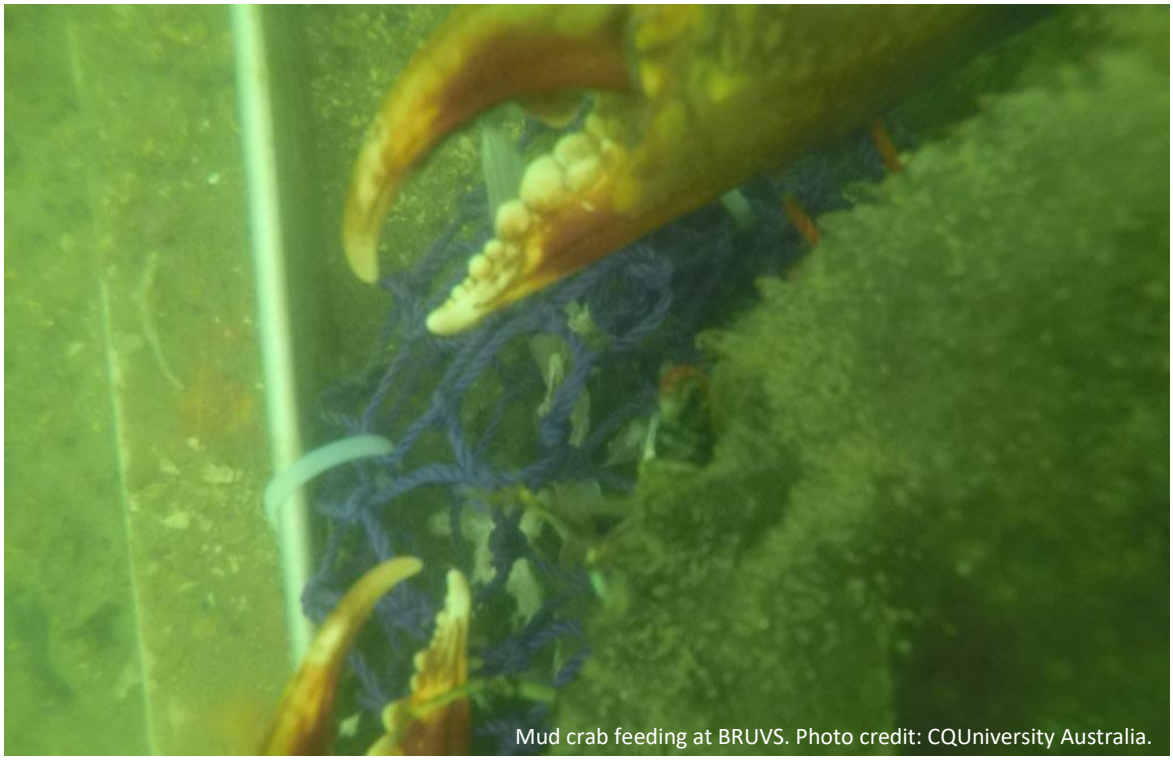
Mud Crab Indicators for the Gladstone Harbour Report Card: Project ISP015-2019



Mud crab feeding at BRUVS. Photo credit: CQUniversity Australia.

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Prepared for the Gladstone Healthy Harbour Partnership

Gladstone Healthy Harbour Partnership

This report should be cited as: Flint, N., De Valck, J., Anastasi, A., and Jackson, E.L. (2019). Mud crab indicators for the Gladstone Harbour Report Card. Report to the Gladstone Healthy Harbour Partnership. CQUniversity Australia, Queensland.

Acknowledgements

This study was funded by the Gladstone Healthy Harbour Partnership (GHHP) and CQUniversity Australia. We thank members of the GHHP Independent Science Panel (ISP), for useful comments and advice. Mac Hansler and Mark Schultz from GHHP provided ongoing project support. Many thanks to Evan Chua, Karl French, Adam Rose, Hayley Threlkeld, Elizabeth Andrews (CQUniversity) and Edward Mallie (Gidarjil Development Corporation Ltd) for conducting field work, and Dylan Charlesworth for on shore operational assistance. Fisheries Queensland scientists provided knowledge and historical data in the development of the indicator in 2017.

The authors would like to take this opportunity to respectfully acknowledge the Traditional Owners of the land on which we live, work and learn, and pay our respects to the Elders, past, present and future for they hold the memories, the traditions, the culture and hopes of Indigenous Australia. In particular, we pay our respects to the peoples on whose Country this research was carried out.

Executive summary

Mud crabs (*Scylla serrata*) are recreationally and commercially important species in Gladstone Harbour, as well as an iconic seafood item, with cultural value to Indigenous Australians. In 2017, CQUniversity proposed four mud crab measures for the Gladstone Harbour Report Card:

- Abundance
- Prevalence of rust lesions
- Sex ratio
- Biomass

In 2019, mud crab monitoring was conducted in seven Gladstone Harbour zones for the third consecutive year, over two sampling events in February and June. Scores and grades were calculated using both data sets for three measures: Abundance, Prevalence of rust lesions, and Sex ratio for each of the seven recommended long-term monitoring zones in Gladstone Harbour. The three measure scores were averaged to reach a Mud Crab Indicator score and grade for each zone, and for the Harbour.

The biomass measure has not previously been reported as three years of data were required to assess its utility as an indicator and potentially develop a benchmark and worst case scenario. The biomass measure was investigated further in 2019, using the three years of available monitoring data. Biomass was found to be influenced by moult stage, missing limbs and crab size. Based on the analysis of three years' worth of data, it was decided that the biomass measure should not be incorporated into the Report Card.

In June 2018 and February 2019, additional sampling was undertaken at Eurimbula Creek, 20 km south of Rodds Bay. The creek is within Queensland Government fisheries regulated waters with no crabbing permitted and was proposed as a comparison site to crabbed zones in Gladstone Harbour, particularly for the sex ratio measure. The results from Eurimbula Creek suggest that the current sex ratio benchmark of 2 males : 1 female is appropriate, and it has been retained for 2019.

The following scores and grades have been calculated for 2019:

Zone	Abundance (CPUE)	Prevalence of rust lesions	Sex ratio	Zone score 2019
1. The Narrows	1	0.90	0	0.63
2. Graham Creek	0.12	1	0.24	0.45
4. Boat Creek	0.46	0.94	0.05	0.48
5. Inner Harbour	0.67	0.70	0.08	0.48
6. Calliope Estuary	0.29	1	0	0.43
7. Auckland Inlet	0	NC	NC	NC
13. Rodds Bay	0.27	0.70	0.12	0.36
Harbour Average				0.47

- NC – not calculable, n < 5

The scores and grades for the mud crab indicator reflect the variety of pressures on mud crabs in Gladstone Harbour, including commercial fishing, recreational fishing and environmental/habitat condition and over short time periods, and are also potentially influenced by biological variability. Low overall scores (D) were recorded at five of the seven zones: Graham Creek, Boat Creek, Inner Harbour, Calliope Estuary and Rodds Bay. Rodds Bay also scored a D in 2017 and 2018. The Narrows was graded C, with a very slightly lower score than in 2018.

As was the case in 2018, no grade has been calculated for Auckland Inlet, as only one mud crab was caught across the two sampling periods. The small sample size (< 5) means it is not appropriate to

calculate grades for this site, except for the abundance measure. We recommend the NC overall grade for Auckland Inlet is treated as an “E” when rolling the mud crab indicator results up into the wider Report Card.

Low grades for abundance of mud crabs (E) were recorded in Auckland Inlet and Graham Creek. Rodds Bay, Boat Creek and Calliope Estuary were all graded D and the Narrows was again graded A. As well as human impacts, there are many other factors that can influence the catchability of mud crabs such as the moult state of crabs, reproductive cycles, lunar and diel cycles, temperature, water motion and habitat quality. In light of this potential for natural variability, the decision was taken in 2018 to allow abundance to be scored based on a moving average technique, of the average of the 75th percentile of scores for current and previous sampling years, up to 10 years. This allows the abundance benchmark to move in response to annual harbour-wide changes in catchability and abundance which are more likely to be natural variations.

The prevalence of rust lesions measure scored an A or B grade in all zones for which it was calculated in 2019, indicating low prevalence of this abnormality during the sampling period. Sex ratios of crabs over the legal size limit (for males) tended towards very high proportions of female mud crabs, a possible reflection of the sex-based fishery operating in Queensland. In 2018 the sex ratio benchmark was updated in light of new information from an unpublished Australian study of mud crab sex ratios in unfished estuaries. In 2019, data from a no-crabbing area (Eurimbula Creek) was collected and analysed and provides confirmation of the benchmark of two males to one female.

Eurimbula Creek is located in the Baffle Catchment, directly south of Gladstone Harbour (the same catchment as Rodds Bay). The restriction on crabbing, along with the generally good environmental condition of the creek, means that the population of mud crabs at Eurimbula is considered to be in relatively pristine condition. The surveys that we conducted bore this out – if Eurimbula Creek was scored in 2018-19 using the GHHP mud crab indicator the creek would score A for abundance, A for prevalence of rust lesions, A for sex ratio and A overall. The results demonstrate the relevance of Eurimbula Creek as a suitable reference site for mud crab surveys.

Contents

Introduction

The mud crab (*Scylla* spp.) is a key fisheries product in Africa, Asia, Australia and the South Pacific (FAO, 2017). In Queensland, the 2018 total catch was approximately 2,000 tonnes (The State of Queensland, 2019). Mud crabs (primarily *Scylla serrata*) are a recreationally and commercially important species in Gladstone Harbour and an iconic seafood item, with cultural value to Indigenous Australian peoples (Brewster, 2015). Because of the importance of mud crabs to the local community and stakeholders, the GHHP Independent Science Panel (ISP) selected mud crabs as an important indicator species to assess the health of Gladstone Harbour, and nominated candidate indicators for Gladstone Harbour including: size distribution of adult mud crabs; abundance of adult mud crabs; and visual health assessment of adult mud crabs (McIntosh *et al.*, 2014). In 2017, the Gladstone Healthy Harbour Partnership (GHHP) commissioned CQUniversity to develop mud crab indicators for the Gladstone Harbour Report Card (Project ISP015-2017). CQUniversity conducted a review and analysis of potential mud crab indicators, and proposed four mud crab measures to be incorporated into the mud crab indicator for the Gladstone Harbour Report Card:

- Abundance (catch per unit effort – CPUE)
- Prevalence of rust lesions
- Sex ratio
- Biomass

These indicators were monitored by CQUniversity in 2018 (ISP015-2018), and by CQUniversity in collaboration with Gidarjil Development Corporation in 2019 (ISP015-2019). Brief overviews of each indicator, as described in Flint et al. (2017), are provided below.

