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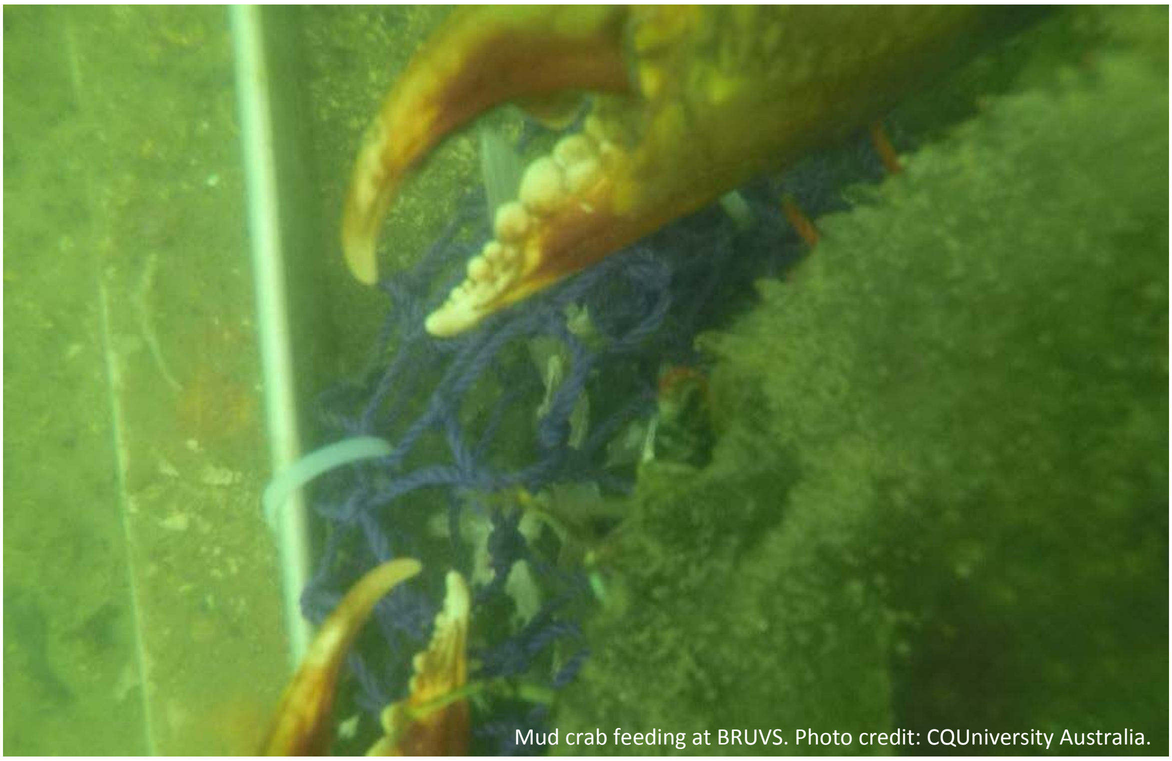
Developing Mud Crab Indicators for the Gladstone Harbour Report Card: Project ISP015-2017



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

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The authors would also 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 their elders, past, present and future.

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)

Executive summary

Mud crabs (primarily *Scylla serrata*) are recreationally and commercially important species in Gladstone Harbour, as well as an iconic seafood item, with cultural value to Indigenous Australian peoples (Brewster, 2015). The total Queensland annual catch in 2015 was approximately 1,000 tonnes worth AUD18.7 million. As a result of their commercial importance to the fishing and aquaculture industries, the biology, ecology and biochemistry of mud crabs is relatively well known. The green mud crab, *S. serrata*, has previously been suggested as a potential biomonitoring species in tropical coastal marine environments of Australia.

Ideal biomonitors are sedentary, easily identifiable, abundant, long-lived, available to sample throughout the year, large enough to provide sufficient tissue for analysis, resistant to handling stress and tolerant to environmental variations in physicochemical parameters. To be regionally relevant, biomonitoring species should also be naturally occurring in the environment to be tested. Mud crabs exhibit all of these characteristics and represent a suitable indicator species.

The objectives of this research are to:

1. Develop mud crab health indicators, baselines and a suitable scoring system for report card use and to design a mud crab monitoring program for the Gladstone Harbour Report Card.
2. Provide report card grades and scores for the 2017 Gladstone Harbour Report Card with an accompanying project report which describes all methods employed and provides an overview of the current status of mud crabs within the Gladstone Healthy Harbour Partnership's (GHHP) reporting area.

Mud crab monitoring was conducted in Gladstone Harbour zones over two sampling events in June and July 2017. Eight zones were sampled in the first event, and six in the second. A set of seven recommended long-term mud crab monitoring sites are recommended, based on their performance against six site selection criteria. The recommended long-term monitoring sites are: Zone 1 – Narrows, Zone 2 – Graham Creek, Zone 4 – Boat Creek, Zone 5 – Inner Harbour, Zone 6 – Calliope Estuary, Zone 7 – Auckland Creek and Zone 13 – Rodds Bay.

Potential mud crab indicators were investigated through a narrative systematic literature review and consideration of historical and current field data from Gladstone Harbour. Potential indicators were scored using a blind-scoring methodology based on ten predefined indicator selection criteria. Four mud crab indicators are proposed for inclusion in the Gladstone Harbour Report Card:

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

An indicator scoring methodology (distance from benchmark) is proposed, and indicators are graded for each sampled zone using the GHHP grading system. The biomass indicator was not able to be scored in 2017, due to insufficient historical weight data for mud crabs in Gladstone Harbour to develop a benchmark. Once three years of monitoring data are available, this indicator will also be able to be scored. For 2017, the mud crab indicator grades for Gladstone Harbour are provided below.

Zone	Abundance (CPUE)	Prevalence of rust lesions	Sex ratio*	Biomass	Zone score 2017
1. The Narrows	1.00	1.00	0.00	NC	0.67
2. Graham Creek	0.52	0.95	0.36	NC	0.61
4. Boat Creek	1.00	1.00	0.11	NC	0.70
5. Inner Harbour	1.00	0.89	0.71	NC	0.87
6. Calliope Estuary	0.14	0.90	0.36	NC	0.47
7. Auckland Inlet	0.12	0.63	0.00	NC	0.25
13. Rodds Bay	0.03	0.67	0.39	NC	0.36
Harbour Average					0.56

* Sex ratio based on legal size limits.
NC = not calculable in 2017.

The scores and grades for mud crab indicators reflect the variety of pressures on mud crabs in Gladstone Harbour, including commercial fishing, recreational condition and environmental/habitat condition. Low overall scores (D) for Calliope Estuary, Auckland Inlet and Rodds Bay were driven by lower grades for abundance of mud crabs (E) in comparison to other zones. In 2017, prevalence of rust lesions scored highly in most sites, and the lowest grade (C) was recorded for Auckland Inlet. Sex ratios tended towards high proportions of female mud crabs, reflecting the sex-based fishery operating in Queensland. As 2017 is the first year this study has been conducted (Pilot Year) the accuracy and reliability of the mud crab grades may improve as more data are collected and all indicators, including biomass, are able to be calculated.

