels and catch rates in the Double-Spined Rock Lobster (*Panulirus penicillatus*), possibly resulting from local variability in predation risks. Decreased activity and catch rates of Blue Swimmer Crabs (*Portunus armatus*) when predation risks are elevated during periods of high luminance have also been linked to depth, with lunar effects greatest in shallow areas where light penetration is least attenuated (Johnston et al. 2021). Elevated Reef Bug catch rates around the full moon may therefore reflect low predation risks on the open sedimental substrates inhabited by Reef Bugs or sufficient depth (most Reef Bug records occurring >30 m) to attenuate light penetration and mitigate predation risks, while possibly gaining some foraging benefit leading to increased activity and catchability.
4 Crew Observer Program

4.1 Introduction

While fishery-independent surveys benefit from scientific design (e.g., randomised and stratified sampling), they are expensive and typically limited in the space and time they can cover. Fishery-dependent data derived from commercial fishing operations are therefore a valuable resource for fisheries management that may capture spatial or seasonal aspects of data beyond the scope of fishery-independent methods. Combinations of both fishery-independent and -dependent data sources can contribute to robust analyses incorporating the best of both worlds.

Fishery-dependent data are often limited to records of catch and effort. At times, scientific observers are accommodated on commercial fishing vessels, increasing the scope and quality of data that can be collected. However, scientific observers are expensive to deploy on commercial vessels, limited in the vessels they can operate on by vessel size and amenities, or may be viewed as a hindrance by crews accustomed to working in tight and sometimes dangerous conditions.

To overcome these limitations, Courtney et al. (2010) used a crew observer approach by asking commercial crews to take photographs and record location data of sea snakes caught incidentally during fishing operations. Because these tasks place little burden on crews, allowing them to remain focused on their primary tasks, and do not require the accommodation of scientific observers, uptake may be increased compared to observer-based studies. Here we employ a Crew Observer Program to record data on Moreton Bay Bug species distributions from commercial trawl catches in the ECOTF.

4.2 Methods

The Crew Observer Program was based on self-reporting of Moreton Bay Bug catches by fishers with data on trawl time and location as well as photographs of the catch, which were later inspected to identify bug species. Data collection kits were designed to be clear and easy to use to avoid imposing any onerous burden on crews during their normal fishing operations and thus promote their use. Kits included a digital camera to capture images of the bug catch, a wheelhouse data book for reporting data on gear, location, time, and target species of trawls, and a waterproof data book for the back deck to link bug photographs with the wheelhouse data (Figure 16). Fishers were asked to photograph bugs placed on their back so that species identification could be made using species-specific markings on the walking legs (Fig. 17). Kits also included information on species identification and the background and need for the project.



Figure 16. Contents of kits distributed to vessels participating in the Crew Observer Program. Kits included a wheelhouse data book for collecting information on location, gear, and time of trawls; a back deck book with dockets to be photographed with bug catches to link photographs with wheelhouse data; and a digital camera.

Kits were distributed to fishers in-person during numerous on site visits to trawler bases along Queensland's east coast or by post, though in-person visits were preferred. These visits were good opportunities to discuss the need for the project and project aims with fishers and build relationships, as well as answer any questions arising from fishers with regard to the project or what the information they provided would be used for. Where large catches were made and it was not feasible to photograph all bugs without severely disrupting fishing operations, fishers were asked to photograph a random subset of their catch (~20–30 bugs). Fishers were asked to take two photographs of each catch in case blurred images caused by movement at sea affected image quality. After deployment, kits were either collected in-person at the wharf or returned by post.



Figure 17. A typical photographic record of Moreton Bay Bug catch from the Crew Observer Program. Bugs are photographed on their backs to allow species identification using the markings on the walking legs. In this photograph, all animals are Mud Bugs except one Reef Bug (second from right in the middle row).

Only legal-sized bugs retained for sale were photgraphed because fishers generally took photographs for the Crew Observer Program after catches had been sorted, with undersized animals having been returned to the water during the sorting process. The amount of data provided (i.e., number of shots) was at the discretion of fishers. Data were entered into a master database and species identities were analysed at the EcoSciences Precinct in Dutton Park using distinguishing features on animals' walking legs.

4.3 Results

The Crew Observer Program ran from September 2020 to May 2022. Data and photographic records of Moreton Bay Bug catches were received for 1,038 sites from 31 vessels that took part in the program. Breakdown of sites by management region (see Figure 1) included: Northern Zone = 384 sites, Central Zone = 230 sites, Southern Inshore Zone = 250 sites, Southern Offshore Zone = 162 sites, and Moreton Bay = 12 sites. Coverage of sites with species distribution data along Queensland's east coast was extended to fill many gaps (Figure 18), though some areas remained data poor (e.g., off Cape York Peninsula and off Mackay).

Target species when bug catches were recorded included Tiger Prawns, Redspot King Prawns, Moreton Bay Bugs, Saucer Scallops, and Eastern King Prawns. The largest bug catches were caught while targeting bugs and Saucer Scallops. Mud Bugs dominated catches where Tiger Prawns were the target species, e.g., in Far North Queensland, Moreton Bay, and inshore areas off Townsville (Figure 18). Mud Bug catches were typically small relative to Reef Bug catches, though some large catches were recorded, particularly in the Princess Charlotte Bay area in Far North Queensland. Reef Bugs tended to dominate catches on the fishing grounds that contribute most Moreton Bay Bug landings off Townsville and Gladstone. Bug catches were typically characterised by the dominance of one species or the other (Figure 19).



Figure 18. Distribution of locations sampled by the Crew Observer Program (left) and corresponding landings of Moreton Bay Bugs (right), where shapes denote dominant Thenus species and colours denote target species recorded by fishers.



Figure 19. Species proportions observed in the Crew Observer Program. 0 = 100% Mud Bugs, 1 = 100% Reef Bugs. Most sampled locations were clearly dominated by one species.

4.4 Discussion

The largest bug catches recorded during the Crew Observer Program were made while targeting bugs, targeting Redspot King Prawns off Townsville, or targeting Saucer Scallops between Gladstone and Hervey Bay. The first kits were produced immediately after the project began and delivered in September and October 2020 to fishers that would be operating in the scallop fishery off Gladstone – Hervey Bay upon its opening in November of that year. However, little data on bug catches was recorded from this region during scallop fishing operations, likely because fishers were busy targeting scallops before the anticipated closure of the fishery. Scallop catches were small by historic standards, and vessels quickly dispersed to other areas. The scallop fishery was not reopened in 2021 and remains no-take in the Gladstone – Hervey Bay area due to stock depletion. Despite this, the small amount of data recorded during the 2020 scallop season (n = 6 shots) included large catches of bugs up to N = 560 (Figure 18) indicating that bugs were likely caught in large numbers in the scallop fishery previously when not targeted specifically. The retention of scallops is now only permitted south of Sandy Cape on Fraser Island, where bug catches are relatively small (Figure 18).

Overall, the Crew Observer Program had a good response from fishers. Some fishers felt that they had endured numerous impositions in recent years ranging from area closures to management restrictions, making it increasingly difficult for them to operate. In this environment it was important to build relationships with fishers in-person to understand their concerns. In addition, fishers were a valuable source of information about the fishery and species' behaviours and their advice was very helpful to the project team.

In summary, the Crew Observer Program was a valuable and cost-effective way to gather useful data over large areas that would not have been possible by fishery-independent means. Future employment of such programs can benefit from lessons learned during the present study. In planning and conducting such programs, consideration should be paid to: 1) building relationships with fishers via in-person visits, 2) streamlining reporting tools to minimise the burden placed on fishers in addition to their normal work, 3) issuing clear instructions and explaining equipment and data collection processes in-person with crews, and 4) informing fishers on the background, need, and aims of the work (i.e., clarifying how their efforts will contribute to project outcomes).

5 Fishery-independent survey of the Townsville bug fishery

5.1 Introduction

Access to suitable habitat is intrinsically linked to population dynamics in marine taxa (Hayes et al., 1996). While abundant fishery-independent data on *Thenus* populations was available from the region of the former scallop fishery off Gladstone (see Section 3.3), no such data was available for bug populations in the other main bug fishing grounds off Townsville. In addition, the data from the Gladstone region were heavily dominated by Reef Bugs, limiting insights into species habitat preferences. The Townsville region is more diverse in terms of habitat and species composition, comprising extensive mud and sand flats, interspersed with coral reefs, islands, and extensive reef flats that may influence the character of nearby sediments (Browne et al., 2010), providing a greater variety of habitats to survey for *Thenus* species distributions.

We designed and conducted a fishery-independent survey of *Thenus* species off Townsville to collect data on population parameters, species composition, and habitat preferences to assist in modelling species distributions.

5.2 Methods

The survey was designed according to the principles of stratified random sampling and is based on methods used to assess scallop abundance (O'Neill et al. 2019). The survey area was selected based on 30' x 30' logbook reporting grids off Townsville that contribute ~ 40% of annual bug landings in Queensland. These grids were used as the basis for the survey strata (Figure 20). To avoid unfished areas with high risk of gear damage and time wastage, the boundary of the survey area was informed by the trawl footprint from 2001–2020 derived from the TrackMapper vessel tracking repository (Good et al. 2007) and boundaries of protected areas in the Great Barrier Reef Marine Park. A 1 km buffer was applied at all survey boundaries to avoid sampling in protected areas.

Sampling effort was based on completing ten 1 nm trawl shots per night for 14 nights (weather and equipment permitting), yielding 140 stations in total. Stations were allocated to strata based on the product of each stratum's area (hectares) and catch per unit effort (CPUE: kilograms of bugs per hour of trawling from 2001–2020) as a proportion of the total for the entire survey area (Table 3). To account for effort with zero bug catches, we considered effort from all records containing the main trawl target species in the survey area, i.e., bugs as well as Tiger-, Redspot King-, Endeavour-, Eastern King-, and Banana Prawns. A minimum of at least three stations (2% of sampling effort: Dichmont et al. 2000) was applied to strata that did not attain this threshold to avoid under

sampling. The allocated number of stations were then randomly distributed throughout each stratum using the ArcMap (ESRI, 2020) random points tool with at least 1 nm separating stations (equivalent to the length of each trawl).



Figure 20. Map of the stratified random survey off Townsville in July 2021. Sediment profiles and trawl transects were sampled at 130 stations and environmental DNA samples taken at 44 stations. *A 1 km buffer was applied at strata boundaries to avoid protected areas.*

Table 3. Calculations from which station allocations to survey strata were derived. Stations were allocated to strata based on the product of each stratum's area and Moreton Bay Bug CPUE as a proportion of the total for the surveyed area. Three stations (indicated in brackets) were allocated to strata where a threshold of 2% of total stations was not met, to avoid under sampling.

| Strata | Area (Ha) | CPUE (kg/h) | Area*CPUE | Stations |
|--------|-----------|-------------|------------|----------|
| I20 | 62,254 | 0.58 | 36,151 | 0 (3) |
| J2W | 57,809 | 1.44 | 83,135 | 1 (3) |
| J21 | 96,539 | 0.89 | 86,134 | 1 (3) |
| I19 | 161,190 | 2.13 | 344,130 | 5 |
| L20 | 52,536 | 8.74 | 459,384 | 6 |
| J19 | 131,050 | 4.34 | 569,041 | 8 |
| L22 | 157,455 | 3.72 | 585,295 | 8 |
| J2E | 125,084 | 4.97 | 622,230 | 9 |
| M22 | 271,004 | 2.85 | 772,879 | 11 |
| M21 | 153,567 | 5.86 | 900,205 | 12 |
| K21 | 222,572 | 5.11 | 1,138,268 | 16 |
| L21 | 259,088 | 8.50 | 2,202,942 | 30 |
| K20 | 298,462 | 7.94 | 2,369,345 | 33 |
| Total | 2,048,611 | | 10,169,139 | 140 |

The survey was conducted on a tendered commercial vessel, the *SS Murchison*, and timed during the period of the full moon. Generalised linear modelling of fishery-independent bug catches from the LTMP survey off Gladstone (Figure 14) and advice from fishers agreed that this lunar phase represents the period of peak catchability for bugs. This may be due to the predatory behaviour of bugs exploiting periods of elevated luminance to visually hunt prey. Sampling was conducted between 5:00 pm and 7:30 am each night in accordance with commercial trawling regulations. Four identical 2.25-inch mesh nets with 5 m head ropes were deployed with otter board apparatuses and stabilizers. Nets were equipped with turtle excluder and bycatch reduction devices.

At each survey station, a sediment sample was collected using a sediment grab (Figure 21), and environmental conditions were recorded using a conductivity, temperature, and depth (CTD) probe. A GoPro camera and light were mounted to the CTD probe to visually confirm homogeneity of the seafloor. Environmental DNA (eDNA) samples were collected from the sediment at 44 sites (Figure 20). Although eDNA samples will not provide information on bug species densities, they may provide a further source of presence/absence information for each species in each area (see Appendix 11.5). Once these tasks were completed, a 1 nm trawl transect intersecting the station was undertaken. This method was devised so that sediment samples could be taken first while sediment was undisturbed by trawl gear. For sediment analyses, sediment samples were labelled and stored in plastic sealed bags. For eDNA trials, subsamples of wet sediment 3 g in weight were stored frozen in sterilised packaging for later analysis in the laboratory. The survey ran broadly to plan with 130 stations sampled over 14 nights due to minor delays caused by shipping, gear issues etc.



Figure 21. Samples of sediment and eDNA were collected with a sediment grab at each site, as well as recordings of depth, temperature and salinity readings taken using a CTD probe before sites were trawled so that sediment was undisturbed by trawl gear prior to sampling.

After each trawl, data were collected on all Moreton Bay Bugs caught, including species, sex, and carapace length. Species was determined by observing the markings on the walking legs of individuals to identify the characteristic 'speckled' markings of Reef Bugs or longitudinal stripes of Mud Bugs (DAF 2022). Sex was determined by the presence/absence of female gonopores at the base of the third set of walking legs (Figure 22). Carapace length was measured using callipers in millimetres.



Figure 22. Female Reef Bug (Thenus australiensis), as indicated by the gonopores at the base of the third walking legs (white arrow) and pigmented 'speckled' markings on forelimbs.

After completion of the survey, sediments were processed in a laboratory to determine grainsize and carbonate content. To measure grain size, samples were prepared with 5% Calgon (Sodium Hexa-Metaphosphate) solution to disaggregate mud particles, then wet sieved through a 63 μ m sieve to isolate the mud fraction of each sample. The mud fraction was diluted, mixed, and three subsamples of 100 ml were removed and dried in an oven at 80 °C until completely dry. The dry weight was used to determine the total mud weight of each sample after applying a Calgon correction and volume correction factor. The remaining coarse fraction (>63 μ m) was dried at 80 °C and then dry-sieved through 63, 125, 250, 500, 1000, 2000, 4000, and 8000 μ m sieves shaken for ten minutes at 60 amps. The weight of sediment in each sieve was recorded and used to determine the proportion of each sample that was gravel (2000–8000 μ m sieves) and sand (63–1000 μ m sieves). The sediment remaining in the pan under the 63 μ m sieve was weighed and added to the total mud weight of the sample.

Calcium carbonate content was determined by acid digestion. Approximately 5–10 g of each original sediment sample was removed and dried in an oven at 80 °C. Dry weight was recorded, and

the sediment was treated with 10% hydrochloric acid solution each day until no reaction occurred. Then samples were rinsed with distilled water and dried again. Dry weight was recorded and subtracted from the dry weight before the acid was applied to determine the percentage of calcium carbonate in each sample. Weights of each sediment grain size class for each sample were analyzed using the 'G2Sd' package in the R statistical environment (R Core Team, 2022). The output provided arithmetic and geometric mean grain size, the distribution of grain size, standard deviations, skewness, kurtosis, the Trask sorting coefficient (a measure of sediment grain size sorting), sediment type descriptions, texture, and other parameters that describe each sediment sample.

Maps of observed species distributions were produced in ESRI ArcGIS (version 10.8.1). Species abundance data were used in association with other variables to assess differences in the distribution of each species. Depth and mean grain size were assessed based on Jones' (1988, 2007) reports suggesting that these parameters are the primary drivers of habitat partitioning of *Thenus* species. A two-factor ANOVA and a frequency analysis were used to test differences in abundance associated with mean grain size between species and depth. Assumptions of normality and homogeneity of variance were checked by Levene's tests and residual plots. The abundance data were square root transformed to better meet the assumptions of ANOVA. Frequency analyses were also used to evaluate differences between the species' distributions across mean grain size by Wentworth classifications, calcium carbonate content, skewness, kurtosis, and Trask sorting coefficient. The assumptions of Pearson's chi square tests were met as the data assessed were count data for each species and the categories used for assessment were mutually exclusive. For sediment kurtosis, there were 12 extreme outliers out of 130 sites, making it impossible to categorize the variable in a continuous manner without masking information in most of the species' distributions, so those outliers were omitted from analyses involving kurtosis of sediment grain size distributions.

Canonical Analyses of Principle coordinates (CAP, Anderson and Willis 2003) were performed via the 'BiodiversityR' package (Kindt and Coe 2005) in R and included distance-based redundancy analyses as designed by Legendre and Anderson (1999). Calcium carbonate content was not included in this analysis due to an unequal number of observations. Seven samples from greater depths (i.e., >50 m) taken during periods of intense wave action did not produce enough sample volume to undergo acid digestion.