Abundance

Abundance of mud crabs caught during standardised independent monitoring gives an estimate of changes in total abundance and population size (Dumas *et al.*, 2012; Meynecke *et al.*, 2012; Alberts-Hubatsch *et al.*, 2016) of mud crabs through time and between areas. Discrepancies in abundance can be due to capture technique, sampling areas and sampling times, so it is necessary to minimise monitoring variation in seasonality, catch and sampling techniques. In the GHHP sampling program, consistent methodologies are employed during each catch period, to ensure opportunity for direct comparison of CPUE and abundance at different zones and between years. Abundance can reflect a wide variety of natural and anthropogenic impacts on a population (Alberts-Hubatsch *et al.*, 2016). Factors influencing abundance of mud crabs may include localised and regional fishing pressure, habitat availability and habitat condition, availability of food and proximity to suitable nursery grounds for the settlement of mud crab megalopae and metamorphosis to immature crabs.

Climate has been shown to impact the abundance of mud crabs (Meynecke *et al.*, 2012) so there is also potential for this indicator to be used to monitor climate effects on mud crabs in the longer term. Expected global warming and rising sea temperatures in the future may benefit some *S. serrata* populations, which tend to thrive in warmer waters. However, global warming is also predicted to increase the occurrence of extreme La Niña events (Cai *et al.*, 2015), and the associated higher rainfall, lower water salinity and ocean acidification could potentially be detrimental to *S. serrata* populations and contribute to habitat degradation. In Australia, *S. serrata* tends to be dominant in mangrove habitats which typically have salinities around 34 ppt (Meynecke *et al.*, 2012). However, such populations are also vulnerable to flooding events, which reduces water salinity and in turn results in increased mortality (Meynecke *et al.*, 2012).

Prevalence of rust lesions

In 1994, the first records of “rust spot” shell lesions in mud crabs were reported by commercial fishers in Gladstone Harbour (Andersen and Norton, 2001). The disease is not infectious and may possibly be related to inhibition of calcium uptake following sublethal copper exposure, although

this has not yet been experimentally confirmed (Andersen and Norton, 2001). The disease has also been reported from the Fitzroy River and (with much lower prevalence) from Moreton Bay, Ayr (Andersen and Norton, 2001) and Stanage Bay (Dennis et al. 2016).

Rust spot lesions initially start as an orange discolouration on the crab's carapace, before progressing to penetration and degradation of the affected carapace area ('perforation'), with resultant exposure of the soft tissues beneath the carapace (Andersen *et al.*, 2000; Andersen and Norton, 2001). In 2011-12, there were reported increases in the incidence of disease in finfish and crabs in Gladstone Harbour. Due to the fact that rust spots are not continuously observed in the Gladstone Harbour region, the prevalence of rust spot at any given time is an overall indicator of environmental state. There is potential for rust spot to impact on the local fishery, as it impacts on the seafood 'grade' of crabs. Recording the presence of rust spot is a relatively straight forward and non-destructive monitoring tool.

Sex ratio

Recreational and commercial fishing are major factors driving the management of Queensland's *S. serrata* population, and exert pressure on fished stocks. In Queensland, only male mud crabs over 150 mm carapace width (measured across the ninth posteriolateral spines, referred to as 'spine width' in this report) may be retained by either commercial or recreational fishers. Female mud crabs, and any mud crabs under 150 mm may not be retained. Identifying the gender of most crabs is quick and easy, and in areas where a sex-based fishery is enforced, changes in the ratio of males to females may be indicative of a change in fishing pressure (Heasman, 1980; Williams and Hill, 1982; Pillans *et al.*, 2005; Alberts-Hubatsch *et al.*, 2016). Shifts in sex ratio not only have implications for dynamics of the crab population and reproductive success, but may also influence ecosystem processes due to the different behaviours of the sexes. For example, male, rather than female mud crabs create burrows (Bonine *et al.*, 2008) which may aid in the process of bioturbation; and female crabs often migrate offshore to spawn (Knuckey, 1999).

Besides fishing pressure, another possible influence on the sex ratio of crabs is seasonal changes, and this needs to be considered when interpreting indicator scores. For example, the mud crab population in Princess Charlotte Bay in northern Queensland was dominated by females only during August (Hill, 1984). Similar results were found between December and February in the Northern Territory (Knuckey, 1999). It is not yet known whether female mud crabs in Gladstone Harbour migrate to spawn.

Finally, the sex of the crab may also affect the probability of capture and recapture, particularly of the sub adult size class. Williams and Hill (1982) showed differences in the vulnerability to capture among *S. serrata* of different sizes, with vulnerability increasing strikingly for males over 130 mm and females over 140 mm. In 1980, Heasman found a difference between the capture of different sexes of moulting sub adults of *S. serrata* in Moreton Bay. The reason proposed was that behavioural differences between the sexes and among size classes led to unequal vulnerability to capture.

Biomass

Similar to abundance, biomass has previously been suggested an important indicator of both species and ecosystem health (Alberts-Hubatsch *et al.*, 2016). The carapace width to body weight ratio (CW:BW) was considered as a possible indicator of health in mud crabs, with high ratios indicating high ecosystem productivity and food densities (Ikhwanuddin *et al.*, 2011). In 2017, there were no historical data with which to determine a benchmark for biomass, so it has not yet been reported. Three years of monitoring data are now available and have been used to assess the use of biomass as a measure within the mud crab indicator.

Objectives

The overall objectives of this project were to:

1. Conduct mud crab surveys of 7 GHHP reporting zones consistent with the survey methods used in 2018 (Flint et al. 2018) and consisting of a summer (warm, wet season) survey in February and a winter (cool, dry season) survey in June.
2. Provide mud crab scores and grades for the 2019 Gladstone Harbour Report Card. The scores and grades will be calculated using the statistical methods developed in the 2017 mud crab monitoring project with the revised thresholds for sex-ratio and abundance used for the calculation of the 2018 mud crab scores and taking into account results from the separate GHHP study being conducted in Eurimbula Creek.

Methods

Field methods

The following permits and approvals are in place for this research:

- General Fisheries Permit (Queensland Department of Agriculture and Fisheries; Permit Number 192151)
- Animal Ethics Approval (CQUniversity Animal Ethics Committee; Approval Number 20633)
- Authorisation for research in the Great Barrier Reef Marine Park (Approval Number G17/05-027)
- Field Work Risk Assessment (CQUniversity Occupational Health and Safety Unit)

Two mud crab surveys were undertaken in 2019 (Table 1), representing a summer (warm, wet season) and winter (cool, dry season) sample. The seven monitoring sites (Figure 1) were previously chosen through a quantitative selection process (Flint *et al.*, 2017) related to the availability of suitable habitat types and the occurrence of previous sampling sites, and were also surveyed in 2018.

Table 1: Gladstone zones/sites sampled during February and June 2019.

Zone/site	Survey 1	Survey 2
Zone 1: Narrows	15 February	29 June
Zone 2: Graham Creek	15 February	29 June
Zone 4: Boat Creek	16 February	28 June
Zone 5: Inner Harbour	14 February	27 June
Zone 6: Calliope Estuary	16 February	28 June
Zone 7: Auckland Inlet	14 February	27 June
Zone 13: Rodds Bay	17 February	30 June

Sampling dates and times were determined by tidal cycles. Surveys were conducted on dates when low tide was between 10.30am and 3.00pm. Pots were set at least three hours before the low tide, and collected at least two hours after the low tide, resulting in soak times of at least five hours per pot. To comply with Animal Ethics Approval pots were placed so that they would still be submerged at low tide (preventing exposure mortality of any fish caught in the pots). Pots were placed as close as possible to mangrove habitats within this limit.

At each site and sampling event, 20 heavy duty 4-entry round collapsible crab pots were set a minimum of 100 m apart. The exception to this was Zone 4 Boat Creek, as fewer pots can be accommodated in this smaller system. Collapsible crab pots were purchased from a local tackle store, as they are easy to transport, assemble on the vessel and replace (Fisheries Queensland, 2009). Each pot was baited with one large sea mullet (*Mugil cephalus*) head, and all floats were attached with 10 m ropes and marked with researcher contact details and the Fisheries Queensland research permit number. Every float had a unique identifying number to allow any missing pots to be identified quickly during retrieval. The opening of each pot was secured with a cable tie, so that if crabs were removed by others this could be detected and recorded on retrieval.
At each sampling site, the following information was recorded:

- Zone and site name;
- GPS location;
- Date;
- Set time and retrieval time for each uniquely identified pot;
- The total number of animals of each species caught in every pot, and the sex of all mud crabs

caught; and
- Water quality parameters (temperature, dissolved oxygen, conductivity, pH, turbidity, total dissolved solids, oxidation reduction potential and salinity) measured using a YSI ProDSS Multiparameter Sampling Instrument, recorded once before setting the first pot and once after retrieving the final pot (not reported but provided to GHHP).

For every mud crab captured at each site, the following information was recorded:
- Species;
- Sex;
- Carapace width (notch width) (mm);
- Weight (g);
- Abnormalities: type, body location, dimensions of rust spot lesions, grade of rust spot lesions (source Andersen, 2003);
- Presence of absence of mating scars on male mud crabs;
- Seafood grade: A, B or C (measured using the Australian Industry Live Mud Crab Grading Scheme, available at: https://www.c-aid.com.au/wp-content/uploads/Live-Mud-Crab_Grading-Scheme_WEB.pdf).

All bycatch species (including blue swimmer crabs, fish and other crabs) were also recorded. Blue swimmer crabs were opportunistically weighed, measured, and checked for abnormalities before release. All catch was released alive at the site of capture. Used baits were kept on board the vessel for later disposal on land, and not discarded at the sampling site, to reduce interference with commercial and recreational mud crabbers in the area.