List of Acronyms

Acronym	Definition
BaP	benzo-a-pyrene
BRUVS	Baited Remote Underwater Video Station
CAT	catalase
ChE	cholinesterase
CPUE	catch per unit effort
CW:BW	carapace width: body weight
DDT	dichlorodiphenyltrichloroethane
DNA	deoxyribonucleic acid
FRDC	Fisheries Research and Development Corporation
GHHP	Gladstone Healthy Harbour Partnership
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	total glutathione
GST	glutathione-S-transferase
ISP	Independent Science Panel
LPO	lipid peroxidation
LTMP	Fisheries Queensland Long Term Monitoring Program for the Mud Crab Fishery
NA	Not available
NC	Not calculable
NSW	New South Wales
NT	Northern Territory
PAHs	polycyclic aromatic hydrocarbons
PCBs	polychlorinated Biphenyls
PHCs	petroleum hydrocarbon compounds
POPs	persistent organic pollutants
ppt	parts per thousand
RNA	ribonucleic acid
SC	selection criteria
SD	standard deviation
SOD	superoxide dismutase
SOI	Southern Oscillation Index
USA	United States of America
WCS	worst case scenario

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 total annual catch was approximately 1,000 tonnes worth AUD18.7 million in 2015 (DAF, 2017). Mud crabs (primarily *Scylla serrata*, and potentially *S. olivacea*) are recreationally and commercially important species in Gladstone Harbour, as well as an iconic seafood item, with cultural value to Indigenous Australian peoples (Brewster, 2015). As a result of their commercial importance to the fishing and aquaculture industries, the biology, ecology and biochemistry of mud crabs is relatively well known. The green mud crab, *S. serrata*, has previously been suggested as a potential biomonitoring species in tropical coastal marine environments of Australia (Negri *et al.*, 2009; van Oosterom *et al.*, 2010).

Ideal biomonitors are sedentary, easily identifiable, abundant, long-lived, available to sample throughout the year, large enough to provide sufficient tissue for analysis, resistant to handling stress and tolerant to environmental variations in physicochemical parameters (Rainbow, 1995). To be regionally relevant, biomonitoring species should also be naturally occurring in the environment to be tested. With the exception of when females are migrating to spawn, *Scylla* spp. exhibit a relatively small foraging range, meaning that compared to finfish, mud crabs may provide better detail of spatial and temporal pollution impacts (Hyland *et al.*, 1984; van Oosterom *et al.*, 2010; Alberts-Hubatsch *et al.*, 2016). The relative ease of capture and recapture of mud crabs makes them a suitable candidate as an indicator species.

Bioindicators provide a time-integrated measure of the effects of changes in water quality (Cooper *et al.*, 2009). Many biomonitoring techniques have been adopted, dependant on the specific needs of the monitoring program. The most widely used include bioaccumulation, biochemical alterations, molecular approaches, observation of morphology and behaviour, and quantitative assessments at the local stock and population levels (Prabhakaran *et al.*, 2017).

The mud crab fishery

The Queensland Government's QFish database provides data on commercial mud crab licenses, catch and effort. In 2014 there were 38 commercial mud crab licenses operating in Grid S30 (includes Gladstone Harbour), seven in Grid S31 (includes Colosseum Inlet) and 16 in Grid T31 (includes Rodds Bay). In 2014 these 61 licenses accounted for more than 16% of the total number of mud crab licenses in Queensland (total of 370) and more than 21% of the total mud crab catch (287 tonnes of a total 1,329 tonnes). Grid S30 has the largest number of licenses in a single Grid area after W37 in Moreton Bay (49 licenses) and equivalent to Grid R30 in the Fitzroy River delta (38 licenses), emphasising the importance of Gladstone Harbour to the fishery.

Recreational and Indigenous catches are not as well understood as commercial catches. Recent estimates for Queensland suggest a combined take of mud crabs by recreational and Indigenous fishers of about 25% of the total harvest (Grubert *et al.*, 2015) but these estimates are not considered highly reliable.

Fisheries Queensland ran a fishery-independent Long Term Monitoring Program (LTMP) for mud crabs from 2000 to 2009. Sampling sites included three sites in Gladstone Harbour (Calliope River, Graham Creek and Gladstone Harbour foreshore in close proximity to the newly constructed Wiggins Island Coal Export Terminal) and two nearby sites to the north of the Narrows (Deception Creek and Conner Creek). Data from the LTMP are available on request from Fisheries Queensland.

Mud crab disease

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 was studied extensively in the early 2000's and data on prevalence of the disease in mud crab populations are available for Gladstone Harbour. The disease has also been reported from the Fitzroy River and (with much lower prevalence) from Moreton Bay, Ayr and Stanage Bay.

In 2011-12, there were reported increases in incidence of disease in fin fish and crabs in Gladstone Harbour. Dennis *et al.* (2016) undertook a veterinary analysis of locally caught fish and crabs in early 2012, including characterisation and assessment of mud crab shell lesions (using the lesion grading system developed at CQUniversity by Andersen *et al.* (2000)). Disease prevalence was recorded at six regions in Gladstone Harbour and control sites including Rodds Bay and Stanage Bay. In addition to rust spot lesions, mud crabs are also susceptible to parasites such as barnacles, and a variety of bacterial, fungal and viral diseases (Jithendran *et al.*, 2010).

Objectives

The Gladstone Healthy Harbour Partnership (GHHP) commissioned CQUniversity to develop mud crab indicators for the Gladstone Harbour Report Card (Project ISP015-2017). Because of the importance of mud crabs to the local community and stakeholders, the GHHP Independent Science Panel (ISP) has 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).

The overall objectives of this project are to:

1. Develop mud crab health indicators, baselines and a suitable scoring system for report card use and to design a mud crab monitoring program for the Gladstone Harbour Report Card.

2. Provide report card grades and scores for the 2017 Gladstone Harbour Report Card with an accompanying project report which describes all methods employed and provides an overview of the current status of mud crabs within the GHHP reporting area.

Project overview

The project includes ten tasks, as follows:

Task 1: Project inception meeting, held on 22 May 2017. Agreement on project tasks, timelines, objectives and integration with other GHHP projects.

Task 2: Detailed program design and work plan, delivered 8 June 2017. Provision of a detailed plan and timeline developed in discussion with the GHHP Science Team.

Task 3: Mud crab monitoring data from the 2017 Gladstone Harbour Report Card, completed 6 July 2017. Two sampling events were conducted from 19-23 June (in eight zones) and 3-6 July (in six zones).

Task 4: Milestone Report “Identification of long-term mud crab monitoring sites and survey design”, delivered 17 July 2017. Included proposed location and number of sampling sites, parameters and methods.

Task 5: Milestone Report “Mud crab indicators for the Gladstone Harbour Report Card”, delivered 27 July 2017. Included indicators selected and methodologies used for their development.

Statistics workshop: Workshop with GHHP ISP members in Brisbane, 8 August 2017.

Task 6: Submission of draft scores/grades for review, delivered 30 August 2017.

Task 7: Submission of a draft report for review, delivered 30 August 2017.

Task 8: Presentation to the ISP in Gladstone, delivered 12 September 2017.

Task 9: Submission of a final report including final report card grades, 30 September 2017.