5.3 Results

A total of 1,215 individuals were sampled, comprising 792 Reef Bugs and 423 Mud Bugs. Reef Bugs were found exclusively at 74 sites, typically offshore, while Mud Bugs were found exclusively at 19 sites, typically inshore (Figure 23). Although there was some spatial overlap between species, usually one species dominated catches at each station (Figure 23). Neither species were found at five sites. Observed size frequencies indicated that Mud Bugs (mean \pm SD: 46.4 \pm 9.3 mm CL) were substantially smaller on average than Reef Bugs (65.5 \pm 11.6 mm CL) in the Townsville region (Figure 7, Table 4).

Biological findings confirmed some expected trends, including the preference of Mud Bugs for shallower depths and finer sediments (Table 4, Figures 24 & 25). The greatest bug densities were found in such shallow areas and dominated by small Mud Bugs (Figure 24). *Thenus* species were found to prefer different depths (ANOVA; $F_{1,43} = 4.965$, p <0.01). Up to 27 m, Mud Bugs were the more abundant species, while in areas >27 m the dominant species shifted to Reef Bugs (Figure 24). Reef Bugs attained greater average sizes and were more widely distributed throughout the survey area, despite attaining lower maximum densities.

In the Townsville area, the greatest frequencies of both species were distributed at sites with mean grain sizes in the coarse sand classification (Figure 25). However, Mud Bugs were also distributed across sites with significantly smaller mean grain sizes compared to Reef Bugs (X^{2}_{7} (N = 1215) = 83.298, p <0.01). Mud Bugs were recorded at sites with mean grain sizes ranging from mud to very coarse sand according to Wentworth classifications (Wentworth 1922). Reef Bugs were observed at sites with medium fine sand to very fine gravel (Figure 25).

Bugs were observed at sites with sediment skewness between 1.54–22.37 μ m (Figure 26A). Sediment skewness describes how the distribution of grain size fractions is skewed around the mean grain size of each sediment sample. Both species preferred sediments characterised by skewness values between 1.75–5.25 μ m. Reef Bugs occurred at greater densities at sites with lower skewness values compared to Mud Bugs (Pearson's chi square: X²₈ (N = 1215) = 36.217, p <0.01).



Figure 23. Graduated symbols of counts and species composition at sites where Moreton Bay Bugs were recorded during the fishery-independent survey off Townsville.

Table 4. Summary statistics of Moreton Bay Bugs sampled during the Townsville bug survey.

| | Mud Bugs | Reef Bugs |
|---------------------------------|-----------------------|----------------------------|
| Total sampled | 425 | 793 |
| Sex ratio M:F | 1.2:1 | 1.6:1 |
| Mean carapace length ± SD | $46.4\pm9.5\ mm$ | $65.5 \pm 11.8 \text{ mm}$ |
| Carapace length range | 23 – 85 mm | 24 – 95 mm |
| Sites where present | 51/130 | 105/130 |
| Mean density where present ± SD | 1.7 ± 2.3 bugs/ha | 1.6 ± 1.2 bugs/ha |
| Max density | 9.3 bugs/ha | 6.3 bugs/ha |
| Mean depth where present | 26 m | 39 m |
| Depth range | 14 - 54 m | 15 – 59 m |



Figure 24. Densities (bugs per hectare) of Thenus species surveyed off Townsville as a function of *depth.*



Figure 25. Frequency distributions of Thenus *species surveyed off Townsville relative to sediment grain size classes.*

Sediment sorting was found to differ between the habitats preferred by each species, with a significant difference in species distributions across Trask sorting coefficients when sorted into bins of $1.75 \oslash (X^{2} (N=1215) = 279.07, p < 0.01)$. Sediment sorting is how similar the grain size of particles in a sample are. Bugs inhabited sites with Trask sorting coefficient values between $1.294 - 8.554 \oslash$. Greatest frequencies of Reef Bugs were observed at sites with Trask sorting values between $1 - 2 \oslash$ (well sorted), while greatest frequencies of Mud Bugs were observed at sites between $3.5 - 5.25 \oslash$ (normal to poorly sorted) (Figure 26B).

Bugs were recorded at sites with sediment kurtosis values between 4.12–736 μ m. Kurtosis of sediment describes how peaked the sediment grain size distribution curve of each sample is. Greatest frequencies of both species occurred at sites with kurtosis values between 11–22 μ m (Figure 26C). These sediment samples generally had meso-platykurtic grain size distributions, meaning the grain size distribution curves of these samples were between a normal peak and a flat curve. When evaluated in bins of 11 μ m, the difference in distributions across kurtosis values was not as visually clear as was seen in the other variables assessed (Figure 26C). However, the difference in distribution between the species was statistically significant (X ²₈ (N= 1215) = 29.3, p <0.01).

Both species were observed throughout the same range of sediment calcium carbonate values (0.95–95.0%), however Mud Bugs were found at greater frequencies at sites with lower CaCO₃ content

compared to Reef Bugs (Figure 26D). This difference was significant when percentages were distributed in bins of 5% (X $^{2}_{18}$ (N= 1215) = 275.9, p <0.01).



Figure 26. Frequency distributions of Thenus species abundance as functions of sediment properties including skewness of grain size distributions (A), Trask sorting coefficient (B), kurtosis of grain size distributions (C), and percent calcium carbonate (D).

A multivariate Canonical Analysis of Principal coordinates analysis of *Thenus* species distributions correctly classified 90% of all individuals using sediment parameters and depth as predictor variables. The CAP model correctly classified 88.7% of mud bugs sampled and 90.7% of Reef

Bugs. There was visible clustering of each species, with limited overlap (Figure 27). Depth, sediment sorting, and sediment mean grain size explained the greatest variance between species, while sediment kurtosis had limited influence.



Figure 27. Canonical Analysis of Principal coordinates (CAP) for Thenus species distributions showing influence of habitat variables assessed during the Townsville survey. Lengths of vectors indicate their relative influence as predictors of species' distributions. Ellipses indicate 95% confidence range around group centroids.

5.4 Discussion

Thenus species in the Townsville region displayed habitat partitioning similar to that observed in the LTMP survey off Gladstone, with some patches of species overlap. Mud Bugs were more prevalent off Townsville than off Gladstone, suggesting greater availability of habitats preferred by this species. However, Reef Bugs were still most prevalent overall. Depth and sediment sorting were the main drivers of species distributions observed in this study, with Mud Bugs inhabiting shallower habitats with poorly sorted sediment compared to Reef Bugs, which inhabited deeper habitats with more well sorted sediments. Co-location with other taxa was observed but not quantified, particularly between Mud Bugs and Tiger Prawns, suggesting ecological relationships between these taxa may be useful predictors of species distributions. Ecological clustering has previously been reported between Mud Bugs and Grooved Tiger Prawns (*Penaeus semisulcatus*) in the Great Barrier Reef region (Pitcher et al. 2007).

Greatest densities of Mud Bugs were found in shallow, inshore areas. These were heavily fished areas where adults of legal size were mostly absent, suggesting high post-release survival of juveniles and/or strong recruitment in these areas. The timing of the survey in July – August followed sustained fishing for Tiger Prawns in the inshore portions of the survey area since the beginning of the trawl season in March. This probably explains the relative lack of legal-size Mud Bugs by the time of the survey late in the season.

A surprising result was the strong influence of sediment sorting (Trask coefficient) on species distributions, with Mud Bugs preferring more poorly sorted sediments compared to Reef Bugs. Sediment sorting is typically poorer in relatively shallow areas where periodic disturbance of the seafloor occurs due to the action of high energy events like storms. By contrast, beaches that are exposed to more constant wave or wind energy are typically composed of well sorted sediments. The sediments in areas preferred by Reef Bugs are likely too deep to be influenced by high energy events; their well sorted character may derive from ancient processes. Sediment sorting has previously been found to influence species distributions in juvenile horseshoe crabs (*Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*), which are similar to *Thenus* in size and burying behaviour (Chen et al., 2015).

Both species were most frequently observed in coarse sands off Townsville, in contrast to previous findings where Mud Bugs clearly preferred finer sediments (Jones 1988). It is unclear whether this phenomenon is unique to the Townsville area. Despite this habitat overlap, finer sediments comprising fine sand to mud sized particles were inhabited exclusively by Mud Bugs. A similar preference of Mud Bugs for fine to medium-fine sands (grain size 63–500 μ m) has been previously reported for the Townsville area (Jones 1993). In that study, there was no significant relationship between Mud Bug abundance and muddy sediments (<63 μ m), although laboratory sediment selection trials indicated some preference for mud (Jones 1988). In this study, mud was not a preferred habitat in the wild, with Mud Bugs instead preferring fine to medium-fine sand (63–500 μ m) as reported in Jones (1993). Reef Bug abundance off Townsville was mostly associated with medium-fine to coarse sand (250–2,000 μ m). Similar findings were reported by Jones (1993), with Reef Bugs preferring medium-fine sand 250–500 μ m (the coarsest sediment class surveyed in that study). The coarsest sediment type inhabited in this study was gravel (2,000–4,000 μ m), where Reef Bugs were exclusively encountered.

6 Predictions of sediment parameters for the Great Barrier Reef

6.1 Introduction

Benthic habitats play key roles in determining the distributions of benthic fauna, which often display strong preferences for certain types of sediment or other seafloor properties (Gray 1974, Auster and Langton 1999, Kostylev et al. 2001). Relationships between benthic animals and their habitats may be driven by factors like the provision of substrates suitable for the burrowing or attachment capabilities (i.e., morphology) of the species in question or that support prey species. Mapping benthic habitats is therefore central to understanding the distributions of benthic species. However, data on benthic habitats are often sparse, typically being limited to sampling of spatially disparate stations and often derived from various sources. To map benthic habitats at large spatial scales it may therefore be necessary to model them by interpolating the likely distribution of seafloor properties between sampling stations. Machine learning techniques provide the computational power to model benthic habitats using complex suites of predictor variables likely to influence seafloor properties over large areas.

Li and Heap (2008) and Li et al. (2011a) classify spatial interpolation methods into five categories: 1) non-geostatistical spatial interpolation methods; 2) deterministic or non-geostatistical methods; 3) stochastic or geostatistical methods; 4) machine learning methods; and 5) 'hybrid' combinations of these methods. Previous studies have considered a wide range of interpolation methods to predict sediment distributions in the marine environment (Li and Heap 2008, Li et al. 2011a, Li et al. 2011b). Li et al. (2011a), in a review of interpolation methods applied to sediment sample data, concluded that machine learning methods generally outperform other spatial interpolation techniques. Li et al. (2011b) concluded that machine learning methods may also introduce artefacts into the interpolated datasets; as a result, visual inspection of resulting datasets is required to ensure they are fit for purpose. An extensive sediment sample dataset exists for the Great Barrier Reef region (Mathews et al. 2007). These sediment samples are publicly available via the Geoscience Australia MARine Sediment database (MARS,

http://dbforms.ga.gov.au/pls/www/npm.mars.search). As extensive as this database is, it is often possible to source additional sediment sample data from theses, research, and scientific reports (Courtney et al. 2021) and improvement to sediment predictions can likely be made by targeted collection of additional datasets in data poor areas.

Here we compile an extensive array of seafloor sample data from various extant and newly sampled sources and use machine learning to model benthic habitats within the range likely to be inhabited by *Thenus* species on Queensland's east coast. The model outputs are then rasterised, producing data layers for seafloor properties that can be interrogated to provide spatially resolved values for predictor variables (e.g., sediment properties) at locations coinciding with biological sampling of *Thenus* species to model their distributions based on each species' habitat preferences.

This section of the report aims to:

- 1) Compile an extensive set of sediment sample analyses for southeast Queensland that extends the previous work of Mathews et al. (2007).
- Compare the performance of two interpolation techniques: 1) a simple Inverse Distance Weighted (IDW) methods, and 2) the Random Forest machine learning technique.
- 3) Develop a series of optimally interpolated maps of sediment grain size statistics for the Great Barrier Reef.

6.2 Methods

6.2.1 Sediment modelling

The focus area of this study is the Great Barrier Reef; however, data outputs extend from the Torres Strait (10°S) to northern New South Wales (-29°S) to inform the extent of *Thenus* species distribution modelling. For the purpose of spatial predictions, this area was split into four smaller domains. Smaller domains were used to reduce computational load during the sediment modelling. The data from these domains would then be mosaicked into a suite of sediment grain size parameter datasets. The domains were Far North Queensland, Townsville Region, Central Region, and Southeast Queensland (Table 5, Figure 28).

Table 5. Spatial domains used for sediment prediction (see Figure 28).

| Domain | North | South | West | East | |
|-----------------------|-------|-------|------|------|--|
| Far North Queensland | -10 | -17 | 142 | 147 | |
| Townsville Region | -16 | -23 | 144 | 149 | |
| Central Region | -18 | -24 | 148 | 154 | |
| Southeast Queensland | -23 | -29 | 150 | 156 | |



Figure 28. The spatial extent of sediment modelling on Queensland's east coast and adjacent areas. Coloured boxes represent sediment modelling sub-domains that were mosaicked to produce the final modelled sediment predictions. The purple domain represents the spatial extent of Thenus species distribution modelling in the 5–80 m depth range.

Sediment grain size is commonly calculated via sieving but can also be quantified by laser diffraction and a range of other methods (Syvitski 1991). Sediment samples are commonly reported as their relative proportions of gravel, sand, and mud. Gravel is sediment with a grain size > 2000 μ m, sand is between > 63–2000 μ m, and mud is \leq 63 μ m (Wentworth 1922). Calcium carbonate (CaCO₃) is reported as the total percentage relative to other mineral grains and is commonly measured through acid dissolution (Siesser and Rogers 1971). Higher resolution sediment grain size analysis can be undertaken but is reported less often. These higher resolution sediment analyses provide a more detailed distribution of sediment grain size and can provide additional statistics on grain size characteristics.

Statistics that were used in this study include:

- 1) % Gravel
- 2) % Sand
- 3) % Mud
- 4) % Carbonate
- 5) Mean grain size (Phi scale)*
- 6) Standard deviation of grain size*
- 7) % Very fine sand (63–125 μm) *
- 8) % Fine sand (125–250 μm) *
- 9) % Medium sand (250–500 μm) *
- 10) % Coarse sand (500–1,000 µm) *
- 11)% Very coarse sand (1,000–2,000 µm) *
- 12) Trask sorting coefficient*

*Indicates statistics derived from high resolution sediment analysis

The sediments of the Great Barrier Reef (GBR) have been surveyed sporadically since 1964. Volumetrically, most of the sediment grain size data that has been published is available through the MARS database (Mathews 2007). Additional sources of sediment grain size data that were used in this study are detailed in Table 6 and summarised in Figure 29 (for detailed information on sample availability for individual sediment parameters see supplementary materials in Appendix 11.4). Samples from outside of the MARS databases added approximately 2000 more samples than previously available. The GBR banks dataset (Harris et al. 2013) provided locations for >1000 submerged banks that are assumed to represent carbonate dominated environments. The geographical centres of these banks were assigned a value of 100% carbonate. **Table 6**. Sources of sediment sample data used in this study. M/G/S denotes samples containing data on sediment mud, gravel, and sand composition. Samples marked * have selected statistics. Samples marked ** are mud only.

| Survey | M/G/S | CaCO ₃ | High-Res | Source |
|------------------------------|-------|-------------------|----------|----------------------------|
| Various | 4725 | 3459 | 4793 | MARS: Matthews (2007) |
| FRDC Project 2017-048 | 166 | 165 | 166 | Courtney et al. (2021) |
| Hervey Bay Coast | 721** | 0 | 742* | Stephens et al. (1988) |
| Hervey Bay and its Estuaries | 47 | 0 | 47* | Ribbe (2014) |
| Southern Great Barrier Reef | 158 | 310 | 184* | Maxwell and Maiklem (1964) |
| Great Sand Strait | 85 | 0 | 89* | Tarabbia (1990) |
| GBR Banks | 0 | 1605 | 0 | Harris et al. (2013) |
| Burdekin Region | 215 | 215 | 215 | Belperio (1978) |
| FRDC Project 2020-020 | 132 | 132 | 132 | This report |
| Bowen/Princess Charlotte Bay | 314 | 307 | 268* | Frankel (PhD) |
| Hinchinbrook and Halifax | 131 | 131 | 131 | Orpin (PhD) |
| Bay | | | | |
| Northern GBR Reefs | 67 | 67 | 0 | Flood Orme Scoffin (1978) |
| Total | 6761 | 5851 | 6767 | |

To improve performance of spatial interpolation, covariates were included in predictive models. Covariates are secondary information sources that correlate with the predictor variables (i.e., sediment grain size parameter) that help improve predictions. Covariates were derived from a regional bathymetry compilation (Beaman 2010), maps of 'geomorphic banks' (Harris et al. 2013), and outputs from the Great Barrier Reef eReefs hydrodynamic model (Herzfeld et al. 2016).