Eurimbula Creek methods

Sampling was also conducted at Eurimbula Creek, located south of Rodds Bay in the Baffle Catchment, on 26 June 2018 and 18 February 2019.

The field methods used at Eurimbula Creek were identical to field sampling at each Gladstone Harbour zone, described above. As the creek is within fisheries regulated waters prohibiting crabbing (map available at: https://www.daf.qld.gov.au/__data/assets/pdf_file/0004/87052/Eurimbula-Creek-FRW-162.pdf), data from Eurimbula Creek were collected as a comparison to crabbed zones in Gladstone Harbour.

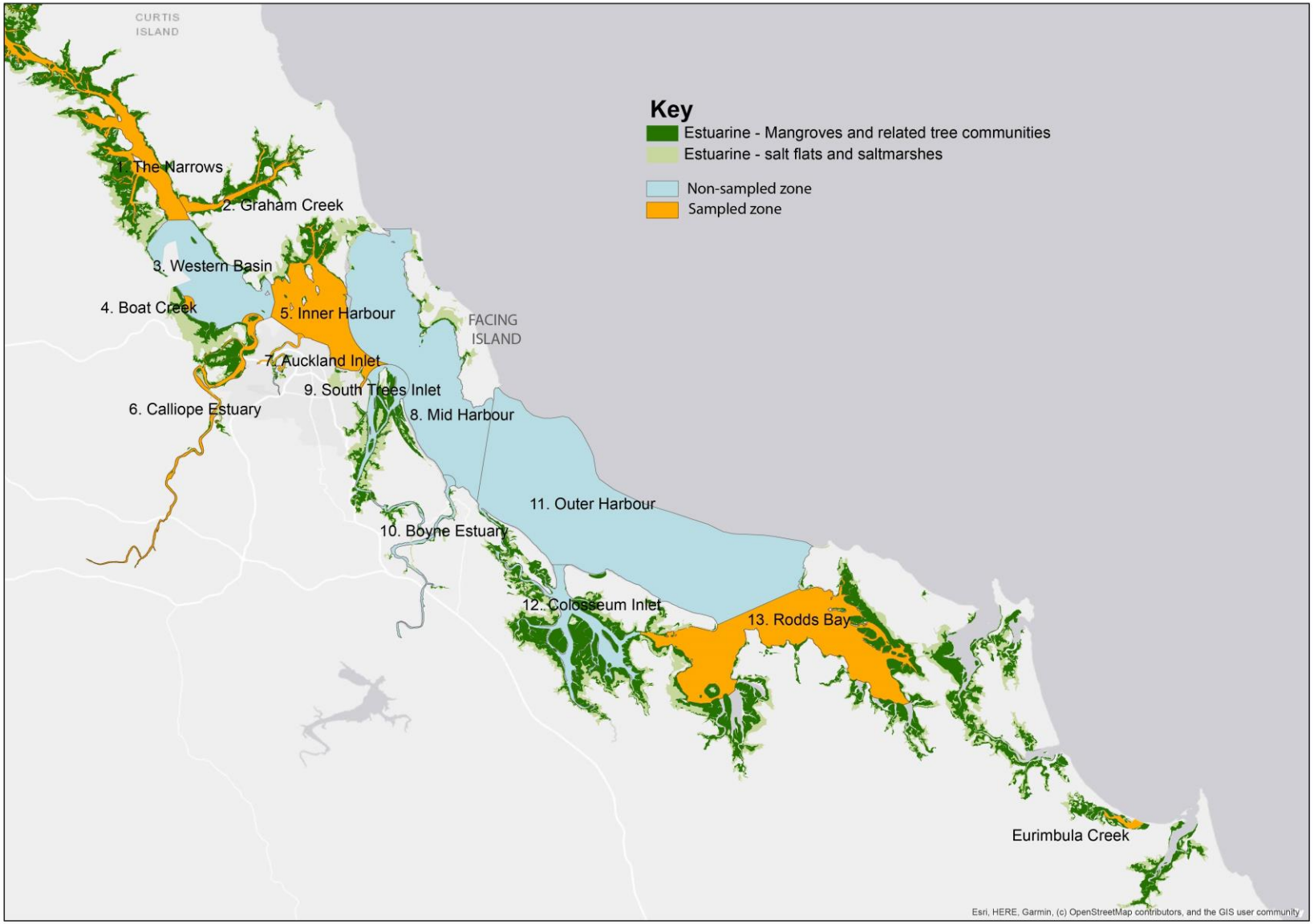

Figure 1: Map of the Gladstone Harbour zones showing long-term monitoring sites surveyed in 2017, 2018 and 2019. The map also shows the location of Eurimbula Creek, sampled in 2018 and 2019 to assess sex ratios.

Data analysis

Data from the first (14-17 February 2019) and second (27-30 June 2019) Gladstone Harbour field surveys were analysed separately and then together. Exploratory analyses included descriptive statistics, for example distribution plots (kernel density), and box plots for visual comparisons of differences and variance around the mean. Welch's unequal variances t-tests were used to test the hypothesis that two populations (of different sample sizes and variances) have equal means.
All analyses were conducted in R version 3.4.1 (https://www.r-project.org/).

Scoring, grading and aggregation

The mud crab measures were calculated for each Zone, as follows:

- **Abundance** (CPUE) in each zone

  $$= \frac{\text{(total number of mud crabs)}}{\text{(number of pots set)}}$$

- **Prevalence of rust lesions** in each zone

  $$= \frac{\text{(number of crabs with rust lesions)}}{\text{(number of crabs assessed for rust lesions)}}$$

- **Sex ratio** based on oversize mud crabs, for each zone

  $$= \frac{\text{(number of male mud crabs > 150 mm)}}{\text{(number of female mud crabs > 150 mm)}}$$

The formulae provided in Table 2 were used to score the mud crab measures, comparing each index value against a pilot benchmark and a worst case scenario (WCS) value. Using this method, index values worse than the WCS score a 0, while index values better than the benchmark score a 1 and all other index values range between these bounds. The method for determining benchmark and WCS values for each measure is described by Flint et al. (2017), with two revisions accounting for new information in 2018. The Gladstone Harbour Report Card grading system is provided in Table 3.

Sex ratio of legal-sized crabs (> 150 mm carapace spine width, which is equivalent to a crab with 143 mm notch width) is calculated against a 'minimally disturbed' benchmark from the international literature. In 2017 a conservative ratio of 3:1 (*sec.* Alberts-Hubatsch *et al*., 2016) was used as the benchmark for sex ratio (Flint *et al.*, 2017). In 2018 an unpublished thesis describing sex ratios in unfished Australian estuaries was made available (Butcher, 2004). The sex ratio in the thesis, 2 males:1 female, was equivalent to results in a separate study from an uncrabbed area in Moreton Bay (Pillans et al., 2005). As a result of this information, the benchmark for sex ratio has been updated to 2:1 for 2018. In 2019, the full set of data are available for an unfished region in Central Queensland (Eurimbula Creek, which is located approximately 20 km south of Rodds Bay), so this benchmark has again been reviewed (see the *Eurimbula Creek results* section of this report).

The benchmark for the abundance measure was also updated in 2018. In 2017, the 75th percentile of the 2017 scores was used as the benchmark. In 2018, a moving average of the 75th percentile of this year (2018) and previous years (2017) scores was applied. In 2019, the moving average of the 75th percentile of this year (2019) and previous years (2018, 2017) scores was applied. It is recommended that this approach continues in 2020 and beyond, using the 10-year moving average methodology.

Table 2: Benchmarks and scoring method for each of the three recommended measures. NC = not calculable.

Measure	**Benchmark** and basis	**Worst case scenario** and basis	**Method of calculation**
Abundance (CPUE)	2017: 3.5 crabs/pot (75th %ile of 2017 scores) 2018: 2.5 crabs/pot (moving average of 75th %ile of 2017 and 2018 scores) **2019: 2.12 crabs/pot (moving average of 75th %ile of 2017, 2018 and 2019 scores)** 2020+: Moving average of 75th %ile of scores for current and previous years, up to 10 years	**0.25** Min of 2017 data (0) 25th percentile of 2017 data (0) Min of LTMP data (0) 25th percentile of LTMP data (0) Catch rate of < 1 crab per allowable 4 pots (0.25)	The function used to calculate scores for abundance is: 1-((x-B)/(WCS-B)) Where: x = recorded CPUE B = benchmark (2.12) WCS = worst case scenario (0.25)
Prevalence of rust lesions	**4% = 0.04** Background level proposed by Sindermann, 1989 (5%) 25th percentile of 2017 data (4%)	**35% = 0.35** Dennis et al. 2016 mean prevalence in Gladstone Harbour (37%)	The function used to calculate scores for prevalence is: 1-((x-B)/(WCS-B)) Where: x = recorded prevalence B = benchmark (0.04) WCS = worst case scenario (0.35)
Sex ratio	2017: 3 (based on unfished tropical mud crab populations from the literature, Alberts-Hubatsch et al., 2016) 2018: 2 (based on new information from Australian estuaries) **2019: 2 (ratio from an unfished Central Queensland population at Eurimbula; see the *Eurimbula Creek results* section of this report)**	**0.25** 25th percentile of LTMP data (0.25) Median of LTMP data (0.26) Mean of LTMP data (0.25)	The function used to calculate scores for sex ratio is: 1-((x-B)/(WCS-B)) Where: x = recorded sex ratio (M:F) B = benchmark (2) WCS = worst case scenario (0.25)

Table 3: Gladstone Harbour Report Card grading scale (Source: GHHP, 2015).

Score	**Grade**
>=0.85	A
>=0.65, <0.85	B
>=0.5, <0.65	C
>=0.25, <0.5	D
0, <0.25	E

Results

Eurimbula Creek results

June 2018

A total of 56 mud crabs were caught during a single day of sampling at Eurimbula Creek on 26 June 2018, using 20 crab pots deployed for at least five hours. There were 35 male mud crabs, 20 female mud crabs and one additional male mud crab which escaped through a hole in the pot and was omitted from further analysis.