Task 10: Submission of a paper to a high quality peer-reviewed international journal, 31 December 2017.

Literature review

A review of the international peer-reviewed literature was undertaken using scientific databases (Scopus, Science Direct, Google Scholar and the CQUniversity Library database). Key words used in the database searches were:

- *Scylla serrata*
- Estuarine crab
- Decapoda
- Indicator
- Biological indicator
- Bioindicator
- Biomonitoring

These key words were used on their own and as a combination of terms. For all databases, publications with a combination of these search terms appearing in any search field were included. Individual search terms (as listed above) were limited in database searches to appearing in the title only. The search protocol was concluded on 14 July 2017. This review addressed English language literature only. No data were collected for non-English literature. This search protocol was used to assemble a bibliography for use in the critical review of the available literature on potential indicators. Results of the database searches can be found in Table 1. Following the removal of duplicates and irrelevant studies, 88 relevant research papers were used to conduct a narrative review.

A recent research project funded by GHHP identified suitable biomarkers to assess fish health in Gladstone Harbour (Kroon *et al.*, 2016). The 2016 review differs from the review conducted for this project, as we have considered papers relating to all possible mud crab indicators, including but not limited to biomarkers. The search methods employed also differed; we have considered all relevant papers in a systematic narrative review, contrasting with the more delimited systematic review methodology employed by Kroon *et al.* (2016).

This review aimed to gather all available information on the suitability of the candidate mud crab indicators initially proposed by GHHP (McIntosh *et al.*, 2014) and other potential indicators for assessing mud crab health and demonstrating change over time. We then considered the suitability of possible mud crab indicators and quantitatively scored them based on a set of ten predefined indicator selection criteria.

Table 1: Results of database searches

	Total number of responses from each database			
Search terms	**Scopus**	**Science Direct**	**Google Scholar**	**CQUniversity Library**
Scylla serrata	409	124	1,860	423
Estuarine crab	270	83	437	293
Decapod	1,365	279	3,860	1,009
Biological indicator	1,137	306	1,110	688
Bioindicator	950	557	1,860	927
Biomonitoring	3,145	1,158	7,980	4,016
Scylla serrata bioindicators	61	37	378	38
Scylla serrata indicators	628	277	2,790	316
Scylla serrata biological indicators	539	188	2,590	263
Scylla serrata biomonitoring	145	35	380	65
Decapoda biological indicators estuarine	2,077	502	13,400	965
Estuarine crab biological indicators estuarine	4,070	2,069	24,500	2,990

The following categories of indicators were identified through the literature review:

- Biomarkers [defined here as “Biomarkers of exposure” and “Biomarkers of effect” as described by Kroon *et al.* (2017)]
- Sex ratios
- Abundance, size and biomass
- Bioaccumulation of toxicants
- Rust spot shell lesions
- Morphology and size at maturity

A summary of reviewed indicators, contributing stressors and benchmarks is provided in Table 3. Detailed descriptions of the indicators are then provided for each category.

Table 2: Indicators identified through the literature review

Indicator	**Stressors**	**Description**	**Benchmarks**	**References**
Biomarkers				
GST induction and ChE inhibition	PAHs and Organophosphates	Detection of biomarkers in *S. serrata* hepatopancreas and haemolymph	*S. serrata* data from Herbert, Normanby, Burdekin and Fitzroy Rivers, Qld.	(van Oosterom *et al.*, 2010)
RNA/DNA ratios	Seasonality and pollution	Estimator of physiological condition	*Perisesarma guttatum* and *Uca annulipes* data from Mozambique.	(Amaral *et al.*, 2009)
Glutathione peroxidase activity and lipid peroxidases	Metal exposure	Variability with metal concentrations.	*Parasesarma erythodactyla* from Lake Macquarie, NSW.	(MacFarlane *et al.*, 2006)
Antioxidant enzymes and oxidative stress parameters	Salinity and seasonality	Detection of enzymes in *S. serrata* tissue	*S. serrata* from Odisha, India	(Paital and Chainy, 2013)
Oxidative stress and antioxidants biomarkers	Seasonality	Tissue and haemolymph	*S. serrata* from Ennore estuary, Tamil Nadu, India	(Ragunathan, 2017)
PCBs and PHCs	Seasonality	Tissue and haemolymph	*S. serrata* from Ennore estuary, Tamil Nadu, India	(Ragunathan, 2017)
Antioxidant biomarkers	Seasonality	Hepatopancreas, gills and abdominal muscle	*Scylla serrata*, sampled from Chilika lagoon of India	(Paital and Chainy, 2013)
Sex ratios				
Male:Female ratio	Fishing pressure	Areas with sex based fishing industries have female dominated populations	Review of *S. serrata* worldwide.	(Alberts-Hubatsch *et al.*, 2016)
Male:Female ratio	Fishing pressure, environmental factors	Spatial patterns for male and female prevalence	*S. serrata* data from New Caledonia.	(Dumas *et al.*, 2012)
Male:Female ratio	Seasonality	Temporal patterns to female dominance	*Scylla* spp. from Malaysian coast	(Ikhwanuddin *et al.*, 2011)
Abundance, size and biomass				
Size ratios	Environmental and fishing pressures, mangrove type	Correlations of size to mangrove type, and environmental and fishing variables.	*S. serrata* data from New Caledonia.	(Dumas *et al.*, 2012)
Abundance	Seasonality, recruitment	Catch per unit effort (CPUE) correlations	*Scylla* spp. in the Philippines	(Walton *et al.*, 2006)
Abundance	Mangrove habitat ecological function	CPUE correlations	*S. olivacea* CPUE data from the Philippines	(Walton *et al.*, 2007)

Indicator	**Stressors**	**Description**	**Benchmarks**	**References**
Abundance	Commercial and recreational fishing	CPUE	Fisheries Queensland long term monitoring program for mud crabs	(Jebreen *et al.*, 2008) (Fisheries Queensland, 2009) *Data provided by Fisheries Queensland in July 2017*
Bioaccumulation of toxicants				
Metal bioaccumulation	Bioaccumulated concentrations of Fe, Cu, Mn, Zn and Cd	Tissue concentrations	*Pachygrapsus marmoratus*, a small crab species from southern Europe.	(Álvaro *et al.*, 2016)
Structural deformities	Bioaccumulated metal concentrations	Gills, muscles and hepatopancreas deformities correlated with metal concentrations	*Scylla serrata* in Pulicat Lake, Chennai	(Arockia Vasanthi *et al.*, 2014)
Metal bioaccumulation	Bioaccumulated metal concentrations	Correlations between tissue and sediment metal concentrations	Red Fingered Marsh Crab, *Parasesarma erythodactyla* from Lake Macquarie, NSW.	(MacFarlane *et al.*, 2006)
Metal bioaccumulation	Bioaccumulated metal concentrations	Correlations between metal uptake and sediment pH and organic content	Red Fingered Marsh Crab, *Parasesarma erythodactyla* from Lake Macquarie, NSW.	(MacFarlane *et al.*, 2006)
Metal bioaccumulation	Bioaccumulated metal concentrations	Positive correlation with metal bioaccumulation and glutathione peroxidase activity	Red Fingered Marsh Crab, *Parasesarma erythodactyla* from Lake Macquarie, NSW.	(MacFarlane *et al.*, 2006)
Cadmium bioaccumulation	Bioaccumulated metal concentrations	Hepatopancreas bioaccumulation of cadmium from food sources	Blue swimmer crabs (*Portunus pelagicus*) from Hawkesbury River, NSW.	(McPherson and Brown, 2001)
POPs and metal bioaccumulation	Bioaccumulated concentrations	Hepatopancreas and tissue.	*S. serrata* from east coast of Queensland	(Mortimer, 2000)
POPs and pesticides bioaccumulation	Bioaccumulated concentrations	Hepatopancreas samples	*S. serrata* from east coast of Queensland	(Negri *et al.*, 2009)
Metal bioaccumulation	Bioaccumulated metal concentrations	Tissue samples found significant differences between male and female for Zn and Ni	The burrowing crab *Neohelice granulata* from Argentina	(Simonetti *et al.*, 2013)
Metal bioaccumulation	Bioaccumulated metal concentrations, seasonality	Seasonal variation of Zn, Ni and Pb concentrations	The burrowing crab *Neohelice granulata* from Argentina	(Simonetti *et al.*, 2013)
Rust spot shell lesions				
Rust and skin lesions	Physiological health	Prevalence of lesions	*S. serrata* caught in Gladstone Harbour during the 2012 sampling period	(Dennis *et al.*, 2016)