An important covariate in the spatial interpolation of seabed sediments is bathymetry (Verfaillie et al. 2006, Li et al. 2011a). Bathymetry data were sourced from Beaman (2010) at 100-metre resolution. The bathymetry dataset is a compilation of multiple individual datasets, acquired from ship-based multibeam and single beam echosounder surveys, airborne LiDAR bathymetry surveys and satellite data. The high-resolution bathymetry dataset has been interpolated to fill in missing data points to provide 100% data coverage over the study area. Eleven derivates of bathymetry were used in sediment modelling:

- Bathymetry (m)
- Aspect (degrees)
- Slope (degrees)
- Standard Deviation of bathymetry within a 3x3 kernel at 100m resolution
- Standard Deviation of bathymetry within a 3x3 kernel at 1000m resolution
- Topographic position index within a 3x3 kernel at 100m resolution

- Topographic position index within a 3x3 kernel at 1000m resolution
- Latitude (degrees)
- Longitude (degrees)
- Distance from the coast (kilometres)
- Distance from the continental shelf (using 200m contour)



Figure 29. Origin of sediment samples for this study. MARS = samples derived from the Geoscience Australia MARine Sediment database. New = samples collected by project staff or compiled from extant data sources including theses and research reports outlined in Table 6. Coloured boxes represent sediment modelling sub-domains (see Figure 28).

Banks represented the presence and absence of geomorphic bank features on the seabed. Geomorphic bank features are features raised more than 15 metres above the surrounding seabed with at least one steep slope greater than approximately 2 degrees. Banks were derived from the 100-metre resolution bathymetry data produced by Beaman (2010) using the method described by Harris et al. (2013). Derivatives from the geomorphic banks dataset were:

- A binary 'bank' or 'not-bank' raster
- Distance from banks (kilometres)

eReefs is a GBR-focused marine modelling and software system that generates models of catchment, hydrodynamic, sediment, wave, optical, and biogeochemical processes (Herzfeld et al. 2016). The marine models have a vertically layered water column for the assessment of biological, biochemical, and oceanographic processes. One year of data from 2020 was used to derive the following variables:

- Average significant wave height (m)
- Average significant wave direction (degrees)
- Average significant wave stress
- Maximum current velocity (m/s)
- Maximum current direction (degrees)
- Mean current velocity (m/s)
- Mean current direction (degrees)
- Average current velocity (m/s)
- Average current direction (degrees)
- Major axis of the current ellipse
- Minor axis of the current ellipse
- Eccentricity of the current ellipse
- Orientation of the current ellipse (degrees)

6.2.2 Interpolation methods

Spatial predictions with the machine learning 'Random Forest' method were compared with the simple inverse distance weighted method of interpolation. The two methods were used to further assess the level of improvement that can be achieved by using machine learning methods. The R package 'SPM' (Spatial Predictive Modelling) was used for spatial predictions (Li 2019a, 2019b).

Inverse Distance Weighting

The Inverse Distance Weighting (IDW) method is a deterministic or non-geostatistical spatial interpolation method (Li et al. 2011). IDW relies on the assumption that an unsampled point within a given neighbourhood has an attribute value equal to the weighted average of known sampled

values within the neighbourhood. The distances between the unsampled point and each sampled point within the neighbourhood is inversely related to the weights for each of the sampled points. This assumes that the values of sampled points close to the unsampled point are more alike than points further away (Lu and Weng 2007). There were two parameters which could be adjusted in the IDW method, the number of points used in the prediction (nmax) and the distance power (weighting) parameter (idp).

As the distance from prediction and the value of the power parameter increases, the weight of each sampled point decreases, giving samples near the prediction a higher weight and a greater influence on the final estimation. The cross-validation function in 'spm' (i.e., idwcv) was used to determine the optimal parameters for IDW, comparing the weights ranging from 1.4 to 3 at increments of 0.2 for idp and the number of samples ranging from 4 to 16 at increments of 2 for nmax.

Random Forest modelling

Random Forest (RF) is a machine learning method based on an ensemble of decision trees (Breiman 2001, Kingsford and Salzberg 2008). Decision trees are formed from a set of sampled points with known attribute values which are analysed and classified based on a set of predictor variables to generate a predicted attribute value for an unsampled point. At each node a decision tree will split the samples into groups that are the most different, or form subgroups with samples that are the least different (Kingsford and Salzberg 2008). RF can produce accurate predictions by taking the predicted attribute values from the ensemble of trees and assigning the most popular predicted attribute value to the unsampled point.

The values used by each decision tree in a RF model are sampled independently from a training set of known sample values and all trees within the ensemble have the same distribution. Advantages of RF are the improvement in classification accuracy through the growth of multiple trees, reduced chance of model overfitting due to random subsampling of the dataset to build each tree, and insensitivity to outliers (Breiman 2001). There were two parameters that could be adjusted in the RF method, the number of trees used in the prediction (ntree) and the number of variables tried at each node (mtry). The number of trees has an influence on the predictive accuracy of the model, while the number of variables tried at each node also influences the predictive accuracy but at the cost of diminishing diversity in individual trees. The cross-validation function in 'spm' (i.e., rfcv) was used to determine the optimal parameters for all RF models, comparing the number of trees ranging from 500 to 5000 at increments of 500 for ntree and the number of variables ranging from 3 to 9 at increments of 1 for mtry. The importance of a predictor variable gives an indication of how much the variable contributes to the predictive accuracy of the model. The importance of predictor variables for RF was based on average variable importance (AVI). A function in 'spm', AVI, was used and iterated 100 times to stabilise the variable importance generated by the Random Forest algorithm. The values for mtry and ntree were set to the default values (a function of the number of remaining predictor variables to use as the mtry parameter and a value of 500 was used for the ntree parameter, Li 2019b).

The performance of the IDW and RF models developed was assessed through ten-fold cross validation. In ten-fold cross validation the sediment sample dataset is randomly divided into ten approximately equally sized data subsets. One of these subsets is retained to validate the predictions produced by the model given a dataset containing the remaining nine subsets. The process is replicated for each of the ten subsets until each subset has been used as a validation dataset, producing ten prediction datasets (Kohavi 1995). Based on the findings of previous studies, the tenfold cross validation process was repeated for 100 iterations (Li 2013b, Li et al. 2014, 2019). The error produced by these predictions was identified to select the optimum model. The error was given as the variance explained by cross validation (VEcv). Categories of model accuracy in terms or VEcv are given in Table 7.

| VEcv range | Model accuracy | |
|------------------------|----------------|--|
| $VEcv \le 10\%$ | Very poor | |
| $10\% < VEcv \le 30\%$ | Poor | |
| $30\% < VEcv \le 50\%$ | Moderate | |
| $50\% < VEcv \le 80\%$ | Good | |
| 80% < VEcv | Excellent | |

Table 7. Classification of the accuracy of predictive models in terms of VEcv.

After model validation, the best ranked modelled outputs of sediment parameters (IDW or Random Forest) were used to produce rasters for use as predictor variables in species distribution models. Rasters were clipped to the spatial extent of the ECOTF where Moreton Bay bugs are primarily distributed (i.e., in the 5–80 m depth range) using ESRI ArcGIS.

6.3 Results

All modelled sediment parameters achieved acceptable cross validation scores (VEcv ratings of moderate to excellent) except Trask sorting coefficient, which achieved a poor rating and was therefore not used in species distribution modelling (Table 8). Random Forest models outperformed IDW models in predicting distributions of all modelled sediment parameters at the scale of the entire Great Barrier Reef (Table 8). At the scale of regional domains (Figure 28), the only case

where IDW outperformed Random Forest models were gravel predictions from the Far North Queensland and Central regions, however the average VEcv for all four domains combined still favoured Random Forest as the superior prediction method. This observation is largely consistent with the results of Li et al. (2011a, 2011b).

Table 8. Comparison of classification accuracy (Variance Explained by cross validation) of modelled sediment parameters between Inverse Distance Weighting (IDW) and Random Forest (RF) techniques. VEcv ratings are described in Table 7.

| Sediment parameter | IDW | RF | VEcv rating |
|----------------------|------|------|-------------|
| Mud content | 58.0 | 63.5 | Good |
| Sand content | 48.3 | 52.7 | Good |
| Gravel content | 31.1 | 31.9 | Moderate |
| CaCO ₃ | 78.6 | 85.9 | Excellent |
| Mean grain size | 53.5 | 57.4 | Good |
| Very fine sand | 33.9 | 38.6 | Moderate |
| Fine sand | 30.0 | 34.2 | Moderate |
| Medium sand | 30.2 | 33.6 | Moderate |
| Coarse sand | 37.5 | 40.9 | Moderate |
| Very coarse sand | 29.3 | 32.2 | Moderate |
| Grain size St. Dev. | 34.0 | 37.3 | Moderate |
| Trask sorting coeff. | 19.6 | 23.5 | Poor |

The top five predictive variables for each Random Forest prediction were tabulated to find the most important covariates for sediment prediction (Table 9). Latitude (y) and longitude (x) were frequently recognised as important variables for the prediction of sediment distribution. Other important variables in the prediction of sediment parameters included distance from the shelf (i.e., from the 200 m depth contour), significant wave height, distance from the coast, and depth (bathymetry). For detailed information on the relative contribution of predictor variables to modelled outputs for all sediment parameters see supplementary materials in Appendix 11.4.

Table 9. Ranking of covariates (i.e., number of times that covariates featured among the top 5important predictors of sediment properties).

| Variable | Count |
|----------------------------|-------|
| Longitude | 39 |
| Shelf Distance | 33 |
| Latitude | 28 |
| Significant Wave Height | 27 |
| Coast Distance | 22 |
| Depth | 20 |
| Significant Wave Direction | 17 |
| Major Axis | 13 |
| Maximum Current Velocity | 10 |
| Significant Wave Stress | 10 |
| Banks Distance | 9 |
| Minor Axis | 4 |
| Eccentricity | 3 |
| Banks | 1 |
| Ellipse Orientation | 1 |
| Maximum Current Direction | 1 |
| Mean Current Velocity | 1 |
| Mean Current Direction | 1 |
| Total | 240 |

Examples of rasterised model outputs for key sediment components including content of mud, sand, gravel, and carbonate as well as mean grain size are provided in Figures 30–34 (for all other sediment rasters see supplementary materials in Appendix 11.4).



Figure 30. Predicted percent mud (grain size $< 63 \mu m$) in the sediment profile for the modelled extent of Queensland's east coast (5–80 m depth).



Figure 31. Predicted percent sand (grain size $63-2,000 \ \mu$ m) in the sediment profile for the modelled extent of Queensland's east coast (5–80 m depth).



Figure 32. Predicted percent gravel (grain size 2,000–4,000 μ m) in the sediment profile for the modelled extent of Queensland's east coast (5–80 m depth).



Figure 33. Predicted percent carbonate in the sediment profile for the modelled extent of *Queensland's east coast (5–80 m depth).*



Figure 34. Predicted mean sediment grain size (Wentworth Phi scale) for the modelled extent of Queensland's east coast (5–80 m depth). Higher Phi values denote finer sediments.

6.4 Discussion

The Geoscience Australia MARine Sediment database was a significant source of data for this project. However, Courtney et al. (2021) showed how significant additional sediment sample data could be accessed through the interrogation of PhD theses and scientific papers. The sediment compilation used for this study benefitted from the addition of approximately 2,000 samples to the ~4,700 samples in the MARS database. These samples tended to be geographically discrete, so samples tended to be clustered in specific geographic areas. Significant numbers of sediment
samples were added to Princess Charlotte Bay and the Townsville region (Figure 35) from Frankel (1974), Orpin et al. (1999), and Belperio (1978).

In total, 1,605 carbonate samples were also added from the mapped submerged banks of Harris et al. (2013) (Figure 36). These banks are elevated and found at significant distances from the coast. They are assumed to be favourable habitats for 'deep' coral reef habitats. As a result, they are assumed to be high in carbonate as are other reefal environments. The banks dataset is inferred only but considered a reasonable assessment based on what is known about the distribution of sediments on the GBR (Harris et al. 2013). The banks dataset is also regional, providing data for all mapped banks within the GBR (Harris et al. 2013).



Figure 35. Added sediment samples for Princess Charlotte Bay (left) and the Townsville region (right).



Figure 36. Carbonate samples from Harris et al. (2013) Great Barrier Reef 'submerged banks' dataset used in sediment modelling. Coloured boxes represent sediment modelling sub-domains (see Figure 28).

Li (2016) classified model predictions using VEcv into five categories (Table 7). Using these categories, the final prediction of calcium carbonate ranked as 'excellent'. Predictions of mud, sand, and mean grain size were 'good'. Most other parameters ranked as 'moderate', while Trask sorting was poor (Table 27). Incorporation of sediment predictions into understanding the distribution of Moreton Bay Bugs or other marine species should be treated with a degree of caution unless the accuracy of the modelled sediment distributions is assessed and considered sufficiently accurate to be included species distribution models.

Due to considerable spatial autocorrelation between samples, samples that were close to one another have a higher likelihood of being similar than samples that were farther apart. This was indicated by the dominance of latitude and longitude. The first law of geography, established by Tobler (1970), is that "everything is related to everything else, but close things are more related than far things". Li et al. (2011a, 2011b) also saw latitude and longitude as important predictive variables and noted significant loss of predictive capability when these variables were not used.

Distance to coast is considered an essential feature for predicting seabed sediments in Australia because it has some influence on the transportation and deposition of mud from onshore to sites with low seabed gradients (Li et al. 2011a, 2011b). This study also included distance from the continental shelf (i.e., the 200m depth contour). While both distance metrics were frequently influential predictors of sediment characteristics, distance from the shelf was more important than distance from the coast. Both distance metrics are likely influenced by processes of sediment transport and deposition. Sediments closer to the coast are likely to contain finer, more poorly sorted and recently deposited material of terrestrial origin, while sediments closer to the shelf break (i.e., further from the coast) are likely to contain coarser, more ancient, and well-sorted material of more uniform consistency (Swift 1976).

Previous studies on the regional predictions of sediment distribution did not include oceanographic variables. Derivatives from the eReefs hydrodynamic model were also consistently considered important with significant wave height and significant wave direction being the two most commonly important variables. It is hypothesised that both variables influence disturbance regimes on the GBR and thus important for the redistribution of sediments on the shelf. The distribution of sediment was found to be influenced by bathymetry, consistent with other studies (Verfaillie et al. 2006, Li and Heap 2008) and is also likely related to disturbance regimes on the GBR.

According to Breiman (2001), Díaz-Uriarte and De Andres (2006), and Elith and Leathwick (2011), Random Forest and other tree-based methods such as Boosted Regression Trees are 'less vulnerable' to noisy and strongly correlated predictors. However, noisy and irrelevant covariates can restrict the accuracy of tree-based approaches. When creating a predictive model, the process of feature selection involves lowering the number of input variables. Lowering the number of variables lowers the computational cost of modelling and, in some situations, improves model performance. Feature selection algorithms only remove variables that are not significant for the prediction process therefore make it easier for the predictive method to choose a variable that is important, rather than just any available variable. Predictions of sediment distribution may be further enhanced by using VSURF (Genuer et al. 2019) or Boruta (Kursa and Rudnicki 2010), both of which are available in the R software landscape (Li et al. 2017).

6.4.1 Conclusions

This study sourced all available data to model the distribution of sediment properties and inform species distribution modelling throughout the area of the Queensland East Coast Otter Trawl Fishery likely to be inhabited by *Thenus* species. In general, Random Forest prediction methods outperformed the Inverse Distance Weighting methods (i.e., in 46 of 48 predictions). This was consistent with previous studies that found machine learning approaches typically outperform distance-based approaches in interpolation of sediment properties (see review in Li et al. 2011a).

Consistent with previous studies, latitude, longitude, depth (bathymetry), and distance from coast were important variables for prediction of sediment parameters (Li et al. 2011a, Li et al. 2011b), likely due to their relationship with transport and deposition of sediments of terrestrial origin, e.g., mud. Distance from the continental shelf was also included in this study and on average was a more important predictive variable than distance from the coast. Both distance metrics were important in predicting sediment properties, highlighting the importance of particle suspension, transport, deposition, and disturbance processes in the formation and evolution of sediments (Swift 1976). The incorporation of oceanographic and hydrodynamic variables also contributed to sediment modelling performance. Significant wave height and significant wave direction were important variables for predicting sediment distributions, possibly due to their influence on disturbance regimes.

The production of high-quality, high-resolution model outputs of sediment properties in this study provides a template for other studies seeking to employ habitat data as predictors in species distribution models extending over large geographic areas where observed habitat data is typically sparse. The resulting rasters of sediment habitat data were used to inform species distribution models for both *Thenus* species throughout the ECOTF.

7 Species distribution modelling

7.1 Introduction

Species distribution modelling (SDM) has become an increasingly important tool for natural resource management. SDM was originally based on relationships between species distributions and abiotic factors like climate, geography, or habitat structure to predict species distributions in data poor regions. Advances in SDM have been closely linked to advances in statistical methods, remote sensing, and computational power, leading to a wide range of SDM applications. A useful application of SDM in fisheries management is to help allocate historical catch data to species level in situations where species composition was not originally recorded (e.g., Northern Prawn Fishery, Venables and Dichmont 2004).

Multi-species complexes are frequently recorded in fisheries logbooks as a single group of closely related species, complicating efforts to assess and manage the effects of fishing on individual species. This is often the case where species were historically caught incidentally until shifts in fishing behaviour or conservation status demand closer attention at the species level. Where historical records from multi-species fisheries lack information on species composition, insights into species' habitat associations and spatial distributions may help allocate records among candidate species. Confidence in such approaches may be reinforced where habitat partitioning is observed among species with minimal spatial overlap.