Of the 55 mud crabs analysed, average size (notch width) was 145.16 mm for the full sample, 143.54 mm for males (which is above the legal size limit of 143 mm notch width / 150 mm spine width), and 148.00 mm for females. Results of a Welch Two sample t-test found that there was no significant difference between the size of females and males caught at Eurimbula Creek ($t = -1.0389$, $df = 32.888$, $p = 0.306$). Average weight of the full sample of mud crabs caught at Eurimbula Creek was 773.64 g. The average male mud crab weight was 838.14 g while the average female weight was 660.75 g. A Welch Two sample t-test found that male mud crabs caught at Eurimbula Creek were significantly heavier than female mud crabs ($t = 2.9166$, $df = 52.572$, $p = 0.005$).

Catch per unit effort was 2.80 crabs per pot, and every pot contained at least one mud crab. In June 2018 the sex ratio of male:female crabs over the legal size limit of 150 mm carapace width (equivalent to 143 mm notch width) was 1.43. That is, there were 1.4 oversize male mud crabs caught for each oversize female mud crab. This was higher than recorded in the Gladstone Harbour zones, where less oversize males than females were caught in June 2018 at all seven zones. Of the 55 crabs analysed from Eurimbula Creek, only one male mud crab had a small rust lesion, a prevalence of 1.82%, similar to the Gladstone Harbour average of 3% in June 2018.

February 2019

On 28 February 2019, 25 mud crabs were caught at Eurimbula Creek using 20 crab pots; 17 were male and eight were female.

Average size (notch width) of the whole sample was 147.28 mm, of the male crabs was 148.88 mm (above the legal size limit) and 143.88 mm for females. A Welch Two sample t-test found that there was no significant difference between the size of females and males caught at Eurimbula Creek ($t = 0.62281$, $df = 8.7931$, $p = 0.5492$). Average weight of the full sample of mud cabs was 841.80 g. The average male mud crab weight was 936.76 g and the average female weight was 640.00 g. A Welch Two sample t-test found that males were again significantly heavier than female mud crabs at Eurimbula ($t = 3.0216$, $df = 14.113$, $p = 0.0091$).

Catch per unit effort was lower than in June 2018, at 1.25 crabs per pot. The sex ratio of male:female crabs over the legal size limit was 2.6 (there were 2.6 males for every female caught) in February 2019. Two of the 25 mud crabs had rust spot lesions, a prevalence of 8%.

Eurimbula Creek scores and grades

For comparison to Gladstone sites, scores and grades have been calculated for Eurimbula Creek using the June 2018 and February 2019 sampling data. These scores and grades are not for inclusion in the 2019 Gladstone Harbour Report Card, as the site lies outside of the GHHP reporting zones. However, they provide an interesting contrast and test case of the scoring system in a relatively environmentally pristine site with no crabbing pressure.

Results for each sampling period and for the combined sampling event (constituting a full year) are provided in Table 4. For all three mud crab measures, and for the mud crab indicator, Eurimbula Creek would be graded A (Table 5).

Table 4. Calculated index values and scores for June 2018 and February 2019 at Eurimbula Creek, by measure.

	Jun-2018				Feb-2019			
	Index value	B	WCS	Score	Index value	B	WCS	Score
Prevalence of rust lesions	0.02	0.04	0.35	1.07	0.08	0.04	0.35	0.87
Sex ratio	1.43	2	0.25	0.67	2.6	2	0.25	1.34
Abundance	2.80	2.12	0.25	1.36	1.25	2.12	0.25	0.53

Table 5. Comparative scores and grades for measures and the overall mud crab indicator, from Eurimbula Creek.

	Scores and grades for June 2018 and February 2019 combined			
Zone	**Abundance (CPUE)**	**Prevalence of rust lesions**	**Sex ratio**	**Overall score**
Eurimbula Creek	0.95	0.97	1.00	0.98

Results of mud crab sampling in Gladstone Harbour, February and June 2019

Abundance and size

A total of 106 mud crabs were caught in the seven Gladstone Harbour zones in February 2019. Of these, 47 were male and 59 were female. A total of 183 mud crabs were caught across all Gladstone Harbour zones sampled in June 2019 including 59 males and 124 females. No mud crabs were caught at Auckland Inlet in February 2019 and only one in June 2019.

The average size of mud crabs caught in February 2019 was 146.53 mm carapace notch width (Table 6), smaller than crabs caught in June 2018 (average 152.84 mm carapace notch width, Table 6). The pattern of catching smaller crabs in February than in June was also identified in 2018 (February 142.53 mm and June 150.37 mm). In June and July 2017, the mean notch widths recorded were 149.16 mm and 155.00 mm respectively. Smaller crabs were also caught in summer during monitoring conducted by the Fisheries Queensland Long Term Monitoring Program (LTMP, average 145.45 mm, details of crabs caught in the LTMP are provided by Flint et al., 2017).

Results of Welch Two sample t-tests found that females caught in February 2019 were significantly larger than males ($t = -3.5616$, $df = 103.85$, $p < 0.01$; Figure 2), as identified in 2018. Females caught in June 2019 were also significantly larger than males ($t = -7.5433$, $df = 113.29$, $p < 0.001$; Figure 3).

The largest average mud crab size in February 2019 was recorded for Rodds Bay (mean notch width 156.00 mm) and the smallest for Boat Creek (121.73 mm) (Figure 4), while the largest average mud crab size in June 2019 sampling was recorded for Calliope Estuary (mean notch width 162.75 mm) and the smallest again at Boat Creek (137.04 mm) (Figure 5). In 2017 samples the smallest average mud crab size was also recorded at Boat Creek, while in 2018 the smallest average size was at Calliope Estuary.

Table 6. Notch width (in mm) of mud crabs caught in February 2019 and June 2019.

	FULL SAMPLE		MALES		FEMALES	
	February 2019 data	**June 2019 data**	**February 2019 data**	**June 2019 data**	**February 2019 data**	**June 2019 data**
Mean	146.53	152.84	138.47	139.85	152.95	159.02
Standard deviation	22.52	18.34	18.14	16.12	23.70	15.98

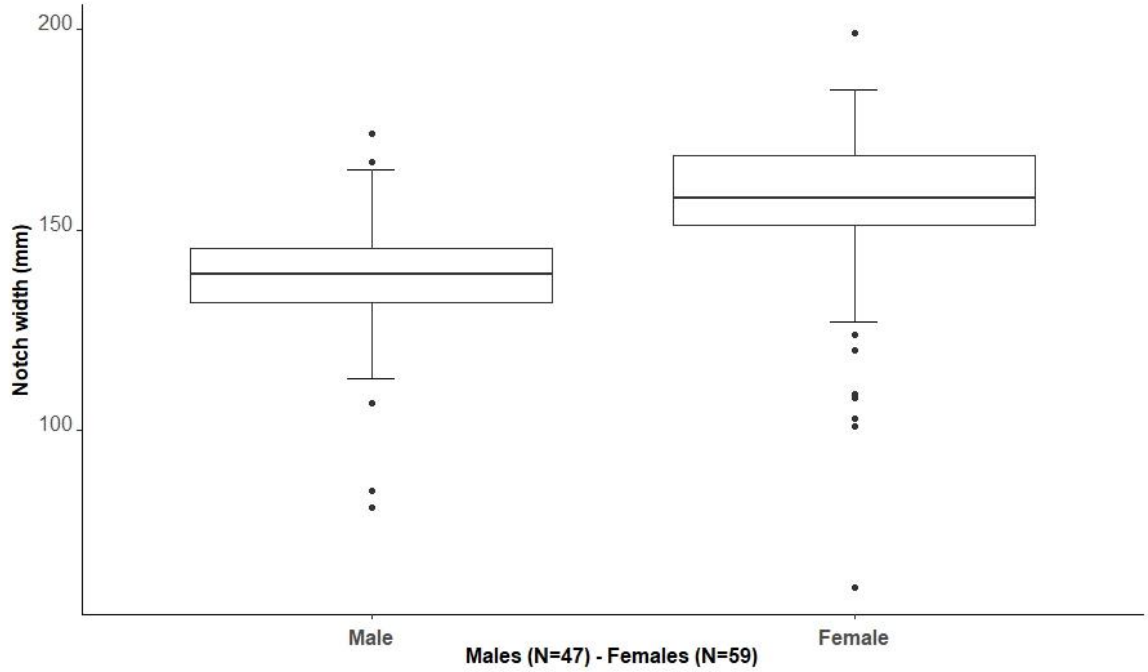

Figure 2: Notch width (mm) distribution of male and female mud crabs caught in February 2019. Centre line is the mean, the lower and upper hinges correspond to the first and third quartiles. Whiskers extend from the hinge to the smallest and largest values no greater than 1.5 times the inter-quartile range from the hinge. Data beyond the end of the whiskers are flagged as outliers and plotted individually as circles.

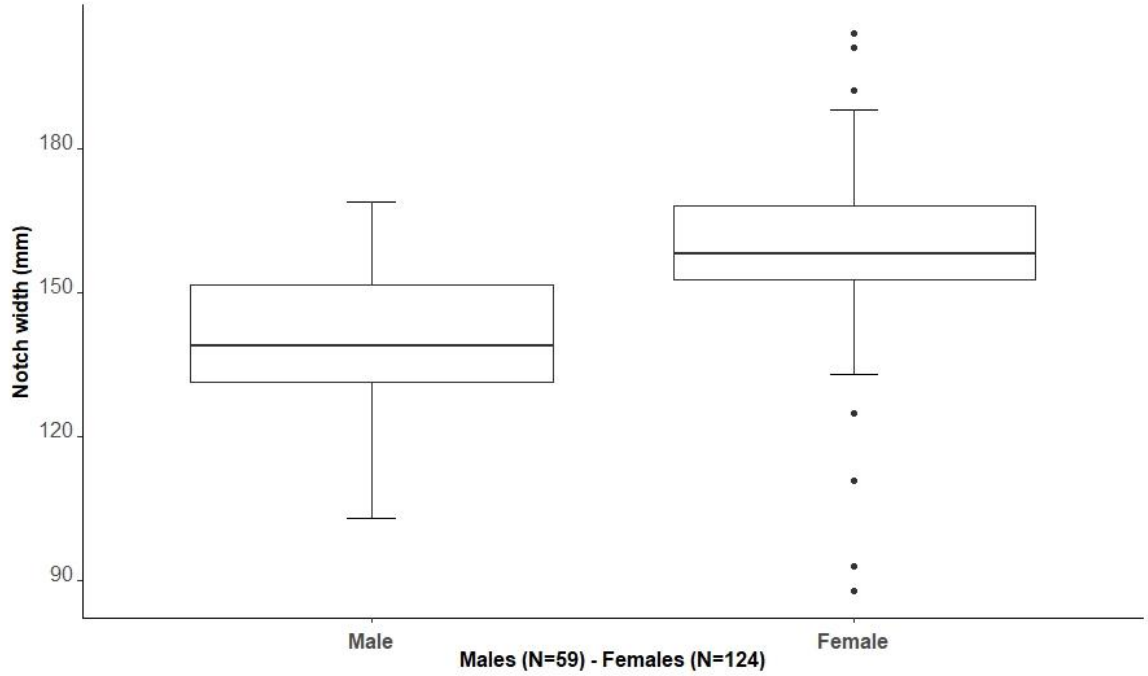

Figure 3: Notch width (mm) distribution of male and female mud crabs caught in June 2019.