Indicator	Stressors	Description	Benchmarks	References
Morphology and size at maturity				
Morphometrics, population densities and behaviours	Environmental health	Claw size ratio and claw loss	*Uca* spp. fiddler crabs from a coastal area of South Carolina	(Giblock and Crain, 2013)
Wet weight and carapace width	Physiological health	Correlated between sexes and shell hardness	*S. serrata* from NT	(Grubert *et al.*, 2012)
Carapace width-body weight relationship	Physiological health	Correlations between CW:BW, biomass and meat recovery	*Scylla* spp. from Malaysia	(Ikhwanuddin *et al.*, 2011)
Body size and sexual maturity	Physiological health	Correlations between size and sexual maturity	*Scylla* spp. from Malaysia	(Waiho *et al.*, 2016)
Residence	Life-stage	Residence and feeding areas vs. life stage	*S. serrata* from tidal flats in Australia	(Hill *et al.*, 1982)

Biomarkers

A study by van Oosterom *et al.* (2010) evaluated a suite of biomarkers from *S. serrata* in four northern Queensland catchments finding the ability to highlight site-specific differences. Adult *S. serrata* were collected from the Normanby, Herbert, Burdekin and Fitzroy Rivers in Queensland, Australia from March to July 2006 (van Oosterom *et al.*, 2010). Adults were defined as having a carapace width of 150 mm or greater (as defined by Hyland *et al.* (1984)). Three of the biomarkers analysed (glutathione-S-transferase (GST) activity, cholinesterase (ChE) activity and benzo-a-pyrene (BaP) urinary metabolites) exhibited site-specific differences in the *S. serrata* collected from the four rivers (van Oosterom *et al.*, 2010). Analysis of these biomarkers indicated possible exposure to polycyclic aromatic hydrocarbons (PAHs) and organophosphate contamination, though the authors state that development of dose-response relationships is required to confirm these results are due to contaminant exposure and not diet or seasonal variability (van Oosterom *et al.*, 2010). While the potential use of biomarkers as indicators is promising, without further research into the specificity of these biomarkers, and development of dose-response relationships, the use of these particular biomarkers as indicators in *S. serrata* would only be preliminary.

Glutathione peroxidase activity is suggested as a biomarker of metal exposure with lipid peroxidases as a secondary marker when bioaccumulated metal concentrations are high (MacFarlane *et al.*, 2006). Total glutathione (GSH), glutathione peroxidase (GPx) and lipid peroxidation (LPO) were measured as indicators of metal contamination and accumulation in the marsh crab *Parasesarma erythodactyla* (MacFarlane *et al.*, 2006). GSH analysed from whole crab homogenate was found to remain similar between males and females across the sites sampled, with GPx elevated in individuals where metals were elevated (MacFarlane *et al.*, 2006). Conversely, females with high concentrations of bioaccumulated metals showed increased lipid peroxidation products (MacFarlane *et al.*, 2006). Increases in these biomarkers suggest exposure to metal contaminated sites, with resultant oxidative stress in the individual (MacFarlane *et al.*, 2006). As with other potential biomarkers, without further research into the dose-response relationships and information about time lags between pollutant exposure and biochemical responses (MacFarlane *et al.*, 2006), the use of glutathione as a biomarker would be premature.

The effects of salinity on antioxidant and oxidative stress parameters in *S. serrata* have been studied in various tissues. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) were measured in the abdominal muscle, hepatopancreas and gills of *S. serrata*. Tissue specific salinity variation of antioxidant and oxidative stress biomarkers was suggested by the findings of the study (Paital and Chainy, 2010). It was also found that higher salinities (>35 ppt) induced increased oxidative stress biomarkers in tissues in comparison with lower salinities (10-17 ppt) (Paital and Chainy, 2010). Variations in antioxidant markers in the hepatopancreas, gills and abdominal muscle homogenates were also found to indicate seasonal and gender specificity (Paital and Chainy, 2013). Male and female *S. serrata* were found to have distinct tissue specific antioxidant strategies against seasonal variation and environmental factors (Paital and Chainy, 2013). Dissolved oxygen concentrations, temperature and salinity have all been shown to alter oxidative stress physiology and biomarkers (Paital and Chainy, 2013). Paital and Chainy (2013) suggest the cautious use of antioxidant and oxidative stress biomarkers as indicators of estuarine environments, due to gender specificity and seasonal variability.

Oxidative stress and antioxidant biomarkers were measured in *S. serrata* in order to understand impacts of seasons on physiological responses in experimental laboratory conditions (Ragunathan, 2017). Gills, hepatopancreas, ovaries and haemolymph of *S. serrata* were analysed for oxidative stress and antioxidant biomarkers, including SOD, CAT, GPx, GR, GST, GSH and LPO (Ragunathan, 2017). Bioaccumulated Polychlorinated Biphenyls (PCBs) and petroleum hydrocarbon compounds (PHCs) were also measured for comparative purposes, as these compounds are known to have seasonal variations in the study area (Ragunathan, 2017). Concentrations of PCBs and PHCs were higher in summer than in the monsoon season in both water and bioaccumulated concentrations (Ragunathan, 2017). Higher oxidative stress with lower antioxidant concentrations were observed in the summer season, with lowered oxidative stress and higher antioxidant concentrations during the monsoon season in conjunction with improved water quality conditions (Ragunathan, 2017).

RNA/DNA ratios are known to be a useful estimator of physiological condition in fish and invertebrates, with the ratio expected to increase when environmental conditions are favourable (Amaral *et al.*, 2009). DNA content remains constant in individuals, but RNA content correlates with the synthesis of protein, which is beneficial to organisms and reflects growth and reproduction (Dahlhoff, 2004; Amaral *et al.*, 2009). Seasonality and mangrove pollution influenced RNA/DNA ratio patterns in the mangrove crabs *Perisesarma guttatum* and *Uca annulipes* suggesting this may be useful an indicator of mangrove habitat quality, especially in areas with clear seasonal variation (Amaral *et al.*, 2009). RNA/DNA ratios however, as far as the authors are aware, have not been tested in *S. serrata*, and responses can be highly variable in the species that have been tested.