Spatial distributions of benthic taxa often reflect species preferences for certain types of habitats (Gray 1974, Auster and Langton 1999). Relationships between benthic animals and their habitats may be driven by factors that support survival and recruitment, like the provision of substrates suited to species morphologies, e.g., attachment or burrowing capabilities (Brand 2006, Courtney et al. 2021). However, benthic habitats are more than substrates and structure alone; they also intersect with species interactions and ideal habitats will support other aspects of an organism's life, such as trophic linkages (Diaz et al. 2004). Combining habitat distributions with species' habitat preferences can therefore help assign multi-species records among species by modelling the likelihood that species occur in certain areas.

Moreton Bay Bugs are well-suited to such a SDM approach. The apparent preference of Reef Bugs (*Thenus australiensis*) and Mud Bugs (*T. Parindicus*) for different habitats lends itself to SDM approaches seeking to allocate historical records based on where each species' preferred habitats are found. Reef Bugs tend to prefer deeper areas \sim 30–60 m, typically offshore and composed of coarser sediments where they were originally caught as bycatch in association with Saucer Scallops

(*Ylistrium balloti*) and Redspot King Prawns (*Melicertus longistylus*) but have been periodically targeted by fishers in recent years. Mud Bugs are generally found in shallow, inshore areas to depths of ~30 m characterised by finer sediments and are typically caught as bycatch in association with Tiger Prawns (*Penaeus esculentus* and *P. semisulcatus*).

We used a SDM approach to split historic records of Moreton Bay Bug landings between both species based on their habitat preferences and habitat characteristics at locations where landings were reported. Input data on species abundance was compiled from several extant and newly implemented surveys, both fishery-dependent and fishery-independent (see Sections 3, 4 and 5). Sediment variables used to predict species distributions were derived using Random Forest models to produce modelled raster layers of sediment parameters for the entire Queensland east coast in the depth range occupied by Moreton Bay Bugs (5–80 m) (see Section 6). Bathymetric and hydrological data were derived from open-source models (Beaman 2010, Herzfeld et al. 2016). Our results will be used to inform reliable long-term indices of abundance for each *Thenus* species for use in stock assessment.

7.2 Methods

7.2.1 Model spatial domain

To inform the area for which model outputs were required for stock assessment, the spatial extent of the trawl footprint for the entire ECOTF was extracted from the TrackMapper vessel monitoring system (Good et al. 2007). To this were added sites with historical logbook catches recorded prior to satellite vessel tracking that occurred predominantly in areas now closed to fishing in the Great Barrier Reef Marine Park (Figure 37). All 6' logbook reporting grids intersecting the trawl footprint were then selected and trimmed in ESRI ArcGIS to retain only areas 5–80 m deep (N = 1,230 grids) to exclude unfished areas and areas unlikely to be inhabited by *Thenus* species that could misinform modelled habitat preferences.



Figure 37. Spatial extent of 6' logbook reporting grids (green) for which Thenus species distributions were modelled based on catch records in the ECOTF.

7.2.2 Data preparation

All available data sources with species-level observations (both historic and those produced over the course of this project, see Sections 3, 4 and 5) were collated (Table 10, Figure 38). These sources included a range of fishery-independent and fishery-dependent datasets that used different sampling equipment (e.g., single vessel vs numerous vessels and gear configurations) and methods (e.g., single transect vs repeat line trawling). For example, the size of recorded bugs differed among data sources, with fishery-independent datasets recording all captured individuals, whereas the fishery-dependent crew observer program recorded only legal-sized bugs (>75 mm carapace width, equivalent to >54 mm carapace length: Milton et al. 2010). As a result, only legal-sized bug observations were retained for analyses. In addition to allowing comparison between datasets, this approach should result in detection of habitat associations of adult bugs and be directly relevant to the fishery, while avoiding any spurious habitat correlations arising from juvenile bug settlement in habitats not relevant to the landed catch. Lastly, two of 3,327 species-level bugs observations were marginally outside the modelled depth range and were therefore removed from the dataset.

In addition, commercial data generated by the crew observer program (see Section 4) included data from line trawling techniques that sweep the same area numerous times to attract prawns to the disturbed plume. Therefore, swept area cannot be confidently calculated and densities (individuals per hectare) are not comparable to bug densities calculated from other survey methods because most bugs likely are caught on the first sweep. We therefore converted all species observations (of legal-size individuals) to proportion of each species per site, resulting in a comparable metric across all input datasets, which also directly addresses our central aim of allocating logbook catches between species at each location. Trawls resulting in catches of ≤ 1 legal-sized bug were excluded from analyses because sites with single animals cause outlying species habitat preferences when modelling species proportions (Table 10).

Table 10. Availability and suitability of input data providing information on Thenus species distributions in the Queensland East Coast Otter Trawl Fishery.

| Data Source | Sites | Sites with >1 legal-size bugs |
|-----------------------|-------|-------------------------------|
| Jones (1988) | 1 | 1 |
| LTMP Scallop Survey | 1,570 | 1,217 |
| Courtney (1997) | 288 | 147 |
| Crew Observer Program | 1,038 | 856 |
| Townsville Survey | 130 | 103 |
| Total observations: | 3,327 | 2,324 |

Initial modelling at the fishery-wide scale revealed issues with spatial autocorrelation. Spatial autocorrelation occurs because spatial data at sites nearer to each other are often more similar when compared to distant sites. When some areas have greater densities of sampled locations than others (e.g., the area of the LTMP scallop survey in our dataset; Figure 38), habitat variables that are important in those locations can be attributed disproportionate importance, affecting the accuracy of model predictions at broader spatial scales. This was addressed by aggregating the input data to the same spatial scale as the intended model outputs (Figure 39). Habitat variables and observed species proportions at all sites were averaged at the 6' reporting grid scale using the "Zonal Statistics" function from ESRI ArcGIS. This resulted in 6' grids which each had a single value for observed

species proportion and each habitat variable. Aggregating data at the scale of 6' reporting grids also had the advantage of allowing the model to output predictions at a spatial scale directly relevant to stock assessment.



Figure 38. Sites where Thenus species information was collected (colours indicate each data source referred to in Table 10).



Figure 39. Thenus species observations aggregated to 6' logbook reporting grids (colour ramp indicates number of sites that informed each 6' grid).

Examination of species compositions at the scale of 6' reporting grids indicated that most grids were clearly dominated by one species or the other, with few grids containing a mix of both species (Figure 40). In consultation with Fisheries Queensland stock assessment scientists, we therefore changed our modelled response variable from proportion of each species to dominant species (i.e., species that constitutes >50% of the catch in each 6' grid). This also simplified the response variable and model distribution family from a zero-and-one-inflated Beta distribution to a binomial regression.



Figure 40. Species proportions in 6' logbook recording grids with species data. Strong habitat partitioning was observed between species, with minimal overlap and most grids clearly dominated by a single species (0 = 100% Mud Bugs, 1 = 100% Reef Bugs).

7.2.3 Variable pre-selection

Species distributions were modelled as the probability of Reef Bug dominance (0 or 1) per 6' logbook reporting grid, using habitat associations based on predictor variables derived as outlined in section 6 (Table 11). For each habitat parameter, the variable's range, distribution, collinearity (statistical redundancy with other habitat parameters), accuracy (VEcv: variance explained by cross validation, see Section 6), and relationship with the response variable were inspected. Habitat parameters without clear relationships with the response variable were excluded *a priori*. Seasonality parameters were not included due to the limited movement of *Thenus* species and the tendency of each species to remain in areas of preferred habitat (Jones 1988, Courtney 1997, Unpubl. Data: see Section 3.2) making it unlikely that species compositions change at the 6' grid scale within or across years.

Strongly collinear variables (r > 0.7) were excluded as they can confound many standard modelling methods (e.g., GLM, GAM) and can lower the accuracy of data predicted by more advanced methods (e.g., random forest, boosted regression trees) if the strength of the collinearity varies

between the training and prediction datasets. When selecting which variables to retain among collinear sets, preference was given to physical measures of habitat type (e.g., sediment characteristics) rather than indirect measures (e.g., climatic, wind, current measures). Habitat variables with low accuracy scores (< 30 VEcv during cross-validation procedure; Table 8) for the entire Queensland east coast were excluded. This resulted in the omission of the Trask sorting coefficient as a predictor variable.

| Variable | Description |
|---------------------------|---|
| Bathymetry | Depth to the seabed in meters |
| Distance from coast | Distance from the mainland (nm) |
| Distance along coast | Distance from origin point at Cape York (nm) |
| Wave Height | Average wave height (m) |
| Wave Direction | Average wave direction (deg) |
| Wave Stress | Average wave stress at the seabed |
| Maximum Current | Maximum tidal current (knots) |
| Mean Current Direction | Mean tidal current direction |
| Relative Exposure Index | A measure of effective wind fetch, direction, and strength |
| Mean Grain Size | Sediment mean grain size in Phi units |
| Sediment SD | Standard deviation of sediment grain size distribution |
| Mud fraction | Percent mud (<0.063mm) in sediment profile |
| Very fine sand fraction | Percent very fine sand (0.63mm–0.125mm) in sediment profile |
| Fine sand fraction | Percent fine sand (0.125mm–0.25mm) in sediment profile |
| Medium sand fraction | Percent medium sand (0.25mm–0.5mm) in sediment profile |
| Coarse sand fraction | Percent coarse sand (0.5mm–1mm) in sediment profile |
| Very coarse sand fraction | Percent very coarse sand (1mm-2mm) in sediment profile |
| Gravel fraction | Percent gravel (>2mm) in sediment profile |
| Calcium carbonate | Percent CaCO ₃ in sediment |

Table 11. Raster variables trialled as predictors in modelling of Thenus species distributions.

We tested but ultimately excluded CPUE (as a proxy for abundance) of various co-occurring prawn species on bug distributions. Possible ecological relationships had been observed during the Townsville survey (Section 5), particularly between Mud Bugs and Tiger Prawns. However, while Tiger Prawn CPUE was very influential in the Townsville area, this relationship lost its influence in subsequent modelling at broader spatial scales, so CPUE was dropped from modelling as a predictor variable.

7.2.4 Model design

Modelling was carried out using binomial Boosted Regression Trees (BRT) in the 'gbm' package (Ridgeway 2006) with supporting diagnostics implemented in the 'dismo' package (Hijmans et al. 2021) in R (v 4.0.5). BRTs have several benefits over generalised models (e.g., GLM, GAM), in particular their ability to capture complex non-linear relationships, detect and model interactions,

iteratively build regression trees from random subsets of the dataset to capture more variance without overfitting, and rank predictor variables by their relative influence (Elith et al. 2008). In addition, BRTs do not require predictor variables to be transformed to accommodate any assumptions of normality or variance distribution. Although BRTs can be robust to collinearity among predictor variables as long as the correlation structure is similar between the training and testing datasets, we opted to use only variables that were not collinear (r < 0.7: Dormann et al. 2012) in each model build.

The 'dismo' package provides a built-in 10-fold cross-validation procedure for BRT, while also identifying the optimal number of boosting trees to use given the input dataset and model parameters. BRTs were parameterised as follows: learning rate (the contribution or weight of each tree towards the final model) of 0.001, tree complexity (maximum order interactions permitted) of 5, and bag fraction (random subset of the dataset used to build each tree) at the default value of 0.75. Smaller bag fractions simulate greater stochasticity, and therefore typically improve model performance (Elith et al. 2008); however, bag fractions smaller than ~0.7–0.75 caused a deterioration in model accuracy on the bug species dominance dataset. This is most likely due to the heavily imbalanced range of values in the response variable (N = 88 Mud Bug-dominant grids, N = 310 Reef Bug-dominant grids), for which low bag fractions (e.g., 0.50) may have resulted in random subsets of the data that did not include any Mud Bug-dominant grids.

Models were built using thematic (e.g., geographical, statistical, and expert opinion) subsets of the habitat parameters, and using both forward and backward stepwise approaches. All models were built from a minimum of 4,000 trees. The top model configuration achieved >99% classification accuracy when applied to the training dataset (N = 398 grids). This model was subsequently used to predict the probability of dominance of each bug species across all 6' grids in the ECOTF trawl footprint (N = 1,230 grids). The probability of dominance of each bug species (0–100%) was then converted to a binomial response variable ("which species is likely dominant") for ease of comparison with the training dataset and for ease of applying model outputs to the catch rate standardisation process.

7.3 Results

The most informative habitat parameters used in the top performing model for predicting *Thenus* species distributions were (in descending order of influence): sediment mean grain size, depth, fraction of medium sand in the sediment profile, fraction of very fine sand, fraction of fine sand, and distance from the coast (Figure 41). The model accounted for very high levels of variance in the dataset ($R^2 = 0.93$) and demonstrated high levels of accuracy at predicting both Mud Bug and Reef

Bug dominance in grids where this was known (100% and 99.68% respectively). Fraction of mudsize particles performed well in models but was highly collinear with mean grain size (r = 0.83), which consistently performed better. As a result, mud was omitted from the optimal model. Fitted functions for the six predictor variables indicated Reef Bug dominance occurs in grids characterised by coarse mean grain sizes, greater depth, and greater distance from coast, higher percentages of medium and fine sands, and lower percentage of very fine sand (Figure 42).

An interaction was found between mean grain size and depth that significantly influenced species dominance, wherein grids with coarser sediments in deeper areas were likely to be dominated by Reef Bugs (Figure 43). Most sites across the ECOTF were predicted to be dominated by Reef Bugs (Figure 44) mirroring the observed dominance of Reef Bugs at the scale of 6' reporting grids (Figure 40). Of the 1,230 grids for which predictions were made, 369 were predicted to be dominated by Mud Bugs and 861 dominated by Reef Bugs. Species distributions in the ECOTF broadly followed a pattern with Mud Bugs distributed in shallower inshore waters, particularly in the central and northern sectors where they are associated with the Tiger Prawn fishery, while Reef Bugs were predominantly distributed in deeper offshore waters from Townsville south to Hervey Bay (Figure 45).



Figure 41. Relative influence of predictor variables used to model spatial distributions of dominant Thenus *species.*



Figure 42. Fitted functions for predictor variables; high fitted values indicate Reef Bug dominance; low fitted values indicate Mud Bug dominance. Fitted functions are centred by subtracting their mean. Panel units: mean grain size in units of Phi (increasing Phi values indicate finer sediments), depth in m, sand fractions in %, coast distance in degrees.



Figure 43. Interaction plot with influence of mean grain size and depth on species dominance. *Higher Phi values indicate finer sediments. Higher fitted values indicate Reef Bug dominance.*



Figure 44. Histogram of modelled probabilities of species dominance of all 1,230 6' grids across the ECOTF.



Figure 45. Modelled outputs for dominant species in each 6' logbook reporting grid with Moreton Bay Bug catch records. Opaque grids indicate observed dominant species; translucent grids indicate modelled dominant species.

7.4 Discussion

In this study we applied a species distribution modelling approach, using species habitat preferences, to predict which of two species of Moreton Bay Bugs are likely dominant in each 6' fishery reporting grid on Queensland's east coast. Our predictions have since been used to split historical logbook records of Moreton Bay Bug landings between Reef Bugs and Mud Bugs to inform stock assessment (see Section 8). This process was assisted by the strong habitat partitioning observed between the species, with a predominantly inshore species preferring finer sediments (Mud Bugs) displaying minimal overlap with an offshore species preferring coarser sediments (Reef Bugs). The Boosted Regression Tree model yielded high classification success (>99%) and explained a high degree of the variance in species dominance at the 6' grid scale (93%). Remaining unexplained variability (~7%) may be due to the influence of unexplored biotic variables such as ecological relationships or behavioural traits, or due to habitat and species heterogeneity at spatial scales finer than the 6' grids used in this study.

The influence of predictor variables on species distributions followed established ecological preferences for both species (Jones 1988). Reef Bugs preferred coarser sediments with larger mean grain size, in particular sediments with high proportions of medium to fine sands typically at greater depths and greater distance from the coast. Conversely, Mud Bugs preferred sediments with finer mean grain size, particularly those with high proportions of very fine grain sizes typically at shallower depths and closer to the coast. There was also a statistically significant and intuitive interaction between sediment mean grain size and depth with Mud Bugs dominating particularly in areas where fine sediments and shallow depths coincided and Reef Bugs dominating most other areas (Figure 42).

In general, sediment characteristics tended to be more influential in determining species distributions than geographic metrics such as depth and distance from shore (Figure 41). Notable "pockets" of surprising species dominance (e.g., Mud Bugs predicted in a deep area off Yeppoon) were influenced by sediment characteristics and have been anecdotally supported by confirmation from local fishers. This suggests that the influence of depth and distance from the coast on *Thenus* distributions may be coincidental to sediment distributions. That is, coarser sediments may generally tend to be associated with greater depths and greater distance from the coast through processes of sediment transport, deposition, and disturbance, but sediment properties are ultimately the most important predictors of *Thenus* species distributions. Similar findings, where substrate was more influential than depth for modelling species distributions, have been reported in crabs (de la Barra et al. 2020), sponges (Rooper et al. 2016), and demersal fish (Chatfield et al. 2010).

Predicted species dominance was consistently corroborated by anecdotal, local knowledge from experienced fishers in data poor areas. The species distribution model built using the top six benthic habitat predictors accurately predicted well-known patterns in species dominance, such as the dominance of Mud Bugs in Moreton Bay and along the northern Queensland coastline and the dominance of Reef Bugs on the main offshore grounds off Townsville and Gladstone (Figure 45). The model also accurately predicted lesser-known patterns of species dominance subsequently confirmed by experienced local operators, e.g., Reef Bug patches offshore of the tip of Cape York, and patches of Mud Bugs offshore of Mackay.