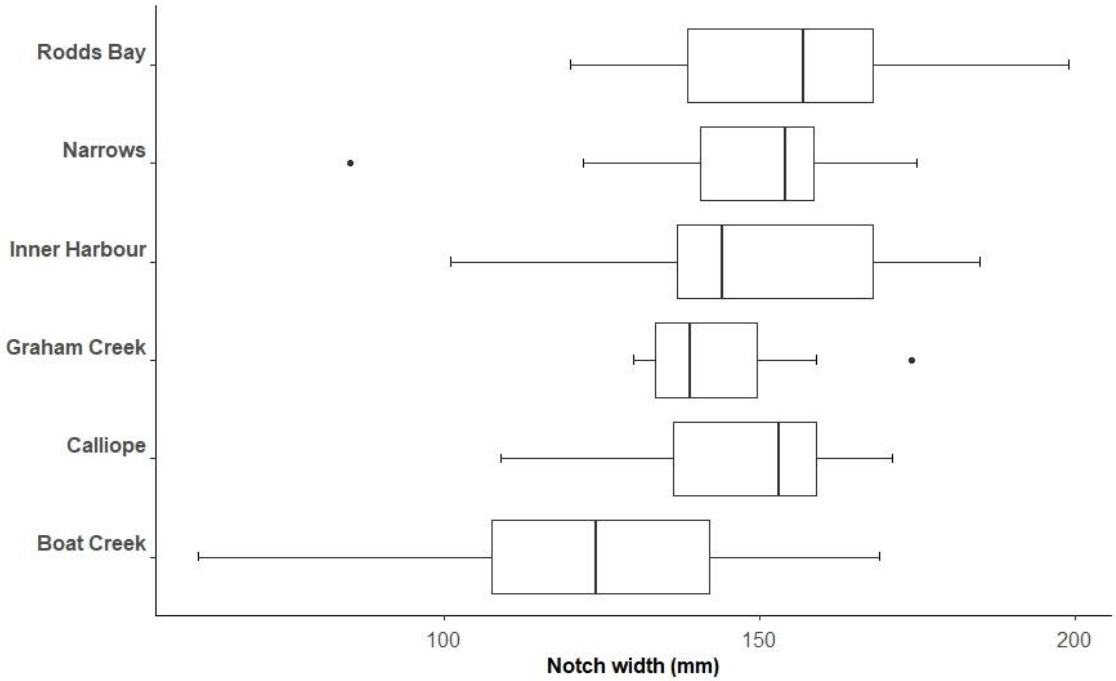

Figure 4: Notch width (mm) of mud crabs caught in February 2019, by zone.

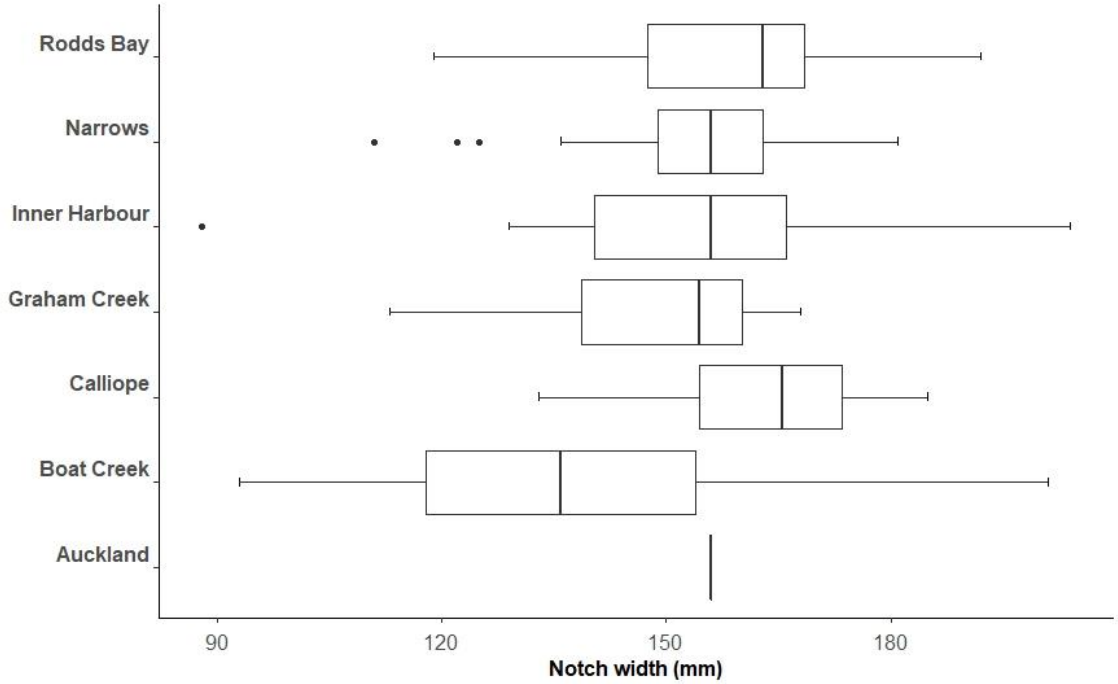

Figure 5: Notch width (mm) of mud crabs caught in June 2019, by zone.

The average weight of mud crabs caught in February 2019 was 718.40 g, and in June 2018 was 732.66 g, similar to the averages of 715.47 g and 762.28 g in February and June 2018, and 718.44 g and 741.28 g in June and July 2017 (Table 7, Figures 6 and 7). Similar to February 2018 (but unlike 2017 and June 2018), results of a Welch Two sample t-test found that there was no significant difference between weight of females and weight of males caught in February 2019 ($t = -0.68423$, $df = 89.454$, $p = 0.496$), or in June 2019 ($t = -1.309$, $df = 84.556$, $p = 0.194$), at the $p < 0.05$ level.

The zone with the highest average mud crab weight in February 2019 was Rodds Bay (mean weight 840.79 g) (Figure 8), substantially lower than the highest average weight in February 2018 at Auckland Inlet (mean weight 1691.00 g – but based on only 2 mud crabs). In June 2019 the zone with the highest average mud crab weight was Auckland Inlet (1100.00 g, but from only one mud crab) and the next highest was recorded at Calliope Estuary (921.75 g) (Figure 9).

Table 7: Weight (g) of mud crabs caught in February and June 2019.

	FULL SAMPLE		MALES		FEMALES	
	February 2019 data	**June 2019 data**	**February 2019 data**	**June 2019 data**	**February 2019 data**	**June 2019 data**
Mean	718.40	732.66	698.94	694.15	733.90	750.98
Standard deviation	255.46	241.52	280.81	301.72	234.64	205.76

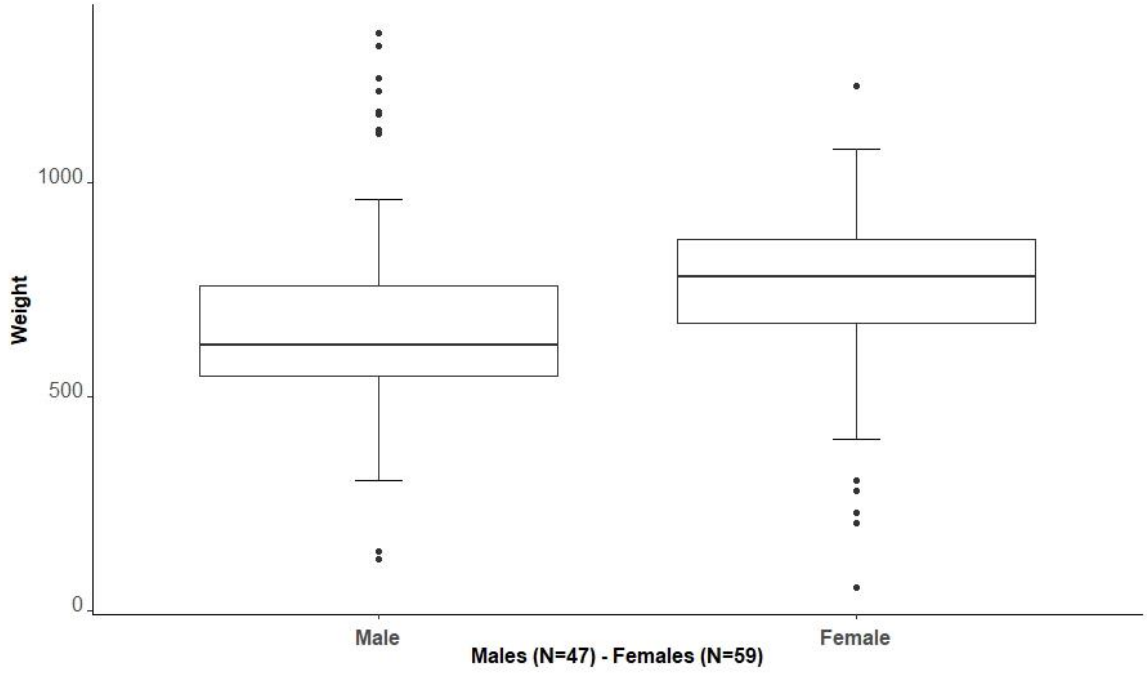

Figure 6: Weight (g) of male and female mud crabs caught in February 2019.