Sex ratios

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. For example, the fishery for *S. serrata* in Queensland is limited to the collection of males, and it has been found that populations are female dominated (Pillans *et al.*, 2005; Alberts-Hubatsch *et al.*, 2016). However, in unfished populations of *S. serrata*, the male:female ratio can be as high as 3:1 (Alberts-Hubatsch *et al.*, 2016). A study of marine reserves in Moreton Bay, South East Queensland, identified a male:female sex ratio of 2:1 in mature crabs (>150 mm) (Pillans *et al.*, 2005). Shifts in sex ratio not only have implications for dynamics of the crab populations, 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.

Besides fishing pressure, another influence on the sex ratio of crabs is seasonal changes, and this would need to be considered during indicator development. In observations on the mature Portunid crab *Callinectes sapidus* in a tributary of Chesapeake Bay, Harding and Mann (2010) found a dominance of male crabs when day lengths were above 13.4 hrs. However, as both day length and water temperature decreased, female crabs were up to 4 times more abundant than males, as males migrated to up-river habitats. In *Scylla* spp. from the Malaysian coast male:female sex ratios were found to change during the monsoon season when females migrate for spawning (Ikhwanuddin *et al.*, 2011). In other areas no temporal patterns were observed, for example in a New Caledonian study of *S. serrata* populations, male dominance was apparent in all sampling periods, with proportions ranging from 0.5 to 1.0 (Dumas *et al.*, 2012).

Dumas *et al.* (2012) also found a spatial difference in the ratio of males to females, with male crabs significantly more likely to be found inside burrows (86.1% of catch), compared to 74.4% of catch caught outside burrows being male (Dumas *et al.*, 2012). Spatially, females were significantly more abundant in coastal mangroves (Dumas *et al.*, 2012). Tagging experiments to assess habitat movement of *S. serrata* in Moreton Bay (Hyland *et al.*, 1984) from 1976 to 1981 identified two types of movement, one of which was sex specific. In an area with direct sea access, males and females moved similar distances, but in a channel environment, female movement (mean 6.6 km) was significantly greater than male movement (mean 3.7 km) (Hyland *et al.*, 1984). Spatial and temporal changes in sex ratio will need to be considered if this indicator is used in monitoring, with sampling needing to be carried out not only at a similar time of year but ideally also at a similar water temperature and habitat.

Finally, the sex of the crab may also affect the probability of capture and recapture. 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 study found that recently moulted females less than 150 mm were not captured in commercial crab pots, whilst recently moulted males of down to 120 mm were captured (Heasman, 1980). The reason proposed was that behavioural differences between the sexes and among size classes led to unequal vulnerability to capture.

Abundance, size and biomass

Abundance and biomass indicators can be important for species health as well as ecosystem health, however, these can be hard to measure comparatively (Alberts-Hubatsch *et al.*, 2016). Discrepancies in abundance can be due to capture technique, sampling areas and sampling times, or due to differences in crab distribution, growth or survival induced by habitat and environmental conditions (Alberts-Hubatsch *et al.*, 2016). By minimising variation due to seasonality, catch and sampling techniques, abundance and biomass indicators can be used as indicators of change in habitat and environment.

A study in New Caledonia conducted by Dumas *et al.* (2012), found *S. serrata* caught averaged a size of 134 mm (87 to 181 mm), this however was potentially confounded by the inclusion of data from a fishery based study during which only individuals over 100 mm in carapace width (the legal size of retention) were collected. Environmental and fishing variables were found to explain 14.9% of the size variability (Dumas *et al.*, 2012). Mangrove type was found to be a major predictor for crab size with larger crabs in coastal areas, compared to estuarine areas (Dumas *et al.*, 2012). Mangrove types were also found to correlate with crab size (Dumas *et al.*, 2012). However, abundance and biomass can be underestimated due to size selectivity during catches (Dumas *et al.*, 2012).

The abundance and distribution of *Scylla* spp. in the Philippines was measured using baited lift nets and cylindrical bamboo traps (Walton *et al.*, 2006). The cylindrical bamboo traps were considered the best estimation of catch per unit effort (CPUE), as the method maintained constant effort (Walton *et al.*, 2006). *S. olivacea* dominated all *Scylla* spp. caught, with no correlations found between CPUE, salinity, temperature or tidal variation (Walton *et al.*, 2006). A variation in CPUE was detected between years, related to a decline in the relative abundance of *S. olivacea* in the area, suggesting variations in recruitment and increased fishing pressures (Walton *et al.*, 2006). In a similar study, abundance of *S. olivacea* was used as an indicator of mangrove habitat ecological function. Abundance was also estimated using CPUE from an experimental standardised trapping grid (Walton *et al.*, 2007). While a similar decrease in CPUE of *S. olivacea* was observed, abundance of other crab species in the area increased, suggesting that the area was still a suitable habitat, but that fishing pressures and recruitment limitations had impacted on the abundance of *S. olivacea* (Walton *et al.*, 2007).

In a study by Meynecke *et al.* (2012), catch and effort data from the Northern Territory (NT; 1990 to 2008) for *S. serrata* was analysed with climatic data and the Southern Oscillation Index (SOI). Four periods of high catch rates were identified, consistent with positive SOI values and La Niña climate phases (Meynecke *et al.*, 2012). Between 30 and 40% of variation in CPUE was explained by periods of high rainfall and SOI alone, with greater freshwater inputs into the estuarine habitats of *S. serrata*, along with warmer water temperatures (Meynecke *et al.*, 2012). Temperatures were also found to affect CPUE rates, with *S. serrata* less active in high (>40 °C) and low (<20 °C) temperatures (Meynecke *et al.*, 2012).

Exposure studies of juvenile *S. serrata* collected from NT, Australia demonstrated their survivability and tolerance of temperatures ranging from 25-35°C, with survival ranging from 94-98%. However, exposure of the crabs to a temperature of 20°C resulted in only a 36% survival rate (Ruscoe *et al.*, 2004), indicating poor tolerance to colder temperatures. Growth rates were also significantly affected by water temperature, with maximum growth observed in individuals exposed to water of 30°C. Meynecke *et al.* (2012) provided an optimal temperature of 28-32°C for *S. serrata* based on a review of previous studies. *S. serrata* is more tolerant of lower temperatures than the black mud crab *S. olivacea* (Keenan *et al.*, 1998).

Chandrasekaran and Natarajan (1994) reported that high rainfall and associated lower water salinity (as low as 1.52 ppt) in an Indian estuary resulted in lowered population densities of juvenile *S. serrata*, and complete absence of juvenile *S. serrata* from sampling sites during monsoonal rains. The same study also reported that juvenile *S. serrata* densities were highest during the post monsoonal months, when water salinity was higher (between 15-30 ppt) (Chandrasekaran and Natarajan, 1994). Within Australia, *S. serrata* tends to be dominant within 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). Whilst previous studies have demonstrated that *S. serrata* is not able to tolerate very low water salinity (<5 ppt (Chandrasekaran and Natarajan, 1994; Ruscoe *et al.*, 2004)), *S. serrata* is able to tolerate a wide range of water salinity, with Williams and Hill (1982) reporting that salinity (ranging from 24-35 ppt) was not correlated with numbers caught. Ruscoe *et al.* (2004) also reported that juvenile *S. serrata* survival was not significantly different in treatment groups exposed to water between 5-40 ppt.