Model predictions for the likelihood of *Thenus* species dominance were extended slightly beyond the extent of the ECOTF across the New South Wales border based on logbook records of 'Moreton Bay Bug' landings (Figure 45). However, records in the extreme southeast of Queensland and northern NSW are likely erroneous and based on misreporting (Figure 46). Prior to 2000 there was no provision in logbooks to record Moreton Bay Bugs (Thenus spp.) and Balmain Bugs (Ibacus spp.) separately; all 'bugs' were recorded as Moreton Bay Bugs. Based on reports from fishers and seafood cooperatives from the Gold Coast to northern NSW, Thenus species are rare in Southeast Queensland from approximately Cape Moreton (outside Moreton Bay) to the NSW border and beyond (Figure 46). Despite provision for logbook reporting of Moreton Bay Bugs and Balmain Bugs separately since 2000, it appears some fishers have continued to report Balmain Bugs as Moreton Bay Bugs through habit. Therefore, while predictions are provided for likely dominant Thenus species in these areas based on habitat properties, it seems unlikely that Thenus occur here in abundance. From the NSW border north to around the Sunshine Coast, Ibacus species are common from the inner shelf to the shelf break. However, northwards from approximately Double Island Point, Thenus species become more prevalent and Ibacus species become sparser on the shelf, transitioning to deeper areas toward the shelf break (Figure 46). This suggests possible preferences for cooler waters in *Ibacus* species and warmer waters in *Thenus* species, which likely explains the relative absence of Thenus in southeast Queensland. Such temperature preferences are supported by laboratory studies, where Thenus species have been reared at water temperatures of 24-27 °C (Mikami and Greenwood 1997, Mikami 2005), while *Ibacus peronii* has been reared at 20-23 °C (Mikami and Kuballa 2006).



Figure 46. In Southeast Queensland, anecdotal advice from fishers suggests Ibacus spp. (Balmain Bugs) comprise a large part of Scyllarid landings on the shelf south from around Double Island Point. North of Double Island Point and in Moreton Bay itself, Thenus spp. dominate catches on the shelf. South of Cape Moreton to the New South Wales border Thenus are rarely caught. Dashed line represents the shelf break.

The modelled spatial scale (6' reporting grids) was found to be appropriate for three reasons: 1) aggregating data at the 6' scale resolved spatial autocorrelation encountered at finer scales; 2) outputs are directly relevant and intuitive to industry, which is accustomed to reporting at the 6' scale; and 3) the length of most commercial trawls (>3.5 nm) means that confidence in where animals were caught diminishes at finer scales. The final model's error rate was very low (<1%) when tested on grids with known species dominance. The lone error occurred near Hinchinbrook

Island north of Townsville (Figure 45), where fishers have anecdotally reported a small patch of Reef Bugs at this inshore location. In rare cases such as this, the 6' grid scale may be too coarse to successfully capture heterogeneity in habitat characteristics and bug species dominance where they occur at fine scales. However, sampling of species occurrence at finer scales is constrained by the sparse availability of fishery-independent data, which is limited by the expense and logistical complexity of such sampling. Commercially derived fishery-dependent data like that from the Crew Observer Program is not available at fine scales due to the longer distances typically trawled by commercial vessels.

The model's predictive accuracy is impossible to quantify for the 6' grids for which bug species dominance has not been recorded. The model's excellent performance at predicting species dominance for 6' grids with known species dominance ($R^2 = 0.93$), together with the strong habitat associations identified for each species, and the robust benthic habitat models developed for the ECOTF footprint, make it highly likely that the model predictions are accurate. However, collection of supplemental data to validate species dominance in data poor areas would provide further confidence in model predictions. Additional observations of bug species dominance would be most beneficial in data poor areas including the Mackay region (inshore and offshore) and Far North Queensland.

The species distribution modelling approach used in this study builds on earlier work in multispecies fisheries in northern Australia (e.g., Northern Prawn Fishery, Venables and Dichmont 2004), identifying and leveraging species-specific habitat preferences to resolve relative species dominance, with the goal of splitting historical catch records for accurate stock assessment. In the current project, adaptability in developing a suitable response variable allowed us to pool observations from a wide range of historical and current datasets. Advances in machine learning algorithms, in conjunction with extensive geological surveys along the Great Barrier Reef, allowed the development of high resolution, high accuracy benthic habitat maps. Advanced statistical and ecological modelling tools such as Boosted Regression Trees generated highly accurate predictive species mapping for the entire ECOTF footprint at a fishery-relevant 6' grid scale. The use of these predictive maps to split historical catch records of Moreton Bay Bugs is described next, in Section 8.

8 Indices of abundance (standardised catch rates)

8.1 Introduction

Fisheries management relies heavily on stock assessments to monitor population trends of fished species and the effects of fishing pressure and management measures on fish stocks. Typically, stock assessment methods share a reliance on inputs including indices of population abundance over time and the extent of extractions (harvest) caused by fishing. Due to the lack of other data sources (e.g., long-term fishery-independent surveys of sufficient scale), indices of abundance are often derived from fishery-dependent data like logbook records based on metrics of catch and effort. However, calculating indices of abundance can be complex because factors other than harvest relative to fishing effort may influence changes in abundance. For example, changes in fishing power (e.g., improvements in gear or vessels), fishing behaviour (e.g., shifts in target species), or management measures (e.g., the introduction of area closures) may need to be considered in addition to catch and effort. For this reason, methods to standardise catch rates are employed that seek to account for the effects of such factors on fished stocks.

Here we use daily Queensland East Coast otter trawl catch records to produce a standardised commercial catch rate time series as an index of abundance for the two Moreton Bay Bug species: Reef Bugs (*Thenus australiensis*) and Mud Bugs (*Thenus parindicus*). The species distribution model (SDM) produced during this project (see Section 7) facilitated the splitting of commercial logbook records into Reef Bugs and Mud Bugs.

8.2 Methods

8.2.1 Data preparation

Compulsory commercial logbooks were introduced in Queensland in 1988 and are updated when necessary to reflect changing data requirements of Fisheries Queensland. Prior to 2000, Moreton Bay Bugs (*Thenus*) and Balmain Bugs (*Ibacus*) were recorded together in otter trawl logbooks under the category 'Bugs'. In 2000, the logbooks were updated to include separate boxes to record Moreton Bay Bugs and Balmain Bugs and also added a third box for 'Bugs – unspecified'. In 2005, 'Bugs – unspecified' was removed from logbooks. The otter trawl logbook released in September 2021 also required the species of Moreton Bay Bugs be recorded as either Reef Bugs or Mud Bugs. As a result, logbook data from January 1988 to December 1999 needed to be separated into Balmain Bugs and Moreton Bay Bugs (genus level) and logbook data from January 1988 to September 2021 needed to be separated into Reef Bugs and Mud Bugs (species level).

The SDM (Section 7) enabled some separation of Moreton Bay Bugs from Balmain Bugs by restricting the spatial scope of Moreton Bay Bug distributions to depths less than 80 m. Moreton Bay Bugs rarely occur in depths greater than 80 m. Therefore, records out of the scope of the SDM (> 80 m depth) were excluded from analyses due to the high potential for these records to be Balmain Bugs.

Anecdotal evidence from industry representatives working with the project noted that while the split of Balmain and Moreton Bay Bugs using the 80 m depth contour was appropriate for most of the fishery, this was likely not the case south of Fraser Island. The industry representatives suggested that in Southeast Queensland Balmain Bugs occur as close as three nautical miles from the coast in waters less than 80 m deep. To address this concern, the species catch composition from logbook entries in 30' CFISH grids south of Fraser Island was examined from 2005 onwards – after the genus level split and removal of 'Bugs – unspecified' in logbook records. For each grid, the average ratio of Moreton Bay Bugs to Balmain Bugs post-2005 was calculated. This ratio was used to allocate a proportion of bug catch pre-2000 to Balmain Bugs which was then subsequently excluded from analyses.

Data recorded as 'Bugs – unspecified' from 2000 to 2005 also needed to be assigned at the genus level. By comparing the Balmain Bug and Moreton Bay Bug commercial logbook records after 2005, once the 'Bugs – unspecified' category was removed, it became apparent that both genera were harvested in approximately equal volumes in the 30' CFISH grids south of Fraser Island. However, prior to 2005 the harvest of Moreton Bay Bugs was greater than the Balmain Bug harvest in the same grid cells. This suggests that from 2000 to 2005 the catch reported as 'Bugs – unspecified' was likely Balmain Bug. Therefore, the catch data reported as 'Bugs – unspecified' in the grid cells south of Fraser Island were allocated to Balmain Bugs and excluded from analyses.

From January 1988 to September 2021, the Moreton Bay Bug commercial logbook records needed to be split at the species level. The SDM allocated dominant species (Reef Bugs or Mud Bugs) in each 6' reporting grid within the spatial scope of the model. The species split for each grid from the SDM was applied to the location reported in daily commercial logbook records to categorise each Moreton Bay Bug record as Reef Bug or Mud Bug (Figures 47 and 48).



Figure 47. Moreton Bay Bug logbook harvest records from 1998–2021 were split between Reef Bugs (blue) and Mud Bugs (pink) using the species distribution model developed in Section 7. Standardised catch rates could then be produced as indices of abundance.



Figure 48. Nominal catch rates (catch per unit effort prior to standardisation) for Reef Bugs (blue) and Mud Bugs (pink) from 1998–2021.

8.2.2 Stock boundary

Industry advice provided to the project suggested that Reef Bugs occur at approximately a 1:2 ratio with Balmain Bugs south of Double Island point. Additionally, the LTMP survey did not extend south of Noosa (26.4° S latitude). Therefore, there were no validated occurrences of Reef Bugs

south of 26.4° S latitude. Since little inference could be made about Reef Bug presence in the southern extent of the fishery and abundance of Balmain Bugs was high, Reef Bug records south of 26° S latitude were excluded from catch rate analyses. Additionally, no observational data south of the southern tip of Stradbroke Island (28° S latitude) was obtained for Mud Bugs. For this reason, Mud Bug records south of 28° S latitude were excluded from catch rate analyses. The Torres Strait region was excluded for both species because the region was outside the scope of the species distribution modelling. Therefore, the spatial extent of the Reef Bug and Mud Bug catch rate analyses was from 10–26°S and 10–28°S, respectively.

8.2.3 Data filtering

To proceed with catch rate analyses, the logbook data required filtering to produce one record per boat-day, with each boat-day including just one location (the 6' reporting grid in which most of the catch by volume was caught).

To produce reliable indices of abundance that avoid confounding influences on catch rates (e.g., fisher experience, vessel specific fishing power, or shifts in fishing behaviour like targeting), the fishers and grid cells that did not substantially contribute to the fishery were removed prior to catch rate analysis in three steps. First, fishers, identified by authority chain number (ACN), who had been fishing for less than two years were excluded from catch rate analyses as their data were deemed not representative of the fishery. Second, fishers were removed from catch rate analyses if their lifetime catch contributed less than 1% of the total harvest from all fishers. These fishers are likely not representative of the fleet. Third, CFISH grids were removed from catch rate analyses if they did not contribute to the top 95% of total landings (ranked by each grid's harvest) (Figure 49). These grids are likely not representative of the fishery.



Figure 49. Average annual harvest (1988-2021) filtered by fishers who harvested the top 99% and grids where top 95% of harvest of Reef Bugs (left panel) and Mud Bugs (right panel).

8.2.4 Handling zero catches

Until recently, Moreton Bay Bugs were typically not a primary target species in the ECOTF, likely resulting in many zero catch records. Zero catches may originate from fishers targeting other species and thereby trawling unsuitable areas for Moreton Bay Bugs. Alternatively, fishers may have tried fishing in a suitable bug area but failed to catch any bugs. The first scenario does not give insight into the abundance of Moreton Bay Bugs, but the second scenario does. In the case that the fisher was operating in a suitable bug area but failed to catch any bugs, the record is deemed a 'true zero' catch. In the case that the fisher was not operating in a suitable bug area and did not catch bugs, the record is deemed a 'false zero'.

Presence or absence of species typically caught or not caught with Moreton Bay Bugs was used to distinguish between true and false zero records of Reef and Mud Bugs. Reef Bugs are typically caught with Redspot King Prawns and Saucer Scallops, and typically not caught with Tiger-, Endeavours-, and Banana Prawns (Figure 50). Zero catches of Reef Bugs were deemed 'false' if the logbook record did not contain catch of Redspot King Prawns or Saucer Scallops but did contain a

significant catch of Tiger-, Endeavour-, or Banana Prawns. A significant catch was defined as being greater than the average catch from instances where no Reef Bugs were caught (Table 12). Using all logbook records (1988–2021) it was found that when no Reef Bugs were caught, fishers caught on average 48.09kg of Tiger Prawns, 28.43kg of Endeavour Prawns, or 14.21kg of Banana Prawns. If the record contained more than these average weights of Tiger-, Endeavour- and Banana Prawns and no Redspot King Prawns or Saucer Scallops, it is likely that the fisher was in an area not suitable for Reef Bugs. Therefore, the record would be deemed a 'false zero'. This method was verified by checking that Tiger-, Endeavour- and Banana Prawn catch decreased when Reef Bugs were reported and increased when prawns were targeted.



Figure 50. Observed (opaque) and modelled (translucent) distribution of Moreton Bay Bug species (left) for comparison with the distributions of the main species harvested in conjunction with Moreton Bay Bugs in the ECOTF (right: adapted from O'Neill and Leigh (2006)). The dashed line in the right panel is the boundary of the Great Barrier Reef world heritage area.

Mud Bugs are typically caught with Tiger-, Endeavour-, and Banana Prawns and typically not caught with Redspot King Prawns and Saucer Scallops (Figure 50). Zero catches of Mud Bugs were deemed 'false' if the logbook record did not contain catch of Tiger-, Endeavour-, or Banana Prawns but did contain a significant catch of Redspot King Prawns and Saucer Scallops. A significant

catch was defined as being greater than the average catch from instances where no Reef Bugs were caught (Table 12). Using all logbook records (1988–2021) it was found that when no Mud Bugs were caught, fishers caught on average 11.04kg of Redspot King Prawns and 17.28kg of scallop meat. If the record contained more than these average weights of Redspot King Prawns and Saucer Scallops, it is likely that the fisher was in an area not suitable for Mud Bugs. Therefore, the record would be deemed a 'false zero'. This method was verified by checking the Redspot King Prawn and Saucer Scallop catch decreased when Mud Bugs were reported and increased when Redspot King Prawns or Saucer Scallops were reported.

An alternate method for characterising true zeroes from false zeroes for Reef and Mud Bugs was explored but is not presented in this report. The method presented herein (for both Reef Bugs and Mud Bugs) provided the most contrast to the catch rate using only catches greater than zero (zero excluded model) to test pattern sensitivity in catch rates.

After removing false zeros, records of true zeros outnumbered catch records greater than zero in the data, creating a problem of overdispersion in the generalised linear model (GLM). The overdispersion was overcome using the two-step "hurdle" method, with one model developed to explain catch vs zero catch situations, and another model developed to explain catch rates in those situations where catch occurred (i.e., excluding zero catch situations). First, the zeros were excluded and a GLM was fitted to the non-zero catch records only, using a negative binomial model for Reef Bugs and a Quasi-Poisson GLM for Mud Bugs. Model diagnostics indicated that the species data were better represented using these different models. Second, a two-component analysis was used whereby each record was converted to presence (1) or absence (0) depending on whether the bug catch was greater than or equal to zero. A binomial logistic model was fitted to the presence/absence data to produce annual probabilities of catching each Moreton Bay Bug species. The binomial logistic model assumes that the presence/absence data (y_i) follow a Binomial distribution, where $p = P(y_i = 1)$ is the probability of bugs present in the catch. The prediction (or expectation) from the binomial logistic model is:

$$\mathcal{E}(y_i) = \mathcal{P}(y_i = 1) = p$$

The binomial logistic model was then combined with the non-zero catch rate model, resulting in a model which accounts for the possibility of not catching any bugs. The non-zero catch rate models assume the catch data in kilograms (z_i) follow a Quasi-Poisson distribution or a negative binomial distribution where the prediction (or expectation) is:

$$E(z_i) = \mu$$

Table 12. Criteria used to distinguish false zeroes from true zeroes for Reef Bugs and Mud Bugs using all logbook data 1988–2021. For Reef Bugs, the catch weights of tiger, endeavour, and Banana Prawns were derived as the mean catch weights for those species when zero kilograms of Reef Bugs were reported. For Mud Bugs, the catch weights of Redspot King Prawns and scallop meat were derived as the mean catch weights for those species when zero kilograms of Mud Bugs were reported.

| Species | False Zero Criteria | True Zero Criteria |
|-----------|---|-------------------------|
| Reef Bugs | Tiger Prawn catch > 48.09kg | All remaining zeroes |
| | OR | are assumed to be true. |
| | Endeavour Prawn catch > 28.43kg | |
| | OR | |
| | Banana Prawn catch > 14.21kg | |
| | AND | |
| | Redspot King Prawn + Scallop meat catch = 0kg | |
| Mud Bugs | Redspot King Prawn catch > 7.28kg | All remaining zeroes |
| | OR | are assumed to be true. |
| | Scallop meat catch > 6.59 kg | |
| | AND | |
| | Tiger-, Endeavour- and Banana Prawn catch = 0kg | |

To include the possibility of not catching any bugs, the expectation of z_i (how many kilograms are caught) is conditioned on y_i (whether bugs are present in the catch), and written as:

 $E(z_i|y_i)$

The law of total expectation states that

$$E(z_i|y_i) = E(z_i|y_i = 1) \times P(y_i = 1) + E(z_i|y_i = 0) \times P(y_i = 0)$$
$$= \mu \times p + 0 \times (1 - p)$$
$$= \mu p$$

Here, $E(z_i | y_i = 0) = 0$ because the expected catch of bugs, given bugs are not present in the catch, is zero kilograms. So, to determine the catch rate which allows for the possibility of not catching any bugs, the expectation of the Quasi-Poisson or negative binomial model (μ) must be multiplied by the expectation of the binomial logistic model (p).