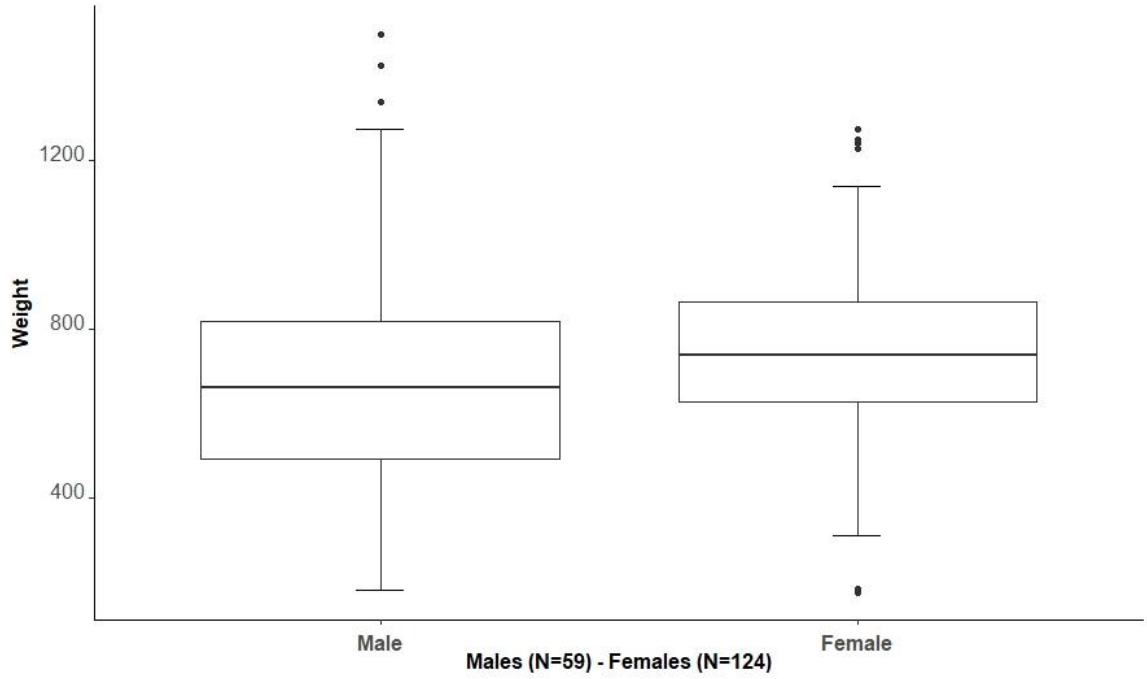

Figure 7: Weight (g) of male and female mud crabs caught in June 2019.

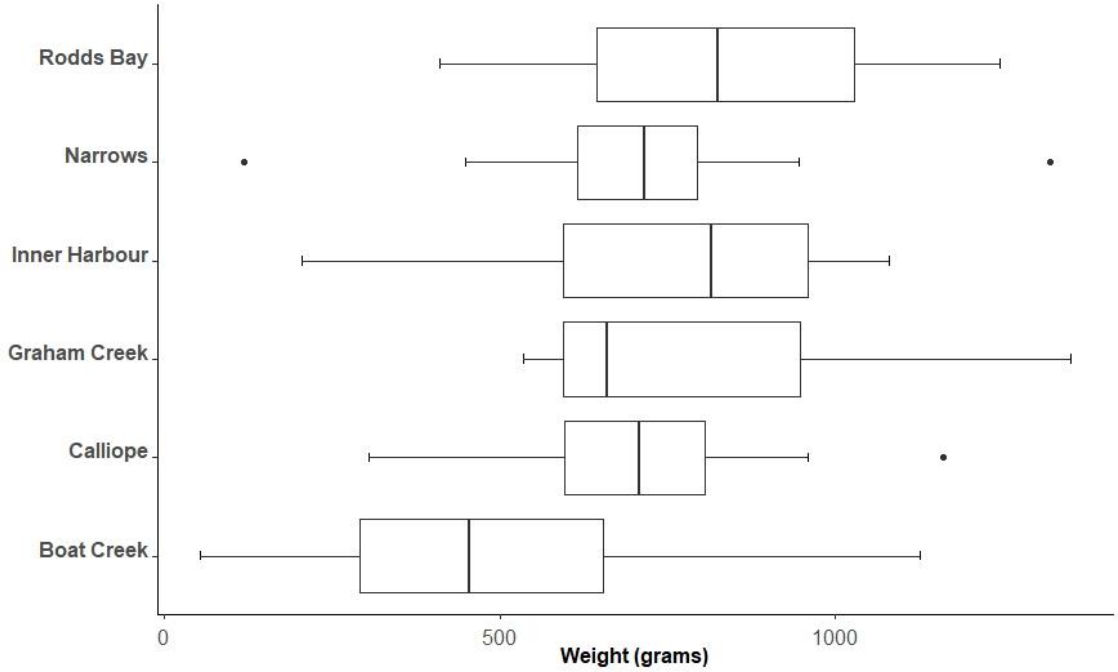

Figure 8: Weight (g) of mud crabs caught in February 2019, by zone. No mud crabs were caught at Auckland Inlet.

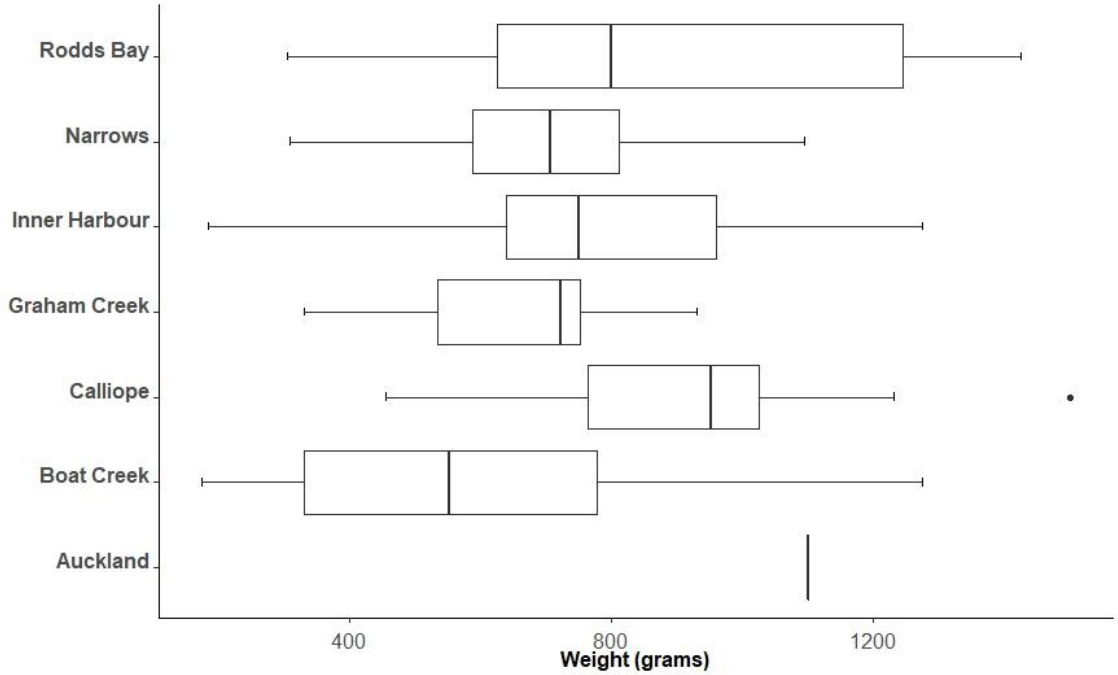

Figure 9: Weight (g) of mud crabs caught in June 2019, by zone. Only one mud crab was caught at Auckland Inlet.

In February 2019, for the third year in a row, total catch per unit effort (CPUE) was again highest at the Narrows (1.60) and lowest at Auckland Inlet (0) (Table 8, Figure 10). CPUE was also highest at the Narrows in June 2019 (4.0) and lowest at Auckland Inlet (0.05) (Table 8, Figure 11).

Table 8. Catch per unit effort in February 2019 and June 2019, by zone.

Zone	Zone name	February 2019 data			June 2019 data		
		# Pots deployed	Total # mud crabs caught	CPUE	# Pots deployed	Total # mud crabs caught	CPUE
1	Narrows	20	32	1.60	20	80	4.00
2	Grahams Creek	20	7	0.35	20	12	0.60
4	Boat Creek	15	11	0.73	16	24	1.50
5	Inner Harbour	20	17	0.85	20	43	2.15
6	Calliope Estuary	20	20	1.00	20	12	0.60
7	Auckland Inlet	20	0	0	20	1	0.05
13	Rodds Bay	20	19	0.95	20	11	0.55
	Harbour average			**0.91**			**1.35**

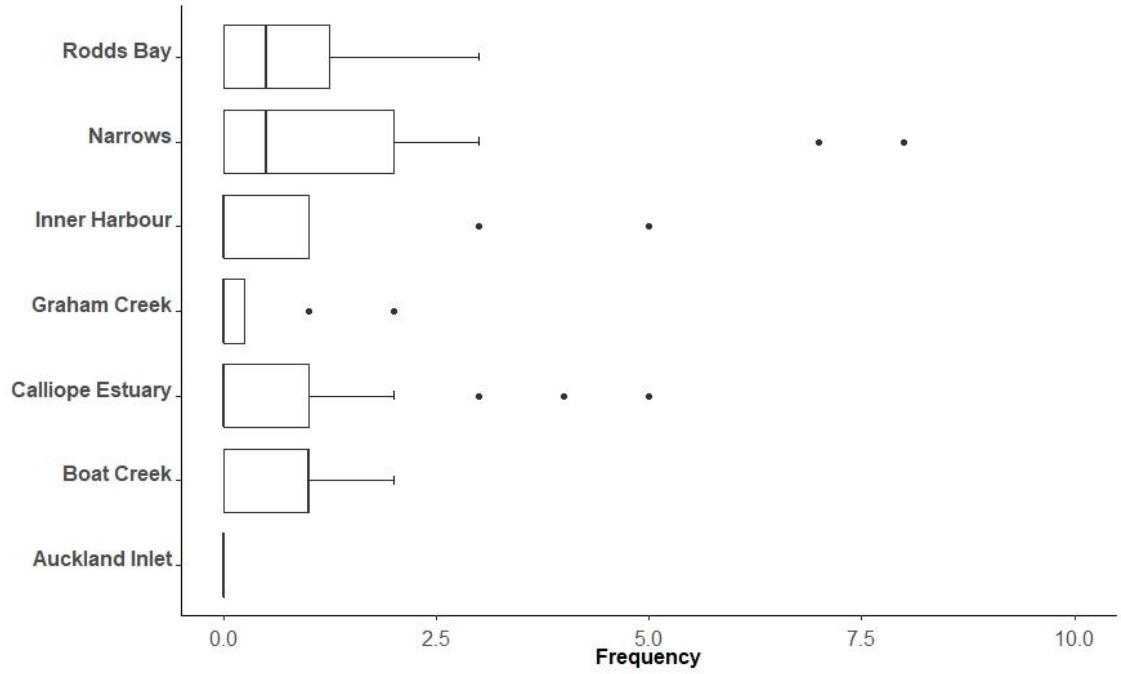

Figure 10: Number of mud crabs in each pot set in February 2019, by zone. No mud crabs were caught in Auckland Inlet.