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.

Meynecke *et al.* (2012) reported that abundance data are often highly variable, due to *S. serrata* behaviour, population biology and dynamics, fishing effort efficiency and magnitude, and environmental conditions (Meynecke *et al.*, 2012). Alberts-Hubatsch *et al.* (2016) also noted that there have been no comparative studies conducted on the three different continents where *S. serrata* reside, therefore it is not known whether abundance, size and biomass variations reported in the literature are due to sampling methods, genetic diversity or phenotypic plasticity.

Bioaccumulation of toxicants

Several studies have been conducted using crabs as bioindicators of heavy metal and toxicant bioaccumulation. The effect of heavy metals on natural populations of organisms needs to take into account tolerance in communities that have been exposed to particular toxicants over several generations (Chiarelli and Roccheri, 2014). The usefulness of an organism as a bioindicator of toxicant also needs to consider if the organism bioaccumulates the toxicant over a measurable time, or if the toxicant in question is regulated by the organism, with no significant bioaccumulation measurable (McPherson and Brown, 2001).

Blue swimmer crabs (*Portunus pelagicus*) have been shown to accumulate cadmium in the hepatopancreas when metal source was food/prey (McPherson and Brown, 2001). In a laboratory based study of *Portunus pelagicus*, crabs were fed contaminated and uncontaminated mussels (*Trichomya hirsuta*) and bioaccumulation was analysed over an eight week period (McPherson and Brown, 2001). Of the six metals and metalloids analysed (Cd, Cu, Zn, As, Al and Fe), only Cd was found to have been significantly bioaccumulated by the crab after an eight week period (McPherson and Brown, 2001).

A study by Álvaro *et al.* (2016), investigated the use of *Pachygrapsus marmoratus*, a small crab species from southern Europe as an indicator of heavy metals. Bioaccumulated concentrations of Fe, Cu, Mn, Zn and Cd in hepatopancreas tissue of *Pachygrapsus marmoratus* separated individual crabs by site (Álvaro *et al.*, 2016). The concentrations of metals bioaccumulated by the crabs were positively correlated with the bioavailable metals at the sites analysed (Álvaro *et al.*, 2016).

A study on the red fingered marsh crab, *Parasesarma erythodactyla*, showed significant relationships between sediment metal concentrations and tissue metals for certain metals (Pb, Cu, Cr, Zn, and Se in males and Cd, Pb, Cr Zn, As, and Se in females), these correlations were also found to be site specific (MacFarlane *et al.*, 2006). Higher pH and lower organic content were also correlated with metal uptake (MacFarlane *et al.*, 2006).

Burrowing crab, *Neohelice granulata*, soft tissues and eggs were analysed for metal bioaccumulation to determine suitability of this species as an indicator of metal pollution (Simonetti *et al.*, 2013). The study found no significant spatial differences for the metals tested (Zn, Ni and Pb), however, significant differences were found between sexes for Zn and Ni accumulation. The authors suggest that male and female *N. granulata* should be treated separately for all future studies. Seasonal variation of Zn, Ni and Pb was also found, with Pb only detected during spring, suggesting that seasonality is a specific consideration, particularly in this species.

Structural deformities of the gills, muscles and hepatopancreas have been observed in *S. serrata* due to metal toxicity, with the degree of damage correlated with elevated bioaccumulated metal concentration (Arockia Vasanthi *et al.*, 2014). Significant metal accumulation and histocytological lesions were evident in the mud crabs (Arockia Vasanthi *et al.*, 2014). Tissue and metal specific bioaccumulation was observed, with Cu, Pb, Cd and Mn highest in the hepatopancreas, lower in the gills and significantly lower in the muscle samples analysed, whereas Zn and Fe were highest in the gills, lower in the hepatopancreas and significantly lower in the muscles (Arockia Vasanthi *et al.*, 2014).

Scylla serrata has the ability to accumulate a range of contaminants including persistent organic pollutants (POPs) and metals (Mortimer, 2000). *S. serrata* were sampled in six estuaries between Brisbane and Cairns, Qld, for analysis of metals, metalloids and pesticides in muscle and hepatopancreas tissue (Mortimer, 2000). Most metals and metalloids were found to be preferentially sequestered to the hepatopancreas. *S. serrata* from industrialised areas such as the Brisbane River and Gladstone showed higher concentrations of Pb, Se and Sn than more unimpacted locations (Mortimer, 2000). Dieldrin residues in *S. serrata* tissue suggested that ambient exposures of dieldrin exceeded national water quality guidelines at all sampling locations, with one of the 77 samples of crab body muscle concentrations of dieldrin (0.31 mg/kg wet weight) exceeding Australian Food Standards (0.1 mg/kg wet weight) (Australia New Zealand Food Authority, 1999; Mortimer, 2000).

Elevated metal (As, Cr, Cu, Fe, Hg, Mn, Ni, U, Zn) concentrations in the hepatopancreas of *S. serrata* were reported from two sites at Spillway Creek in Gladstone Harbour; 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 on *S. serrata* 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.

Scylla serrata hepatopancreas samples from 11 Queensland rivers were analysed for pesticides and POPs with Dichlorodiphenyltrichloroethane (DDT) and dieldrin detected along with other banned organochlorines (Negri *et al.*, 2009). As the rivers sampled were similar to the Mortimer (2000) study, the authors compared results directly. Concentrations of DDT and dieldrin measured in the earlier Mortimer (2000) study were found to be around one order of magnitude greater than those measured in the Negri *et al.* (2009) study. Concentrations of DDT and dieldrin measured in the Negri *et al.* (2009) study were found to be too low to pose a threat to human health. Contemporary pesticides were also analysed in *S. serrata* hepatopancreas by Negri *et al.* (2009), however, only endosulfan and chlorpyrifos were detected. The authors noted that while other contemporary pesticides (e.g. diuron, atrazine, simazine etc.) do not appear to be bioaccumulated by *S. serrata*, this does not mean that there are no effects from them (Negri *et al.*, 2009).

Rust spot shell 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 and zinc exposure although this has not yet been experimentally confirmed (Andersen *et al.*, 2000; 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). This disease has the potential to damage the mud crab industry around Gladstone Harbour, thus making it a potential indicator of high interest to members of the public. A method to objectively measure and grade the severity of lesions on crab shell carapace was developed and described by Andersen *et al.* (2000), and is based on the size of the lesion, and whether the carapace has been perforated.

In 2011-12, there were reported increases in the incidence of disease in finfish and crabs in Gladstone Harbour. A veterinary analysis conducted by Dennis *et al.* (2016) in early 2012 found rust spot lesions on 37% of *S. serrata* caught in Gladstone Harbour. This is compared with a prevalence of 14% of the crabs caught in Stanage Bay and 7% of the crabs caught in Rodds Bay and Turkey Beach during the same period (Dennis *et al.*, 2016). Prevalence of rust spot lesions measured during the Dennis *et al.* (2016) study was greater than previously recorded prevalence in the same region. Andersen *et al.* (2000) recorded a rust spot prevalence of 22% from Gladstone Harbour in the late 1990s.