8.2.5 Model design

Catch rates were standardised for effects of year, month, region, ACN, lunar effects, hours trawled, gear fishing power improvements, marine park rezoning and targeting behaviour.

Hours trawled provided effort information after records had been filtered to one fisher-day. Some boat-day records had missing hours trawled data, making those records incomplete. Therefore, the missing values were predicted by fitting a Quasi-Poisson GLM to the complete logbook records with region, month and bug catch weight as covariates.

Fishing power refers to how adoption of technology and gear advancements improve bug catchability through time. Changes in fishing power are real world effects and must be considered. An annual change in fishing power relative to 1989 was calculated using the uptake of computer mapping, GPS, Bycatch Reduction Devices, Turtle Excluder Devices, as well as the type of otter board, type of ground gear, number of nets, trawl speed, and horsepower. Prior to 2004, gear information was collated by O'Neill et al. (2005). In 2006, gear description sheets were introduced in the ECOTF. Fishing power in 2005 was taken as the average of 2004 and 2006 fishing power estimates. Fishing power was included in the GLMs as a log-transformed offset. ACN (Authority Chain Number) is the unique anonymous identifier for an authority-vessel combination. By including ACN in the model, the analysis accounted for vessel operating differences and fleet dynamics that were not gear related, e.g., consolidation of licenses among the most efficient fishers after reductions in license numbers.

Changes in marine park zoning over time could also influence catch rates. For example, the Great Barrier Reef marine park was rezoned in 2004, limiting spatial access to trawling (Hand 2003). The fraction of each CFISH grid open to fishing in each year was calculated and included in the GLMs to account for the loss of area open to fishing.

Moreton Bay Bug standardised catch rates published in 2020 identified an upward trend in standardised catch rates from 2005 onwards (Helidoniotis 2020). This upward trend in catch rates was likely a result of shifts in fishing behaviour and reporting, as Moreton Bay Bugs have become increasingly targeted due to a rise in market value and depletion of the scallop fishery. To capture this effect, a time series of wholesale price per kilogram for species prevalent in landings from the ECOTF was compiled using price data from offload receipts supplied by industry members. This market value time series was applied to catch records to identify effort targeted at Moreton Bay Bugs. Industry representatives overseeing the analysis (as part of a separate stock assessment project team) submitted a set of rules to classify targeting behaviour. Each catch record was classified as `target' if the expected profit of Moreton Bay Bugs was at least two times greater than the second most profitable species caught that day. If the expected profit was less than half the expected profit of the most profitable species, then the catch record was classified as `non-target'. All other records were classified as `seafood salad' to represent a catch record with at least two similarly profitable species.

Year, month, region, ACN, and targeting factors were included as fixed factors. Interactions between year and month, year and region and month and region were added to the model. Lunar effects were included as a numeric measure of luminance in addition to a 7-day advanced measure of luminance to capture the lunar phase. The fraction of each CFISH grid open to fishing each year was included as a numeric value between 0 and 1. The logarithm of the hours fished was also included as a numeric value. Fishing power was used as a log offset in the model.

Standardised catch rates were analysed for Reef Bugs using a negative binomial GLM with a logarithmic link using the R statistical environment:

catch ~ year × month + year × region + month × region + target + log(hours) +
lunar + lunar advanced + ACN + fraction trawlable + offset(log(fishing power))
Standardised catch rates were analysed for Mud Bugs using a Quasi-Poisson GLM with a
logarithmic link using R software:

 $catch \sim year \times month + year \times region + month \times region + log(hours) + lunar + lunar advanced + ACN + fraction trawlable + offset(log(fishing power))$

The annual catch rate values shown in Figure 50 were predicted using the last five years of active ACNs (169 total for Reef Bugs, 239 total for Mud Bugs), the mean hours spent trawling from the last five years (11.4 hours for Reef Bugs, 10.8 hours for Mud Bugs), the mean fishing power offset from the last five years (1.051 for Reef Bugs, 1.004 for Mud Bugs), the mean fraction of each grid cell open to fishing since 2004 (0.71 for Reef Bugs, 0.43 for Mud Bugs) and was weighted by the month, region, target factor and active ACNs.

8.3 Results

At the scale of the entire ECOTF, catch rates for Reef Bugs showed little change through time for both the zero excluded and zero included models (Figure 51.A). Trends between the zero excluded and zero included models were similar, but inclusion of zero catches resulted in approximately 30% lower catch rates in kilograms per boat day. From 1988, Reef Bug catch rates generally decreased until 2000, followed by a general rise until they peaked in 2013. From 2013 to 2021, the Reef Bug catch rate declined again but remained above 1988 levels.

Catch rates for Mud Bugs across the entire ECOTF have remained relatively constant for both the zero included and the zero excluded models (Figure 51.B). Both models show an increasing trend in the early 1990s with a decrease to 1996. Following 1996, both catch rate models indicate a slow increase for a period of 12 years (1996–2008). A stronger increasing trend occurs between 2008 and

2013, after which the catch rates decrease and stabilise at levels similar to those prior to the peak in 2013. The zero included model reduced the magnitude of the catch rate by approximately 70%.

At the scale of ECOTF trawl management regions (Figure 52), regional catch rates for Reef Bugs remained constant between 1988 and 2021 for the Central, Northern, and Southern Offshore management regions (Figure 53.A). In the Southern Inshore management region, there are noticeable pattern changes. From 1988, Reef Bug catch rates in the Southern Inshore region generally decreased until 2000, followed by a rise until 2012. Between 2012 and 2021 the Reef Bug catch rate in the Southern Inshore region declined again but remained above 1988 levels. Reef Bug catch rates are considerably higher in the Central and Southern Inshore regions when compared to the Northern and Southern Offshore regions.

Regional catch rates for Mud Bugs have slowly increased between 1988 and 2021 in the Southern Inshore, Southern Offshore, and Moreton Bay management areas (Figure 53.B). Catch rates in the Northern and Central regions show a different trend. Catch rates in the Northern region increase marginally until 1994, then generally declined until 2021. Catch rates in the Central trawl region had two noticeable peaks in 1992 and 2012, with lower and flatter trends before and after the peaks. Mud Bug catch rates are considerably higher in the Central and Northern regions when compared to the Southern Inshore, Southern Offshore and Moreton Bay regions.



Figure 51. Standardised catch rates for Reef Bugs (A) and Mud Bugs (B) from 1988–2021 in the East Coast Otter Trawl Fishery. Shaded ribbons indicate 95% CI. Zero excluded models use only logbook records where the relevant species were present in the catch. Zero included models use presence/absence to model the probability that logbook records include the relevant species then model the catch rate in situations where it was present using the zero excluded model. Both models exclude records where candidate species were unlikely to occur.



Figure 52. Management regions of Queensland's East Coast Otter Trawl Fishery with regional colour coding used in Figure 53.



Figure 53. Regional standardised catch rates of Reef Bugs (A) and Mud Bugs (B) from 1988–2021 in the East Coast Otter Trawl Fishery. Line colours correspond to management regions in Figure 52.

Two model terms had clear effects on Reef Bug catch rates as shown by the influence plot in Figure 54. ACN and targeting behaviour had the largest influences on Reef Bug catch rates, transforming an increasing trend to a more stable trend through time. The lunar, lunar advanced, fishing power, hours trawled, and fraction fishable model terms had little influence on the Reef Bug catch rate model but were statistically significant. Unlike Reef Bugs, none of the model terms had a clear effect on Mud Bug catch rates when added sequentially despite being statistically significant (Figure 55).



🔶 region, month, year, lunar, lunar advanced, acn, fishing power, hours, fraction fishable, target

Figure 54. Influence of model terms on Reef Bug standardised catch rates using only logbook records where catches were greater than zero. For visual comparison of model trends, all catch rate indices were standardised to equal 1 in 1988 when logbook records began.


Figure 55. Influence of model terms on Mud Bug standardised catch rates using only logbook records where catches were greater than zero. For visual comparison of model trends, all catch rate indices were standardised to equal 1 in 1988 when logbook records began.

8.4 Discussion

Comparing the current catch rate models for Reef Bugs and Mud Bugs to Helidoniotis (2020), several key differences were apparent. Helidoniotis (2020) found that annual standardised catch rates were relatively flat until 2005, then increased until 2015 and slightly decreased between 2015 and 2019. By allocating commercial logbook records between Reef- and Mud Bugs, further insight into species-specific abundance trends has been gained. Reef Bugs have become increasingly targeted through time (Figure 53), while Mud Bugs have not been targeted. Historic market value data revealed that the wholesale price for Reef Bugs has increased from approximately \$15 per kg in 2002 to more than \$40 per kg in 2022 as domestic consumer demand increased. This is likely a driver of increased targeting of Reef Bugs. The causes of increasing domestic consumer demand for Reef Bugs over this period remain unclear but may be linked to improved marketing and/or a shift in consumer demand to Reef Bugs as an alternative to increasingly expensive Rock Lobsters directed mostly to the lucrative export market. Additionally, in 2021 it was estimated that Saucer Scallop biomass had declined to 15% of unfished levels (DAF 2021), likely contributing to a shift to targeting of Reef Bugs in recent years. Differences in targeting behaviour through time were

acknowledged in Helidoniotis (2020) but were not modelled in that report due to a lack of market value time series and species-specific catch and effort data at the time.

Secondly, in the current Mud Bug catch rate model (Figure 50.B), there are two peaks occurring around 1992 and 2013. In Helidoniotis (2020), the first peak is present but less distinctive over the same period. It is likely that the combined Moreton Bay Bug catch rate model diluted the species-specific trends that the current model has been able to capture.

The catch rates presented in Helidoniotis (2020) were split into two fleets grouping the Central and Northern trawl management regions together north of 22° S and the Southern Inshore, Southern Offshore and Moreton Bay regions together south of 22° S (Figure 51). Catch rates of the northern and southern fleets diverged around 2014, with higher catch rates in the south. Due to the dominance of Reef Bugs south of 22° S, the increased catch rate there likely results from increased targeting behaviour reflecting a shift in effort from scallops to bugs as the Saucer Scallop stock declined. By including targeting behaviour in the current Reef Bug model, standardised catch rates are distinctly flattened from approximately 2002 onwards (Figure 53). Targeting behaviour was also considered as a possible term in the Mud Bug model but was found to have an insignificant impact and omitted. This result corroborated feedback from industry and confirmed that fishers typically do not target Mud Bugs.

The stock-wide catch rates provided in the present study appear to be largely driven by one or two management regions within the fishery. For example, the patterns of change for Reef Bugs in the Southern Inshore management region closely reflect the stock wide catch rate, whilst the Central, Northern, and Southern Offshore region catch rates remain relatively constant. This is likely because the Southern Inshore region has a large proportion of the fishing effort and therefore its associated catch rate is weighted higher when combining all regions. Effort shifts from the depleted Saucer Scallop stock to Reef Bugs in the Southern Inshore region may also contribute to this trend.

Future work will aim to better quantify advances in gear efficiencies such as the transition to trawl nets with 'lead ahead'. Historically, trawl nets tended to have equal headline (i.e., top leading edge of the net) and footline (i.e., bottom leading edge of the net) lengths, causing the headline to be directly above the footline. However, trawl nets set up with lead ahead have a shorter headline, causing the headline to act as a 'ceiling' as it travels in front of the footline. This ceiling likely decreases the ability of a Moreton Bay Bugs to swim over the headline and avoid capture, improving efficiency when targeting Moreton Bay Bugs.

Another suggested improvement to the catch rate standardisation model is to differentiate between scallop and prawn gear to account for different trawl efficiencies. Scallop gear, by regulation, is allowed a larger swept area than prawn gear and with larger mesh size. Therefore, scallop gear reduces the amount of bycatch (which causes clogging) and the frequency that nets need to be emptied. By sweeping larger areas and spending more time fishing, the use of scallop gear may result in larger Moreton Bay Bug catches than prawn gear. The standardisation of gear types requires additional computation that will not be completed by the time of this report but will be considered in the stock assessment currently being undertaken by Fisheries Queensland, which is the first ever stock assessment for Moreton Bay Bugs.

Additionally, future work should also better quantify the effects of increased fishing power through new technologies such as modern charting and mapping technologies. Trawl fishing is heavily dependent on seabed characteristics with different species preferencing different habitats. Multiple sources of bathymetric mapping are now available on charting devices, in addition to the capability for fishers to create their own personalised maps at finer scales specific to their area. This has likely improved fishers' ability to better target specific species.

This study provides an updated assessment of commercial catch rates for Reef Bugs and Mud Bugs, building on the work done by Helidoniotis (2020). Catch rates are a key indicator of species abundance used in stock assessment models for Queensland commercial fisheries (see e.g., Wortmann et al. 2020, Helidoniotis et al. 2022). These data will be used as an index of abundance to inform the first stock assessments of Reef Bugs and Mud Bugs in Queensland.

9 Conclusion

This project synthesised all available data sources to model the spatial distribution of the two Moreton Bay Bug species and facilitate allocation of historic logbook records between species. Long-term indices of abundance in the form of standardised catch rates were then produced for both species to inform the first stock assessments for Moreton Bay Bugs in Queensland's East Coast Otter Trawl Fishery.

An important trend observed in all surveys of Moreton Bay Bug species distributions considered in this study, including the LTMP survey off Gladstone, the Crew Observer Program, and the Townsville bug survey, was that catches were characterised by strong habitat partitioning between Reef Bugs and Mud Bugs in all areas, with very little spatial overlap in species distributions. This characteristic contributed to the success of the project's objective to model, predict and map the spatial distribution of the two Moreton Bay Bug species based on each species' habitat preferences.

Machine learning methods were applied, combining extensive marine sediment survey data with a suite of geographic and hydrographic covariates to model sediment distributions throughout the Moreton Bay Bug fishery on Queensland's east coast. For this task, Random Forest models were found during cross-validation trials to consistently outperform simpler Inverse Distance Weighting models. High quality raster layers of habitat data were produced for a range of sediment parameters to help predict *Thenus* species distributions.

Species distribution modelling was then performed using a Boosted Regression Tree machine learning approach. Because of the observed extent of species habitat partitioning, dominant species at the scale of 6' fisheries reporting grids was modelled as the response variable. This allowed outputs of dominant species composition at a scale directly relevant to fisheries management.

The most influential predictors of Moreton Bay Bug species distributions were (in order of influence): sediment mean grain size, depth, fractions of medium-, very fine- and fine sands, and distance from the coast. These six predictors explained 93% of the variance in dominant species distribution. Of 398 sites (6' grids) in the training dataset where dominant species was known, the model made correct predictions in 100% of cases where Mud Bugs were the dominant species and in 99.68% of cases for Reef Bugs, providing a high degree of confidence in the model's performance. Anecdotal advice from fishers has also validated many of the model's species distribution predictions.

Using the species distribution model to allocate historical catch and effort records between species, long-term standardised catch rates for each Moreton Bay Bug species were produced as indices of abundance for stock assessment. When standardised to account for various factors including changes in fishing power, vessel effects, and shifts in fishing behaviour to periodic targeting (in the case of Reef Bugs), catch rates for both species were similar to 1988 levels, indicating relatively stable populations for both species over this timeframe.

Population stability despite increased targeting of Reef Bugs may result from management measures leading to reductions in fishing effort (license reductions) and area closures (e.g., the Great Barrier Reef Marine Park) from which unfished populations likely contribute to the fished stock by way of spill over or pelagic larval dispersal. The catch rates produced herein should be used as indices of abundance to inform future stock assessment and management.

10 Implications

Moreton Bay Bugs are a key component of Queensland's most valuable fishery, the East Coast Otter Trawl Fishery (ECOTF). This is particularly the case for the larger Reef Bugs that now comprise >90% of Moreton Bay Bug landings and have rapidly increased in value in recent years. Shifts in fishing behaviour towards periodic targeting of Reef Bugs have made it necessary to conduct the first stock assessment on these species. This project facilitates stock assessment by enabling the allocation of historic logbook records between Moreton Bay Bug species using a robust evidence-based approach.

The importance of this project is underscored by the importance of the bug fishery to local communities supported by the trawl industry, particularly in areas where the co-located Saucer Scallop stock is depleted.

The standardised catch rates produced during this project, based on a highly accurate species distribution model, provide reliable estimates of trends in abundance over time for both species. These catch rates will inform stock assessment and management in Queensland's ECOTF.

Standardised catch rate trends indicated that both species were being landed in 2021 at similar rates (per unit effort) as at the beginning of logbook records in 1988 after accounting for changes in targeting behaviour, vessel effects, temporal (month and year) and spatial (region) effects, lunar phase, effort (hours fished), and area closures.