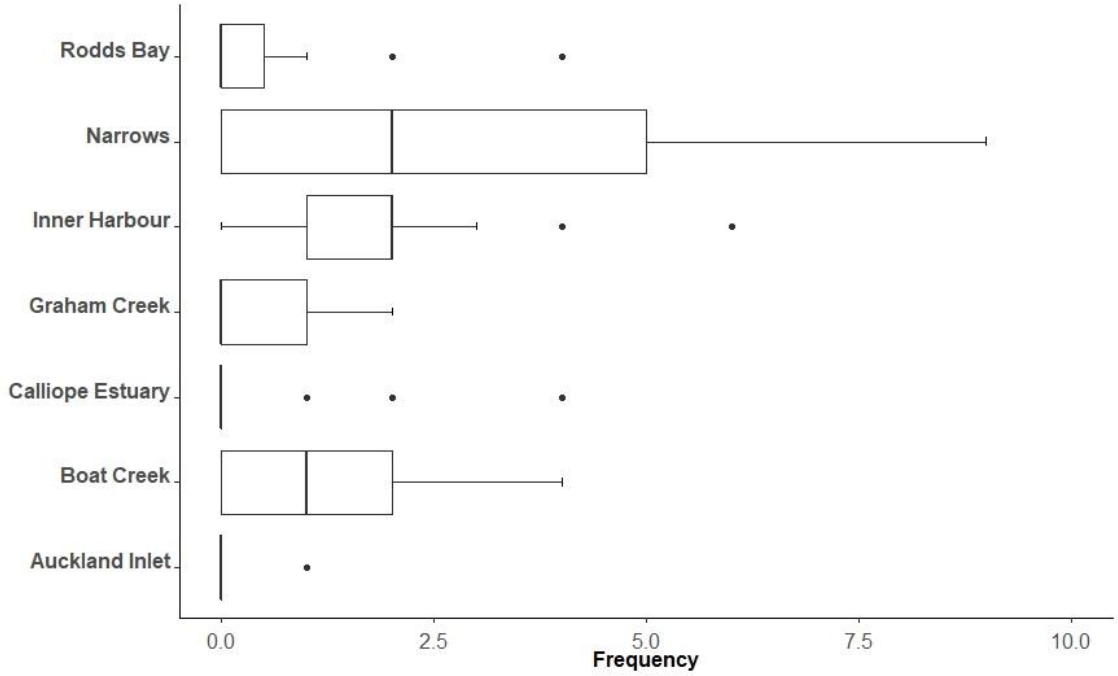

Figure 11: Number of mud crabs in each pot set in June 2019, by zone. Only one mud crab was caught in Auckland Inlet.

Sex ratio

In February and June 2019, many more oversize female crabs were caught than oversize male crabs in Gladstone Harbour. Sex ratios were therefore low across the harbour in both February and June (Table 9). A total of 197 mud crabs over the legal size limit of 150 mm carapace width (equivalent to 143 mm notch width) were caught in 2019, and only 37 were male. This result is similar to 2018, when 201 mud crabs over legal size were caught and only 38 were male. All zones had more than 2 females to every 1 male when the two sampling periods were combined, compared to the benchmark from unfished populations of 2 males to every 1 female.

Table 9: Sex ratios of mud crabs in February and June 2019, by zone. NC = not calculable.

Zone	Zone name	February 2019 data			June 2019 data		
		Males > 143mm notch width	**Females > 143mm notch width**	**Sex ratio**	**Males > 143mm notch width**	**Females > 143mm notch width**	**Sex ratio**
1	Narrows	4	17	**0.24**	3	66	**0.05**
2	Grahams Creek	2	0	**NC**	2	6	**0.33**
4	Boat Creek	1	2	**0.5**	2	7	**0.29**
5	Inner Harbour	2	7	**0.29**	9	21	**0.43**
6	Calliope Estuary	2	11	**0.18**	2	8	**0.25**
7	Auckland Inlet	0	0	**NC**	1	0	**NC**
13	Rodds Bay	5	9	**0.56**	2	6	**0.33**
	Harbour average			**0.45**			**0.28**

Rust lesions

Of the 106 mud crabs captured in February 2019, only six had rust lesions and 103 did not. During February 2019, crabs with lesions were caught at the Narrows (12.5%; from four mud crabs), Inner Harbour (5.88%; from one mud crab) and Calliope Estuary (5%; from 1 mud crab). Prevalence was zero at all other zones (Table 10). Prevalence of rust lesions was higher in June 2019, with 17 of 183 crabs presenting with rust spot lesions. The percentage of crabs with lesions in June 2019 was highest at Rodds Bay (36.4% from four mud crabs), Inner Harbour (16.3% from seven mud crabs), Boat Creek (8.33% from two mud crabs) and the Narrows (5% from four mud crabs) (Table 10). These results are substantially different to June 2018, when only four of 162 mud crabs displayed rust spot lesions. During June 2018, the percentage of crabs with lesions was also highest at Rodds Bay (11.76%; from two mud crabs), Boat Creek (7.69%; from one mud crab) and the Narrows (1.67%, from one mud crab).

Table 10: Percentage of mud crabs caught in February and June 2019 with and without rust spot lesions, by zone. NC = not calculable.

Zone	Zone name	February 2019 data				June 2018 data			
		Mud crabs with lesions	Mud crabs without lesions	% without lesions	% with lesions	Mud crabs with lesions	Mud crabs without lesions	% without lesions	% with lesions
1	Narrows	4	28	87.50	**12.50**	4	76	95.00	**5.00**
2	Grahams Creek	0	7	100	**0**	0	12	100	**0**
4	Boat Creek	0	11	100	**0**	2	22	91.66	**8.33**
5	Inner Harbour	1	16	94.12	**5.88**	7	36	83.7	**16.30**
6	Calliope Estuary	1	19	95.00	**5.00**	0	12	100	**0**
7	Auckland Inlet	0	0	NC	**NC**	0	1	100	**0**
13	Rodds Bay	0	19	100	**0**	4	7	63.6	**36.4**
	Harbour average				**3.90%**				**9.43%**

Biomass

Three years of mud crab data have now been collected in Gladstone Harbour by CQUniversity (June/July 2017, February and June 2018, and February and June 2019). Exploratory data analysis conducted using these data identified a series of covariates and outliers that influence the biomass measure:

- *Seafood grade* (A, B or C, determined using the FRDC mud crab grading scheme). As expected, there were significant differences in biomass between grades.
- *Missing limbs.* Missing limbs, particularly the large claws, result in a lower body weight for size. It was decided to retain crabs with missing limbs in the dataset because this provides information on the overall condition of the mud crab population. A significant difference in biomass between crabs with and without missing limbs was identified.
- *Outliers.* Several outliers were identified, and visual inspection of the data showed that biomass was very high in small, sub-adult crabs (< 100 mm carapace width) of both sexes.

A reduced dataset, adjusted for seafood grade and with outliers removed, was then investigated. Biomass is a function of carapace width, so it is not surprising that these two parameters are highly

correlated. However, the pattern in biomass that was identified is one of decreasing biomass with increasing carapace width. That is, as crabs grow larger, their width-weight ratio declines. This means a benchmark and WCS for the CW/BW measure cannot be set, because a sample of large healthy crabs would potentially score the same as a sample of smaller crabs in poor condition. To confirm that this size/condition relationship also exists in an unfished crab population, data from Eurimbula Creek mud crabs were plotted in the same way, and a similar decline in biomass with increasing size was identified.

On the basis of these analyses, and in discussion with the GHHP ISP, the biomass measure has been removed from consideration for future inclusion in the mud crab indicator.

Mud crab measure results by zone

The mud crab data set used to score each selected zone for the 2019 Gladstone Harbour Report Card included combined data from both February and June 2019. Results for each measure are provided by zone in Table 11.

Table 11: Calculated index values for 2019, for each of the four recommended measures in each of the seven recommended long term monitoring sites. NC = not calculable (Auckland Inlet, < 5 crabs caught in 2019; Biomass measure currently under development).

Zone	Zone name	Abundance (CPUE)	Prevalence of rust lesions	Sex ratio
1	Narrows	2.80	0.07	0.08
2	Graham Creek	0.48	0	0.67
4	Boat Creek	1.12	0.06	0.33
5	Inner Harbour	1.50	0.13	0.39
6	Calliope Estuary	0.80	0.03	0.21
7	Auckland Inlet	0	NC	NC
13	Rodds Bay	0.75	0.13	0.47
	Harbour wide average	**1.23**	**0.02**	**0.37**

Indicator grades

Scores and grades for the mud crab measures for the 2019 Report Card are provided in Table 12. Scores > 1 and < 0 have been bounded by [1, 0] in line with GHHP standard methods (GHHP, 2014). A combined score for the Mud Crab Indicator has been calculated as the average of the three measure scores, and an overall grade is provided for each zone and for the Harbour. Only one mud crab was caught in Zone 7 – Auckland Creek. Given the very small sample size (< 5 mud crabs from 40 pots) it is not appropriate to calculate scores and grades for the prevalence of rust lesions or sex ratio measures in this zone.