In addition to rust spot lesions, *Scylla* spp. are susceptible to parasites such as barnacles, and a variety of bacterial, fungal and viral diseases (Jithendran *et al.*, 2010). However in comparison to rust spot lesions, other diseases and parasites are not well understood for Gladstone Harbour.

Morphology and size at maturity

Morphometrics of estuarine crab species have been suggested as indicators of environmental health elsewhere (Giblock and Crain, 2013). Fiddler crabs in the USA for example, have been shown to be influenced by natural and anthropogenic disturbances (Giblock and Crain, 2013). Crabs can be negatively affected by oil pollution, resulting in significantly lower population densities and feeding rates in pollution affected areas (Culbertson *et al.*, 2007). As well as reducing population size and affecting behaviour, the degree of industrialisation and intensity of pollution can affect crab size. For example a study in New Jersey, USA, found that fiddler crabs (*U. pugnax*) were significantly larger in the polluted sites compared to the less-polluted sites (Bergey and Weis, 2008).

Scylla spp. individuals have a positive relationship between body size and sexual maturity (Waiho *et al.*, 2016). In Australian populations, mature female *S. serrata* range in carapace width (CW) from 138 to 204 mm, and mature males (identified based on the presence of spermatophores in their anterior vas deferens) have CWs ranging from 108 to 187 mm, based on a study of *S. serrata* populations around Moreton Bay, Queensland (Heasman, 1980). Measurements of a crab's body size, such as CW can provide a means of assessing if a crab has reached maturity. This has potential use in estimating population and ratio of juveniles to adults, providing a possible indicator of overfishing.

The relationship between carapace width and body weight (CW:BW) has previously been used as a condition indicator. The body weight to length ratio provides a measure of how well-fed an individual is, with high ratios typically indicating that an ecosystem has high productivity and food density (Bolger and Connolly, 1989). *S. serrata* CW and BW are highly correlated with each other (r^2=0.825-0.904) in both sexes, even in recently-moulted 'soft-shelled' crab populations (Grubert *et al.*, 2012). CW:BW measurements can also be used to calculate biomass and estimate the amount of obtainable edible meat (Ikhwanuddin *et al.*, 2011). Other morphometric indicators such as claw size ratio, dominant and subordinate claws loss, and claw regeneration could provide potentially useful indicators of environmental stressors, with a study by Giblock and Crain (2013) on estuarine crabs in the USA showing that such morphometric indicators were significantly influenced by site (e.g. reference vs. urbanised locations) and date of sampling. However, Giblock and Crain (2013) also noted that there were no clear patterns on the influences of such environmental factors to those indicators.

The different life stages and size classes of *S. serrata* tend to reside separately (Hill *et al.*, 1982). A study on *S. serrata* populations in Moreton Bay, Queensland reported that juveniles mostly resided in the upper inter-tidal zone, sub-adults mostly resided in the sub-tidal zone, though moved up into the intertidal zone to feed during flood tides, whereas adults mostly resided and fed within the sub-tidal zone (Hill *et al.*, 1982). As adult mud crabs face the most fishing pressure due to legislated catch size limits in Queensland (Pillans *et al.*, 2005), monitoring and sampling that is more focused on the subtidal zone could better target and monitor the adult *S. serrata* populations, and provide a potential indicator of overfishing. Monitoring that targets juvenile crab populations on the other hand could provide an indicator of recruitment rates, and this is in turn sensitive to pollution (Giblock and Crain, 2013) and other environmental stressors such as climate (Meynecke *et al.*, 2012).

Indicator development

Following the literature review, a list of potential mud crab indicators was compiled. The potential indicators included the candidate indicators proposed by McIntosh *et al.* (2014) (size distribution, abundance, and visual health assessment of adult mud crabs), indicators identified through the literature review, and additional indicators based on monitoring data collected in Gladstone Harbour in June and July 2017.

Indicators were compared and scored against a set of ten predefined indicator selection criteria (Table 3). Selection criteria were modified from criteria used in other relevant studies (Rainbow, 1995; Cooper *et al.*, 2009; Kroon *et al.*, 2016; Flint *et al.*, 2017). The indicator scoring process involved four sequential phases:

1. Three of the project team members independently and without discussion or sharing of results (‘blind scoring’), scored the indicators against selection criteria using the criteria and scores defined in Table 3. All three scorers had access to the completed literature review;
2. Two different project team members then checked the three sets of independent scores and highlighted any discrepancies in the three total scores for each indicator (defined as a difference of ≥ 5 points between two total scores);
3. For indicators that were scored similarly (< 5 points difference between any two scores) a total score was calculated as the average of the three independent scores; and
4. Indicators that were scored differently (≥ 5 points difference between any two scores) were workshopped by all team members, and a consensus score reached.

Table 3: Indicator selection criteria used in the scoring process.

Criterion	Description and scoring key
SC1: Ability to account for spatial variability in ecosystem condition (spatial variability)	Is the indicator score likely to change if ecosystem condition is different between zones? Score: 3 = highly likely to account for variability Score: 2 = moderately likely to account for variability Score: 1 = possible that it may account for variability Score: 0 = unlikely to account for variability
SC2: Scientific interpretation is straightforward and meaningful for public communication	Is the indicator so complex as to be difficult to communicate in plain English? Score: 3 = easy to explain and highly meaningful to the public Score: 2 = moderately easy to explain / moderately meaningful Score: 1 = slightly difficult to explain / less meaningful Score: 0 = difficult to explain and/or not meaningful
SC3: Ability to identify change within a site through long term monitoring (temporal variability)	Will the indicator be useful for monitoring long term (e.g. >3 year) trends in the Gladstone mud crab population (based on annual capture:recapture monitoring)? Score: 3 = highly likely to identify long term trends Score: 2 = moderately likely to identify long term trends Score: 1 = possible that it may identify long term trends Score: 0 = unlikely to identify long term trends
SC4: Benchmarks and worst case scenarios can be defined	Can benchmarks be defined based on currently available data/literature? Score: 3 = high level of confidence in benchmarks Score: 2 = moderate level of confidence in benchmarks Score: 1 = low level of confidence in benchmarks Score: 0 = no benchmarks currently available
SC5: Practical – cost effective, easy to measure, non-destructive and observer independent	Is the indicator cost effective, easy to measure, non-destructive and unlikely to be affected by observer bias? Score: 3 = highly practical Score: 2 = moderately practical Score: 1 = less practical Score: 0 = significant issues identified with cost, measurement, destructiveness and/or observer bias
SC6: Does not require further research in order to implement	Is additional research required before using the indicator in Gladstone Harbour? Score: 3 = no further research required before implementation Score: 2 = some (low cost, short term) research required Score: 1 = moderate research required Score: 0 = extensive research required
SC7: Biological response is ecologically relevant	Does the indicator suggest other ecological impacts are occurring (e.g. at higher trophic levels, habitat level) Score: 3 = demonstrable link to other ecological impacts Score: 2 = some evidence of links to other ecological impacts Score: 1 = little/inconclusive evidence of links to other ecological impacts Score: 0 = does not suggest ecological impacts