This stability of catch rates was observed despite increased targeting of Reef Bugs over the same period. The effects of increased targeting on Reef Bug populations may be offset to some degree by an observed decrease in overall fishing effort in the ECOTF since the 1990s driven by management measures like trawl management planning and licence buy-back schemes. The introduction of large Marine Protected Areas from 2004 in the Great Barrier Reef Marine Park rezoning may also support source populations that contribute to the fished stock via spill over or larval dispersal from areas with no fishing mortality. Together these management measures may be contributing to the sustainability of Moreton Bay Bug stocks.

11 Recommendations

Recommendations for future research arising from this study include:

- Continue species composition sampling to validate modelled species distributions in data poor areas, particularly off Mackay and Cape York Peninsula north of Lockhart River. Such sampling could be implemented through extension of the Crew Observer Program from this study or a similar program.
- 2. Undertake fine scale modelling of Moreton Bay Bug species densities. Previous work (Pitcher et al. 2007) has estimated that large proportions of *Thenus* biomass reside in protected areas closed to fishing. However, these estimates date from the time when the Great Barrier Reef Marine Park rezoning was newly implemented. Benthic communities and habitats may have since changed in character in the absence of trawling activity. Numerous fishers stated a belief that bug populations in MPAs spill over into adjacent areas and thus contribute to the fished stock. Fine scale modelling of species densities using habitat data produced during the present study could help produce updated estimates of the proportion of Moreton Bay Bug populations exposed to fishing.
- 3. Consider fishery-independent or observer-based sampling to support fine scale density modelling (as per Recommendation 2). Unfortunately, because the Crew Observer Program surveyed only legal-sized bugs, the resulting data are not suited to quantifying population densities. Sampling should include all size classes over a large spatial range within the ECOTF to produce robust models of species densities.
- 4. Explore future options to sample bug populations in MPAs to validate estimates of the proportion of *Thenus* biomass exposed to fishing and quantify the contribution of unfished populations to the fished stock. Such methods may need to be non-extractive to operate in MPAs, e.g., night-time towed camera surveys. Efficacy of these methods to survey bug populations should be trialled.

12 Extension and Adoption

This project was, and continues to be, extended to stakeholders in the following ways:

- 1. The need and objectives for this project have been widely disseminated to industry throughout the execution of the study. In particular, implementation of the Crew Observer Program provided numerous opportunities to meet fishers at ports along Queensland's east coast. To maximise extension opportunities, these port visits were timed for periods when a large proportion of the fleet was expected to be in port, e.g., prior to season openings, or around the full moon or bad weather. Port visits served the purpose of both promoting the project as a whole and soliciting participation in the Crew Observer Program in particular. In addition, these visits and follow up phone calls with fishers provided excellent extension opportunities and facilitated regular exchanges of knowledge between industry and project staff.
- 2. The splitting of historic logbook records between Moreton Bay Bug species facilitated by this study allowed the production of long-term indices of abundance (standardised catch rates). These catch rates are now available to Fisheries Queensland. A stock assessment of Reef Bugs (*Thenus australiensis*) is currently being undertaken by Fisheries Queensland using this information.
- Dr M. McMillan sits on the Stock Assessment Project Team for Moreton Bay Bugs, a multidisciplinary advisory group comprising Fisheries Queensland stock assessment scientists, independent scientists, and industry representatives. Meetings have been held on 26/07/2022, 19/08/2022, and 5/10/2022. Further meetings will be held in the future.
- Dr M. McMillan presented the project in a presentation entitled "Habitat partitioning and machine learning help map species distributions in an iconic crustacean" at the Australian Society for Fish Biology (ASFB) conference on the Gold Coast on 9/11/2022.
- Nora Louw successfully submitted a Masters Thesis to James Cook University entitled "Habitat partitioning in Moreton Bay Bug species to inform fisheries management" on 22/11/2021.
- 6. Nora Louw leads a paper based on the 2021 Townsville bug survey entitled "Habitat partitioning in Moreton Bay Bug species to inform fisheries management" that was submitted for publication in a peer reviewed marine science journal in December 2022 and is currently in review.
- Dr M. McMillan leads a paper on Reef Bug population structure entitled "Broad-scale genetic population connectivity in the Moreton Bay Bug (*Thenus australiensis*) on Australia's east coast" submitted for publication in March 2023 that is currently in review.

- 8. At least one further paper is planned for publication in a peer reviewed journal on the species distribution modelling component of this work.
- 9. Post-project extension is planned to outline key findings to industry members, particularly those that participated in the Crew Observer Program. A 1-page fact sheet is planned that will be posted to fishers and followed up with one-on-one phone calls designed to outline how their contribution supported the project and answer any questions arising.

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14 Project materials developed

14.1 Publications

McMillan et al. (2024) Untangling multi-species fisheries data with species distribution models. *Reviews in Fish Biology and Fisheries* <u>https://doi.org/10.1007/s11160-024-09863-1</u>

Louw et al. (2024) Habitat partitioning in Moreton Bay bug species to inform fisheries management. *Fisheries Research* <u>https://doi.org/10.1016/j.fishres.2024.106956</u>

McMillan et al. (2024) Broad-scale genetic population connectivity in the Moreton Bay Bug (*Thenus australiensis*) on Australia's east coast. *Hydrobiologia* <u>https://doi.org/10.1007/s10750-023-05460-8</u>

15 Appendices

15.1 List of researchers and project staff

Dr Matthew McMillan, Fisheries Biologist, Queensland Department of Agriculture and Fisheries.

Dr Susannah Leahy, Senior Fisheries Scientist, Queensland Department of Agriculture and Fisheries.

Dr James Daniell, Adjunct Lecturer, Earth and Environmental Sciences, James Cook University.

Ms Nora Louw, Masters Student, James Cook University.

Prof Eric Roberts, Head of Earth and Environmental Sciences, James Cook University.

Ms Montana Wickens, Fisheries Scientist, Queensland Department of Agriculture and Fisheries.

Dr Kyle Hillcoat, Fisheries Scientist, Queensland Department of Agriculture and Fisheries.

Dr Michael O'Neill, Principal Fisheries Scientist, Queensland Department of Agriculture and Fisheries.

Dr Matthew Campbell, Senior Fisheries Biologist, Queensland Department of Agriculture and Fisheries.

15.2 Intellectual property

No intellectual property arose from this work.

15.3 Species biological information (Supplementary)

In this appendix we provide sex-specific histograms of size frequencies measured in three sources: Courtney 1997 mark-recapture study (Figure 56), LTMP survey (Figure 57), and Townsville 2021 survey (Figure 58).



Figure 56. Length frequency histograms for Mud Bugs (top, pink) and Reef Bugs (bottom, blue) sampled from the Courtney 1997 mark-recapture study. Female (left) and male (right) size frequencies are given at the bottom of each panel. Mean CL = mean carapace length, MLS = Minimum Legal Size (75 mm Carapace Width ~ 54 mm Carapace Length).



Figure 57. Length frequency histograms for Mud Bugs (top, pink) and Reef Bugs (bottom, blue) sampled from LTMP survey off Gladstone. Female (left) and male (right) size frequencies are given at the bottom of each panel. Mean CL = mean carapace length, MLS = Minimum Legal Size (75 mm Carapace Width ~ 54 mm Carapace Length).

Mud bugs T. parindicus Mean CL Frequency MLS Ē Carapace length (mm) Female T. parindicus Male T. parindicus Frequency Frequency Carapace length (mm) Carapace length (mm) Reef bugs T. australiensis Mean CL Frequency MLS Carapace length (mm) Female T. australiensis Male T. australiensis Frequency Frequency

Figure 58. Length frequency histograms for Mud Bugs (top, pink) and Reef Bugs (bottom, blue) sampled from Townsville bug survey 2021. Female (left) and male (right) size frequencies are given at the bottom of each panel. Mean CL = mean carapace length, MLS = Minimum Legal Size (75 mm Carapace Width ~ 54 mm Carapace Length).

Carapace length (mm)

Carapace length (mm)

15.4 Sediment modelling of the Great Barrier Reef (Supplementary)

In this appendix we provide more detailed information on sediment modelling methods and results from Section 6.

Mud

Random Forest (RF) predictions of mud content outperformed Inverse Distance Weighting (IDW) in all geographic areas (Table 13). RF predictions of mud ranged from a VEcv 49.3-74.5 with an average of 63.5 indicating that the models had a good level of predictive accuracy overall. The predictive accuracy of the model for the Brisbane regional is significantly lower than for the other models. Distance from the shelf, latitude, and longitude are important predictive variables for most mud in geographical areas (Table 14). See Figure 30 for raster of modelled mud distribution in the GBR.

Table 13. Comparison of models for predicting sediment percentage comprising mud along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 63.8 | 64.7 | Good |
| Townsville Region | 60.8 | 63.5 | Good |
| Central Region | 73.3 | 74.5 | Good |
| Southeast Queensland | 34.3 | 49.3 | Moderate |
| Average | 58.0 | 63.5 | Good |

Table 14. Summary of top-ranked covariates for modelling the distribution of mud.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|------------|--------------|--------------|------------|
| Far North Queensland | Longitude | Shelf dist | Latitude | Max Cur | Depth |
| Townsville Region | Minor | Sig Wave h | Latitude | Longitude | Shelf dist |
| Central Region | Shelf dist | Longitude | Sig wave str | Depth | Latitude |
| Southeast Queensland | Shelf dist | Latitude | Sig wave h | Sig wave str | Banks dist |

Banks dist = distance to nearest submerged bank

Minor = minor direction of current ellipse

Max cur = Maximum current velocity

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sand

Random Forest predictions of sand content outperformed IDW in all geographic areas (Table 15). RF predictions of sand ranged from a VEcv 39.5-60.8 with an average of 52.7 indicating that the models had a good level of predictive accuracy overall. The predictive accuracy of the model for Southeast Queensland is significantly lower than for the other models. Significant wave height, latitude, longitude are important predictive variables for sand in most geographical areas (Table 16). See Figure 31 for raster of modelled sand distribution in the GBR.

Table 15. Comparison of models for predicting sediment percentage comprising sand along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 47.4 | 50.0 | Good |
| Townsville Region | 56.3 | 60.8 | Good |
| Central Region | 57.9 | 60.6 | Good |
| Southeast Queensland | 31.8 | 39.5 | Moderate |
| Average | 48.3 | 52.7 | Good |

Table 16. Summary of top-ranked covariates for modelling the distribution of sand.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|-----------|------------|------------|------------|--------------|
| Far North Queensland | Longitude | Depth | Latitude | Major | Sig wave h |
| Townsville Region | Longitude | Latitude | Minor | Shelf dist | Sig wave h |
| Central Region | Longitude | Shelf-dist | Depth | Latitude | Sig wave str |
| Southeast Queensland | Longitude | Sig wave h | Banks dist | Coast dist | Latitude |

Coast dist = distance from coast

Major = major direction of current ellipse

Minor = minor direction of current ellipse

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Gravel

Random Forest predictions of gravel content outperformed IDW in Far North Queensland and Southeast Queensland only, however, the RF models performed better on average (Table 17). RF predictions of gravel ranged from a VEcv 24.1-39.0 with an average of 31.9 indicating that the models had a moderate level of predictive accuracy overall. The predictive accuracy of the model for the Southeast Queensland region is significantly lower than for the other models. Longitude and distance from coast are important predictive variables for gravel in most geographical areas (Table 18). See Figure 32 for raster of modelled gravel distribution in the GBR.

Table 17. Comparison of models for predicting sediment percentage comprising gravel along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 27.4 | 31.4 | Moderate |
| Townsville Region | 28.2 | 24.1 | Poor |
| Central Region | 39.9 | 39.0 | Moderate |
| Southeast Queensland | 29.8 | 33.3 | Moderate |
| Average | 31.1 | 31.9 | Moderate |

Table 18. Summary of top-ranked covariates for modelling the distribution of gravel.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|------------|------------|------------|--------------|
| Far North Queensland | Shelf dist | Banks dist | Longitude | Sig wave h | Major |
| Townsville Region | Major | Max cur | Latitude | Coast dist | Sig wave dir |
| Central Region | Max cur | Major | Coast dist | Longitude | Sig wave str |
| Southeast Queensland | Coast dist | Max dir | Latitude | Sig wave h | Longitude |

Banks dist = distance to nearest submerged bank

Coast dist = distance from coast

Major = major direction of current ellipse

Max cur = Maximum current velocity

Max dir = maximum current direction

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction

Carbonate

Random Forest predictions of carbonate content outperformed IDW in all geographic areas (Table 19). RF predictions of carbonate ranged from a VEcv 81.9-90.8 with an average of 85.9 indicating that the models had an excellent level of predictive accuracy overall. All geographic areas showed similar VEcv values and rated excellent. Distance from coast, longitude, significant wave height, and significant wave direction are important predictive variables for carbonate in most geographical areas (Table 20). See Figure 33 for raster of modelled carbonate distribution in the GBR.

Table 19. Comparison of models for predicting sediment percentage comprising carbonate along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 72.1 | 83.2 | Excellent |
| Townsville Region | 89.1 | 90.8 | Excellent |
| Central Region | 81.7 | 87.9 | Excellent |
| Southeast Queensland | 71.5 | 81.9 | Excellent |
| Average | 78.6 | 85.9 | Excellent |

Table 20. Summary of top-ranked covariates for modelling the distribution of carbonate.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|------------|-----------|--------------|--------------|
| Far North Queensland | Coast dist | Banks dist | Longitude | Sig wave dir | Banks dist |
| Townsville Region | Coast dist | Sig wave h | Depth | Sig wave dir | Latitude |
| Central Region | Coast dist | Banks dist | Longitude | Sig wave h | Sig wave dir |
| Southeast Queensland | Coast dist | Sig wave h | Longitude | Depth | Sig wave dir |

Banks dist = distance to nearest submerged bank

Coast dist = distance from coast

Sig Wave h = significant wave height

Mean Grain Size

Random Forest predictions of sediment mean grain size outperformed IDW in all geographic areas (Table 21). RF predictions of mean grain size ranged from a VEcv 49.0-66.4 with an average of 57.4 indicating that the models had a good level of predictive accuracy overall. All RF predictions rated good except for Far North Queensland which rated moderate. Longitude was an important predictive variable for mean grain size in most geographical areas (Table 22). See Figure 34 for raster of modelled mean grain size distribution in the GBR.

Table 21. Comparison of models for predicting sediment mean grain size along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 45.0 | 49.0 | Moderate |
| Townsville Region | 57.5 | 61.3 | Good |
| Central Region | 64.9 | 66.4 | Good |
| Southeast Queensland | 46.7 | 52.9 | Good |
| Average | 53.5 | 57.4 | Good |

Table 22. Summary of top-ranked covariates for modelling sediment mean grain size.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|--------------|------------|--------------|------------|
| Far North Queensland | Max cur | Shelf dist | Sig wave h | Depth | Longitude |
| Townsville Region | Coast dist | Sig wave dir | Longitude | Major | Latitude |
| Central Region | Shelf dist | Depth | Major | Sig wave str | Longitude |
| Southeast Queensland | Latitude | Sig wave dir | Longitude | Coast dist | Banks dist |

Coast dist = distance from coast

Major = major direction of current ellipse

Max cur = Maximum current velocity

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction

Very fine sand

Random Forest predictions of very fine sand content outperformed IDW in all geographic areas (Table 23). RF predictions of very fine sand ranged from a VEcv 27.8-45.4 with an average of 38.6 indicating that the models had a moderate level of predictive accuracy overall. All RF predictions rated moderate except for Southeast Queensland which rated poor. Longitude, latitude, and distance to shelf edge were important predictive variables for very fine sand in most geographical areas (Table 24). See Figure 59 for raster of modelled very fine sand distribution in the GBR.

Table 23. Comparison of models for predicting sediment percentage comprising very fine sand along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 32.1 | 39.0 | Moderate |
| Townsville Region | 40.1 | 42.5 | Moderate |
| Central Region | 42.2 | 45.4 | Moderate |
| Southeast Queensland | 21.5 | 27.8 | Poor |
| Average | 33.9 | 38.6 | Moderate |

Table 24. Summary of top-ranked covariates for modelling the distribution of very fine sand.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|--------------|------------|-----------|--------------|------------|
| Far North Queensland | Max cur | Longitude | Latitude | Shelf dist | Sig wave h |
| Townsville Region | Latitude | Shelf dist | Longitude | Sig wave dir | Coast dist |
| Central Region | Major | Shelf dist | Longitude | Sig wave str | Max cur |
| Southeast Queensland | Sig wave str | Shelf dist | Longitude | Depth | Latitude |

Coast dist = distance from coast

Major = major direction of current ellipse

Max cur = Maximum current velocity

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction



Figure 59. Predicted sediment percentage comprising very fine sand $(63-125 \ \mu m)$ for the modelled extent of Queensland's east coast (5–80 m depth).

Fine sand

Random Forest predictions of fine sand content outperformed IDW in all geographic areas (Table 25). RF predictions of fine sand ranged from a VEcv 19.6-48.7 with an average of 34.2 indicating that the models had a moderate level of predictive accuracy overall. Far North Queensland and the Townsville Region rated poor while the Central Region and Southeast Queensland rated moderate. There is a significant drop in VEcv for the predictive models as latitude increases. Longitude, latitude, and bathymetry were important predictive variables for fine sand in most geographical areas (Table 26). See Figure 60 for raster of modelled fine sand distribution in the GBR.