Table 12: Scores and grades for mud crab measures and the mud crab indicator by Zone. NC = Not calculable, n < 5.

Zone	Abundance (CPUE)	Prevalence of rust lesions	Sex ratio	Zone score 2019
1. The Narrows	1	0.90	0	0.63
2. Graham Creek	0.12	1	0.24	0.45
4. Boat Creek	0.46	0.94	0.05	0.48
5. Inner Harbour	0.67	0.70	0.08	0.48
6. Calliope Estuary	0.29	1	0	0.43
7. Auckland Inlet	0	NC	NC	NC
13. Rodds Bay	0.27	0.70	0.12	0.36
Harbour Average				0.47

Discussion

Indicator scores and grades

The overall grades for the Mud Crab Indicator for Gladstone Harbour in 2019 are as follows:

A: No zones.

B: No zones.

C: Zone 1 – Narrows.

D: Zone 2 – Graham Creek, Zone 4 – Boat Creek, Zone 5 – Inner Harbour, Zone 6 – Calliope Estuary, Zone 13 – Rodds Bay.

E: No zones.

Not Calculable (< 5 mud crabs caught): Zone 7 – Auckland Creek.

The Harbour Average was graded a D. The zone grades and the Harbour Average are slightly lower than those reported in 2018.

Changes in data collection and scoring

While the 2017 pilot year only included winter sampling (June/July), in 2018 and 2019 both summer and winter samples were collected, in February and June respectively. The inclusion of both summer and winter sampling allows for the detection of seasonal changes in the local mud crab population and provides a more representative assessment of the conditions in the harbour.

In June 2018 and February 2019, GHHP also commissioned CQUniversity to undertake mud crab surveys at Eurimbula Creek. In this creek in the Baffle Catchment, directly south of Gladstone Harbour (the same catchment as Rodds Bay), recreational and commercial crabbing is prohibited under Queensland legislation. This regulation, along with the generally good environmental condition of the creek, means that the population of mud crabs at Eurimbula Creek is in relatively pristine condition.

The surveys that we conducted bore this out – if Eurimbula Creek was scored in 2018/19 using the GHHP mud crab indicator the creek would score A for abundance, A for prevalence of rust lesions, A for sex ratio and A overall. These results demonstrate the relevance of Eurimbula Creek as a reference site for mud crab surveys.

The primary reason for collecting data from Eurimbula Creek was to test the benchmark for the sex ratio measure. The sex ratio measure compares the number of male crabs over the legal size limit (150 mm carapace width, equivalent to 143 mm notch width) to female crabs over the (male) legal size limit. A benchmark for the sex ratio measure was originally established using international scientific literature from unfished mud crab populations in the 2017 pilot year and adjusted in 2018 in response to results of a previously unavailable unpublished thesis from unfished estuaries in northern New South Wales. The results of the thesis (Butcher, 2004) aligned with results from a study in a small uncrabbed region in Moreton Bay, southern Queensland (Pillans et al., 2005). Both studies found a ratio of two male mud crabs to one female mud crab.

The results from Eurimbula Creek confirm a sex ratio of two male to one female mud crab, so the benchmark used in 2018 has been retained in 2019.

Measure scores and grades

Scores have decreased slightly across all zones in 2019, and some grades have also fallen as a result. Zone 1 – the Narrows, scored a C in 2019, compared to a B in both 2017 and 2018, but the difference was marginal (0.67 in 2017 and 2018, vs 0.63 in 2019) and driven by a 0.1 decrease in the score for prevalence of rust lesions. The grade for Zone 2 – Graham Creek is the same as in 2018 (D) and abundance was lower in that zone in 2019. Grades for Zone 4 – Boat Creek, Zone 5 – Inner Harbour and Zone 6 – Calliope Estuary have both dropped in 2019, falling from C grades to D, again with only very slight decreases in overall scores, driven by poor abundance and sex ratio scores. Zone 13 – Rodds Bay has remained the same as 2017 and 2018 with a score of D.

As in 2018, an overall score for Zone 7 – Auckland Inlet was again incalculable in 2019 due to very low catches (n = 1 across February and June 2019). In situations where less than five mud crabs are caught in a zone, abundance can be scored but the sample size is insufficient to give a reliable indication of the prevalence of rust lesions or sex ratio.

Lower mud crab catches were achieved in most zones in 2019, except the Narrows, despite the use of a moving average benchmark. Abundance can indicate pressures such as habitat availability, extraction (fishing) and recruitment limitation, although natural biological variation can also be a factor, so the lower grade is not necessarily a cause for concern based on a single year. Some of the possible (non-anthropogenic) factors affecting catchability of mud crabs include moult state of crabs, reproductive cycles, lunar and diel cycles, temperature and water motion (Knuckey, 1999). A more worrying trend would be a long-term decline in numbers over a number of years, which may indicate impacts such as a reduction in suitable habitat or overfishing. It is currently too early in the development and grading of the Gladstone Harbour mud crab indicator to establish trends, but the incorporation of a 10-year moving average methodology in setting the abundance benchmark from 2018 onwards helps to allow for these natural variations. It is relevant to note that the reference, unfished and high-quality site at Eurimbula Creek would have been graded an A for abundance.

The abundance measure was scored in 2019 with the benchmark of a moving average of the 75th percentile of abundance scores across the harbour in 2017, 2018 and 2019. A 10-year moving average benchmark will continue to be calculated for abundance, to allow for natural variations in catchability and abundance while still providing for the analysis of trends through time. In the 2017 pilot year, the 75th percentile of the 2017 abundance scores was used as the benchmark. In 2017 and 2018, a catch rate of less than one crab in four pots has been used as a WCS. The WCS of 0.25 crabs/pot is based on assumed recreational fisher preferences of catching a crab (Flint et al., 2017). An alternative approach would be to apply commercial fisher preferences. Knuckey (1999) suggested crabbers in the Northern Territory tend to cease crabbing when the saleable catch is <0.1 kg/pot.day, which in Queensland equates to about one oversized male crab in every 8-9 pots.

The prevalence of rust lesions measure scored an A grade in four zones in 2019 and B in two zones, while in 2018 it was rated A in the same six zones. This measure is based on a moderately-high confidence benchmark and WCS developed using research data published by Andersen and Norton (2001) and Dennis et al. (2016), and data collected in June 2017. Rust lesions were still recorded at relatively low prevalence in Gladstone Harbour in 2019.

In areas such as Queensland, where a sex-based fishery is enforced, changes in sex ratio (the ratio of oversize males to females) that can't be explained by biological factors such as spawning migrations, are likely to be indicative of a change in fishing pressure. The sex ratio measure scored an E in every zone in 2019, a similar result to 2018 when all zones except Boat Creek (D) also received Es. It is also worth noting, as mentioned in Flint et al. (2017) that the pattern observed suggests that fishers are observing regulations regarding the release of females.

Shifts in sex ratio caused by overfishing have implications for population dynamics and may also influence ecosystem processes through gender-biased behaviours such as burrow digging. Research is required to determine whether remaining small males are as successful at mating as larger males.

Biomass (CW:BW) was considered as an additional measure to incorporate into the mud crab indicator once three years of length and weight data were available in 2019. Based on the analysis of the three years of data and in discussion with the GHHP ISP, the biomass measure has been removed from consideration for future inclusion in the mud crab indicator. However, it would be useful to continue to monitor mud crab weight during biannual sampling, as these data may prove useful to answer future research questions, for example relating to reproduction or growth patterns. If in future GHHP decided to revisit a biomass measure, the data would be available for analysis.

In addition to the above measures, two potential indicators were identified by Flint et al. (2017) as potentially useful. Both would require additional research and/or monitoring costs. These two indicators were bioaccumulation of metals and recruitment to nursery grounds of juvenile crabs.

Of these, bioaccumulation is likely to be of higher interest and could be incorporated into the existing adult mud crab monitoring program. It requires lethal sampling of mud crabs to measure toxicant (e.g. metal) concentrations in the hepatopancreas, muscle tissue or gills, with additional costs for dissection and analysis. Bioaccumulation of toxicants can be a particularly relevant indicator for urban and industrialised areas such as ports and is measured using established methods.

Several studies have used mud crabs as bioindicators of contaminants including persistent organic pollutants (POPs) and metals (Mortimer, 2000), and pesticides (Negri *et al.*, 2009). In Gladstone, elevated metal (As, Cr, Cu, Fe, Hg, Mn, Ni, U, Zn) concentrations have previously been reported from the hepatopancreas mud crabs collected at two sites at Spillway Creek, in comparison to two additional sites in the same creek, and to sites in Wild Cattle Creek (Gladstone Harbour), Baffle Creek (south of Rodds Bay) and Ayr (North Queensland) (Andersen *et al.*, 2001). A subsequent study identified only Fe and Se of ten metals tested in hepatopancreas tissues (Andersen *et al.*, 2003), and both were recorded at concentrations below food safety guidelines.

Recommendations

The following additional recommendations are provided for the application of the mud crab indicator in future years. A review of the measures, and the benchmarks and WCSs used for each measure, should be conducted in 2019, when three years' worth of monitoring data are available.

1. **GRADING LOW CATCHES:** As in 2018, an overall score for Zone 7 – Auckland Inlet was again incalculable in 2019 due to very low catches (n = 1 across February and June 2019). In situations where less than five mud crabs are caught in a zone, abundance can be scored but the sample size is insufficient to give a reliable indication of the prevalence of rust lesions or sex ratio. We recommend that for the purposes of the report card, zones with catches of less than five mud crabs are reported as an 'E' grade for the mud crab indicator.
2. **SEX RATIO BENCHMARK:** Sampling at Eurimbula Creek confirmed an unfished sex ratio of two males to every one female over 150 mm carapace width (143 mm notch width). We recommend GHHP retains the existing benchmark of 2.
3. **OTHER MEASURES:** Bioaccumulation would potentially be useful to include if future budgets allowed. The bioaccumulation results would be of particular interest as an additional indication of water and sediment quality, and uses established methods.

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