Table 25. Comparison of models for predicting sediment percentage comprising fine sand along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 20.1 | 19.6 | Poor |
| Townsville Region | 27.5 | 29.5 | Poor |
| Central Region | 32.5 | 39.0 | Moderate |
| Southeast Queensland | 40.1 | 48.7 | Moderate |
| Average | 30.0 | 34.2 | Moderate |

Table 26. Summary of top-ranked covariates for modelling the distribution of fine sand.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|-----------|------------|------------|--------------|------------|
| Far North Queensland | Major | Depth | Latitude | Sig wave dir | Sig wave h |
| Townsville Region | Longitude | Latitude | Coast dist | Minor | Shelf dist |
| Central Region | Longitude | Sig wave h | Depth | Ecc | Banks dist |
| Southeast Queensland | Longitude | Latitude | Coast dist | Depth | Shelf dist |

Banks dist = distance to nearest submerged bank

Coast dist = distance from coast

Ecc = eccentricity of current ellipse

Major = major direction of current ellipse

Minor = minor direction of current ellipse

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction



Figure 60. Predicted sediment percentage comprising fine sand $(125-250 \ \mu m)$ for the modelled extent of Queensland's east coast (5–80 m depth).

Medium sand

Random Forest predictions of medium sand content outperformed IDW in all geographic areas (Table 27). RF predictions of medium sand ranged from a VEcv 28.5-46.2 with an average of 33.6 indicating that the models had a moderate level of predictive accuracy overall. North Queensland and the Townsville Region rated poor while the Central Region and Southeast Queensland rated moderate. Longitude, shelf distance were important predictive variables for medium sand in most geographical areas (Table 28). See Figure 61 for raster of modelled medium sand distribution in the GBR.

Table 27. Comparison of models for predicting sediment percentage comprising medium sand along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 27.7 | 28.5 | Poor |
| Townsville Region | 25.6 | 29.5 | Poor |
| Central Region | 40.1 | 46.2 | Moderate |
| Southeast Queensland | 27.5 | 30.2 | Moderate |
| Average | 30.2 | 33.6 | Moderate |

Table 28. Summary of top-ranked covariates for modelling the distribution of medium sand.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|------------|-------------|------------|--------------|
| Far North Queensland | Max cur | Major | Orientation | Ecc | Longitude |
| Townsville Region | Longitude | Latitude | Coast dist | Minor | Shelf dist |
| Central Region | Longitude | Shelf dist | Latitude | Sig wave h | Depth |
| Southeast Queensland | Shelf dist | Sig wave h | Longitude | Coast dist | Sig wave dir |

Coast dist = distance from coast

Ecc = eccentricity of current ellipse

Orientation = orientation of current ellipse

Major = major direction of current ellipse

Minor = minor direction of current ellipse

Max cur = Maximum current velocity

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction



Figure 61. Predicted sediment percentage comprising medium sand $(250-500 \ \mu m)$ for the modelled extent of Queensland's east coast (5–80 m depth).

Coarse sand

Random Forest predictions of coarse sand content outperformed IDW in all geographic areas (Table 29). RF predictions of coarse sand ranged from a VEcv 34.4-46.1 with an average of 40.9 indicating that the models had a moderate level of predictive accuracy overall. Far North Queensland and the Townsville Region rated poor while the Central Region and Southeast Queensland rated moderate. Distance to the shelf, longitude, and significant wave height were important predictive variables for coarse sand in most geographical areas (Table 30). See Figure 62 for raster of modelled coarse sand distribution in the GBR.

Table 29. Comparison of models for predicting sediment percentage comprising coarse sand along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 32.4 | 34.4 | Moderate |
| Townsville Region | 39.1 | 46.1 | Moderate |
| Central Region | 36.6 | 37.3 | Moderate |
| Southeast Queensland | 42.0 | 46.1 | Moderate |
| Average | 37.5 | 40.9 | Moderate |

Table 30. Summary of top-ranked covariates for modelling the distribution of coarse sand.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|--------------|------------|------------|------------|
| Far North Queensland | Major | Max cur | Depth | Longitude | Sig wave h |
| Townsville Region | Shelf dist | Sig wave dir | Longitude | Sig wave h | Major |
| Central Region | Shelf dist | Sig wave str | Depth | Max cur | Longitude |
| Southeast Queensland | Latitude | Sig wave dir | Shelf dist | Sig wave h | Coast dist |

Coast dist = distance from coast

Major = major direction of current ellipse

Max cur = Maximum current velocity

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction



Figure 62. Predicted sediment percentage comprising coarse sand $(500-1000 \ \mu m)$ for the modelled extent of Queensland's east coast (5–80 m depth).

Very coarse sand

Random Forest predictions of very coarse sand content outperformed IDW in all geographic areas (Table 31). RF predictions of very coarse sand ranged from a VEcv 27.9-36.7 with an average of 32.2 indicating that the models had a moderate level of predictive accuracy overall. Far North Queensland and Southeast Queensland rated poor while the Townsville Region and Southeast Queensland rated moderate. Distance to the shelf, bathymetry, and significant wave height were important predictive variables for very coarse sand in most geographical areas (Table 32). See Figure 63 for raster of modelled very coarse sand distribution in the GBR.

Table 31. Comparison of models for predicting sediment percentage comprising very coarse sand along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 32.5 | 35.7 | Moderate |
| Townsville Region | 28.7 | 29.2 | Poor |
| Central Region | 26.1 | 27.9 | Poor |
| Southeast Queensland | 30.2 | 36.7 | Moderate |
| Average | 29.3 | 32.2 | Moderate |

Table 32. Summary of top-ranked covariates for modelling the distribution of very coarse sand.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|--------------|------------|------------|------------|
| Far North Queensland | Max cur | Mean dir | Sig wave h | Major | Longitude |
| Townsville Region | Major | Shelf dist | Longitude | Sig wave h | Depth |
| Central Region | Shelf dist | Sig wave h | Depth | Coast dist | Longitude |
| Southeast Queensland | Latitude | Sig wave dir | Depth | Coast dist | Shelf dist |

Coast dist = distance from coast

Major = major direction of current ellipse

Max cur = Maximum current velocity

Mean dir = mean current direction

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction



Figure 63. Predicted sediment percentage comprising very coarse sand $(1000-2000 \ \mu m)$ for the modelled extent of Queensland's east coast (5–80 m depth).
Grain size standard deviation

Random Forest predictions of grain size standard deviation content outperformed IDW in all geographic areas (Table 33). RF predictions of standard deviation ranged from a VEcv 25.4-44.7 with an average of 37.3 indicating that the models had a moderate level of predictive accuracy overall. North Queensland rated poor while the Townsville Region, the Central Region and Southeast Queensland rated moderate. Distance to the coast, distance to the shelf, longitude, and significant wave height were important predictive variables for grain size standard deviation in most geographical areas (Table 34). See Figure 64 for raster of modelled sediment grain size standard deviation distribution in the GBR.

Table 33. Comparison of models for predicting sediment grain size standard deviation along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 22.5 | 25.4 | Poor |
| Townsville Region | 39.7 | 42.1 | Moderate |
| Central Region | 41.9 | 44.7 | Moderate |
| Southeast Queensland | 32.2 | 37.1 | Moderate |
| Average | 34.0 | 37.3 | Moderate |

Table 34. Summary of top-ranked covariates for modelling sediment grain size standard deviation.

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|--------------|--------------|--------------|------------|------------|
| Far North Queensland | Sig wave str | Sig wave h | Shelf dist | Latitude | Longitude |
| Townsville Region | Coast dist | Longitude | Sig wave h | Shelf dist | Latitude |
| Central Region | Depth | Sig wave dir | Shelf dist | Coast dist | Longitude |
| Southeast Queensland | Longitude | Latitude | Sig wave str | Shelf dist | Coast dist |

Coast dist = distance from coast

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction

Sig Wave str = significant wave stress



Figure 64. Predicted sediment grain size standard deviation for the modelled extent of *Queensland's east coast (5–80 m depth).*

Trask sorting coefficient

Random Forest predictions of Trask sorting coefficient outperformed IDW in all geographic areas (Table 35). RF predictions of Trask sorting coefficient ranged from a VEcv 17.9-34.2 with an average of 23.5 indicating that the models had a poor level of predictive accuracy overall. Far North Queensland, the Townsville Region, and Southeast Queensland rated poor while the Central Region rated moderate. Distance to the shelf, latitude, significant wave direction, and significant wave height were important predictive variables for Trask sorting in most geographical areas (Table 36). See Figure 65 for raster of modelled Trask sorting distribution in the GBR.

Table 35. Comparison of models for predicting Trask sediment sorting coefficient along the Queensland east coast. Optimum model is in bold. IDW and RF VEcv = Variance Explained by cross validation for Inverse Distance Weighting and Random Forest models respectively.

| Location | IDW VEcv | RF VEcv | VEcv rating |
|----------------------|----------|---------|-------------|
| Far North Queensland | 17.7 | 17.9 | Poor |
| Townsville Region | 20.1 | 23.7 | Poor |
| Central Region | 28.6 | 34.2 | Moderate |
| Southeast Queensland | 12.2 | 23.6 | Poor |
| Average | 19.6 | 23.5 | Poor |
| Average | 19.6 | 23.5 | Poor |

Table 36. Summary of top-ranked covariates for modelling distribution of sediment sorting (Trask coefficient).

| Location | 1 | 2 | 3 | 4 | 5 |
|----------------------|------------|--------------|--------------|------------|--------------|
| Far North Queensland | Banks dist | Sig wave h | Sig wave dir | Latitude | Shelf dist |
| Townsville Region | Shelf dist | Sig wave dir | Mean cur | Banks dist | Ecc |
| Central Region | Shelf dist | Depth | Longitude | Latitude | Sig wave h |
| Southeast Queensland | Shelf dist | Sig wave h | Latitude | Longitude | Sig wave dir |

Banks dist = distance to nearest submerged bank

Ecc = eccentricity of current ellipse

Mean cur = Mean current velocity

Shelf dist = distance from shelf break (200 m depth)

Sig Wave h = significant wave height

Sig wave dir = Significant wave direction



Figure 65. Predicted sediment Trask Sorting Coefficient for the modelled extent of Queensland's east coast (5–80 m depth).

15.5 Trial of environmental DNA to detect Thenus species presence

MBB eDNA Progress Report 2022

17/11/2022

Researchers:

Julie Goldsbury and Nora Louw Overseen by Dean Jerry Consulted with Scott Morrissey and TropWater team

Overview:

In May 2022 a project analysing sediment for Moreton Bay Bug eDNA commenced at the Molecular Ecology and Evolution Lab of the Australian Tropical Science and Innovation Precinct at James Cook University in Townsville, QLD. The aim of this project is to determine the practicality and validity of extracting eDNA of commercially valuable species from marine sediments. The target species are *Thenus australiensis* and *Thenus parindicus*. For this study, marine sediment samples taken during a FDRC-funded survey run by Queensland Department of Agriculture and Fisheries (QDAF) in July 2021 were processed, purified, and analysed using qPCR to determine presence and absence of the target species with species-specific primers. The qPCR results from each sample location will be compared with the actual catch of the target species at the same locations to determine the strength of eDNA assessment as a method of determining species presence on a fishery scale.

Methods:

1. Sample procurement

In July-August 2021, samples were taken at 48 of 131 survey sites during the FRDC/QDAF Moreton Bay Bug survey off Townsville. At each of these 48 eDNA sites, a 2kg sediment grab was lowered to the seafloor, deployed, and retrieved to the vessel. A site control was taken by placing the working spatula into a tube containing DESS buffer and sealed. From the grab, a one-gram sediment sample was taken and stored in a 15 ml tube containing DESS buffer. This was repeated two more times, resulting in a control and three sediment samples at each site. Samples were stored in a freezer until processing.

2. Sample processing

The samples were processed in the eDNA lab where DESS buffer was removed from each sample tube by cycles of spinning in a centrifuge and decanting/pipetting. eDNA was extracted from each sample following MEEL Mu-DNA sediment extraction protocols. Site controls were treated as sample tubes and additionally, an extraction blank was processed for every 24 tubes extracted. Extracted DNA was purified using Zymo inhibitor removal kits, then stored in a refrigerator. A tank water sample was procured from a MARFU tank containing multiple *T. australiensis* and processed using PPLPP extraction protocols to get a positive control of eDNA for verification.

3. Tissue sample extraction

Tissue samples of each target species were taken from pleopods of individuals caught during the Townsville survey and preserved in ethanol. In the lab, DNA was extracted from the tissue following CTAB protocols and sequenced. Due to unclear result sequences and potential interspecies contamination in the nets of the trawler, further tissue samples were procured from other sources. *T. australiensis* was sampled from MARFU facilities at JCU and *T. parindicus* was sampled from a green, frozen individual from NQ Marina Fresh Seafood.

4. Primer design

Species-specific primers for the COI gene were designed based on consensus sequences from all GenBank entries for each target species. Primers were designed using Geneious software and assessed using IDTDNA OligoAnalyzer (Table 37).

| Table 37. Primers a | leveloped fo | or Thenus s | species eDNA | trials. |
|---------------------|--------------|-------------|--------------|---------|
|---------------------|--------------|-------------|--------------|---------|

| SPECIES | PRIMER NAME | SEQUENCE | Primer b.p. | Tm | HAIRPIN | PRODUCT SIZE |
|------------------|----------------|----------------------------|----------------|------|---------|-----------------|
| T. australiensis | ReefSSCO1_F | GATTACTTCCTCCTTCTCTAATACTA | 26 | 55.1 | 0 | 149 |
| T. australiensis | ReefSSCO1_R | TAGATGAAGTGAAAAGATACCG | 22 | 53.4 | 0 | 149 |
| T. parindicus | MudSSCO1_F | TCTATCGGCCGCTGTTGC | 18 | 60.2 | 0 | 203 |
| T. parindicus | MudSSCO1_R | GTGATAGAAGTAAGAGGACGGC | 22 | 58 | 0 | 203 |

5. Primer optimization and qPCR

The species-specific primer pairs were tested and optimized using positive controls from tissue samples via endpoint PCR.

For *T. australiensis* primers, the optimal annealing temperature was determined, leading to this recipe for qPCR:

| Step | Time | Temp | |
|-----------------------------|---------|------|-----|
| UDG Activation | 2 mins | 50 | |
| Dual-Lock DNA Polymerase | 2 mins | 95 | |
| Denature | 15 secs | 95 | X50 |
| Anneal/Extend | 1 min | 55 | |
| | 15 secs | 95 | |
| Melt Curve | 1 min | 55 | |
| | 15 secs | 95 | |

The master mix for 20 microliter qPCR reactions was based on Sybr Green protocols:

For *T. australiensis* primers:

| Master Mix | x1 |
|-------------------|-----|
| H2O | 1.4 |
| SYBR | 10 |
| Forward Primer | 1.8 |
| Reverse Primer | 1.8 |
| Total | 15 |
| DNA Template | 5 |
| Final Total | 20 |

The primers set for *T. parindicus* need further optimization. Due to the high degree of relatedness between the two species, there are limited variable loci on which primers can be designed. When *T. parindicus* gDNA was extracted, it identified as *T. orientalis*, the name previously used for *T. australiensis* before the genus revision in 2007 by Burton and Davie. Due to the previous taxonomical ambiguity, it is likely that the sequences in GenBank used to verify our samples genetic sequences are incorrectly entered, or not revised after the genus revision. This creates

difficulty using these sequences and therefore designing species-specific primers for *T. parindicus* to use on our sediment sample extractions.

Initial Summary of Results/Discussion:

At this point, we can report that the qPCR reactions are working to pick up both gDNA and eDNA of our target species. Using the *T. australiensis* COI primer set, our positive control tissue samples for this species have shown clear and strong amplifications on all runs. The tank water eDNA from MARFU also has shown strong amplifications, indicating that our methods of sampling, processing, and extracting were successful. However, it should be noted that the tank water sample was so highly concentrated with eDNA that a dilution was necessary to avoid overwhelming the qPCR. Dilutions of 1:20, 1:50, and 1:100 were all successful and showed strong amplifications. This highlights one limitation of qPCR. Thus far, two replicate well reactions of site sediment samples have successfully shown amplifications. These were both sites where *T. australiensis* was caught. However, there were many sites that had very large catches of this species that the samples did not amplify on qPCR. This could be due to several reasons. Initially it seems probable that the small sample of sediment we procured just happened to be outside of the path of travel of the target species. The trawling area of each site spanned one nautical mile, so it is likely that the bugs caught there had not visited the spot we grabbed.

The *T. parindicus* COI primer set still needs optimization. While it appeared to be complete on the endpoint PCR where there were no signals of primer dimers or non-specific bands, the sensitive qPCR showed otherwise. While many sample wells amplified for this site with a large catch, there were quite messy amplifications in all our controls (site, extraction, non-template) towards the end of the run, producing unreliable results. The same controls were tested with *T. australiensis* COI primers and there were no amplifications. Once this set of primers are optimized for qPCR, all sites and controls can be analysed for *T. parindicus* presence.

When all sites and controls are run with both COI primer sets for both species, we can compare the results of presence/absence to the catch log at each site. From there, we can also investigate the sediment characteristics of the sites and determine if there are patterns in the qPCR results and benthic composition. It is possible that muddy sediments hold eDNA differently than gravely sediments, or that other environmental factors are at play.