Criterion	Description and scoring key
SC8: Relevant to local and state management goals	Is the indicator relevant to local and statement management goals (e.g. availability of healthy adult male mud crabs for recreational and commercial fisheries; strong spawning cohort and recruitment to ensure long term sustainability of the population)? Score: 3 = highly relevant to management goals Score: 2 = moderately relevant to management goals Score: 1 = slightly relevant to management goals Score: 0 = not linked to management goals
SC9: Provides an early warning of ecosystem health decline	Does the indicator react quickly to changes in the environment/ecosystem in order to provide an early warning of system decline? Score: 3 = highly reactive (short term; 1 year or less) Score: 2 = moderately reactive (medium term; 2-3 years) Score: 1 = slightly reactive (long term; > 3 years) Score: 0 = unreactive
SC10: Evidence of causation	Does current understanding suggest that the indicator reacts to specific causes, or does it provide a non-specific indication that something is wrong? Score: 3 = demonstrable link to a specific cause/impact Score: 2 = some evidence of links to a specific cause/impact Score: 1 = limited evidence of links to specific causes/impacts Score: 0 = non-specific

Prioritisation of possible indicators

Nine mud crab indicators were identified as potentially useful for inclusion in the Gladstone Harbour Report Card. The nine potential mud crab indicators, descriptions and either average or consensus total scores for each indicator are provided in Table 4. Appendix 1 includes the complete scoring table, including the three independent (blind) scores for each indicator against the ten selection criteria, and consensus or average scores.

Of the nine potential indicators, three were scored similarly (< 5 points difference between any two total scores) by the three independent scorers. The final scores for each of these three indicators were calculated as the average of the independent scores. Six potential indicators had ≥ 5 points difference in total scores, and were workshopped to determine a final consensus score based on shared understandings.

The four highest scoring indicators were: Size:Sex Ratio; Total Abundance (CPUE); Prevalence of Rust Lesions; and Biomass (calculated separately for male and female crabs). Higher total scores signified better indicators.

Table 4: Potential mud crab indicators for the Gladstone Harbour Report Card. Key: Total scores represent either a consensus (˜) or an averaged (*) score. Total scores indicated in bold are the four highest scoring indicators. The complete scoring table is provided at Appendix 1.

Potential Indicator	**Description**	**Total score /30**
Biomarkers, e.g. • GST induction and ChE inhibition • RNA/DNA ratios • Glutathione peroxidase activity and lipid peroxidases • Antioxidant enzymes and oxidative stress parameters	• Three biomarkers (glutathione-S-transferase (GST) activity, cholinesterase (ChE) activity and benzo-a-pyrene (BaP) urinary metabolites) indicate possible exposure to PAHs and organophosphate contamination. • RNA/DNA ratios are known to be a useful estimator of physiological condition in fish and invertebrates, with the ratio expected to increase when environmental conditions are favourable • Glutathione peroxidase activity is suggested as a biomarker of metal exposure with lipid peroxidases as a secondary marker when bioaccumulated metal concentrations are high. Increases in these biomarkers suggest exposure to metal contaminated sites, with resultant oxidative stress in the individual. • Higher oxidative stress with lower antioxidant concentrations are observed during periods of poor water quality conditions. *[Sometimes requires collection and dissection of crabs for analysis]*	14˜
Bioaccumulation of toxicants, e.g. • Bioaccumulation of metals in tissues • Structural deformities of organs (associated with metal bioaccumulation) • POPs bioaccumulation • Pesticides bioaccumulation	Several studies have been conducted using crabs as bioindicators of heavy metal and toxicant bioaccumulation. Structural deformities of the gills, muscles and hepatopancreas have been observed in *S. serrata* due to metal toxicity, with the degree of damage correlated with elevated bioaccumulated metal concentration. Tissue and metal-specific bioaccumulation was observed, with Cu, Pb, Cd and Mn highest in the hepatopancreas, lower in the gills and significantly lower in the muscle samples analysed, whereas Zn and Fe were highest in the gills, lower in the hepatopancreas and significantly lower in the muscles (Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014). In addition to metals, *S. serrata* can accumulate a range of other contaminants including persistent organic pollutants (POPs) and pesticides. *S. serrata* were sampled in six estuaries in Qld, for analysis of metals, metalloids and pesticides in muscle and hepatopancreas tissue. Most metals and metalloids were found to be preferentially sequestered to the hepatopancreas, and industrialised areas showed higher concentrations of Pb, Se and Sn than less impacted locations. DDT and dieldrin were detected in tissues along with other banned organochlorines. Dieldrin concentrations suggested that ambient exposures of dieldrin exceeded national water quality guidelines at all sampling locations, with one of the 77 samples of crab body muscle concentrations of dieldrin exceeding Australian Food Standards. Contemporary pesticides were also analysed in the hepatopancreas, endosulfan and chlorpyrifos were detected, while diuron, atrazine, simazine were not detected. *[Requires collection and dissection of crabs for analysis]*	21.3*
Size: Sex ratio	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. For example, the fishery for *S. serrata* in Queensland is limited to the collection of males, and it has been found that populations are female dominated. However, in unfished populations of mud crabs, the male: female ratio can be as high as 3:1. Seasonal changes in the sex ratio of crabs have been demonstrated in other portunid species. While not previously suggested in the literature, a size:sex ratio differentiating between both fished (male) and unfished (female) and fished (>150mm) and unfished (<150mm) *S. serrata* populations, would provide an indicator of fishing pressure. There is not conclusive evidence to determine times when spawning females are offshore in this region. *[Catch and release]*	**26.5˜**

Potential Indicator	**Description**	**Total score /30**
Nursery value (juvenile crabs; CPUE)	Mature female *S. serrata* range from 138 to 204 mm and mature males range from 108 to 187 mm. Measurements of a crab's body size, such as CW thus provides a means of assessing if a crab has reached maturity. This could potentially be useful in estimating the population and ratio of juveniles to adults, providing a potential indicator of overfishing and also of important nursery habitats. The different life stages of *S. serrata* tend to reside separately; juveniles are reported to reside mostly in the upper inter-tidal zone, sub-adults mostly reside in the sub-tidal zone, though move into the intertidal zone to feed during flood tides, and adults mostly reside and feed within the sub-tidal zone. As adult mud crabs face the most fishing pressure due to legislated catch size limits in Queensland, monitoring and sampling that is more focused on the subtidal zone could better target and monitor the adult *S. serrata* populations, and provide a potential indicator of overfishing. Monitoring that targets juvenile crab populations on the other hand could provide an indicator of recruitment rates, and this is in turn sensitive to pollution and other environmental stressors such as climate. *[Catch and release using different methods/locations]*	18˜
Total abundance (number of crabs; CPUE)	Estimating total harbour abundance (population size) requires repeated mark-recapture experiments. Trends in relative abundance between areas or through time are more straightforward, and can be measured using consistent monitoring methodologies to reduce variability. Discrepancies in abundance can be due to catch method, sampling areas and sampling times, or due to differences induced by habitat and environmental conditions. CPUE and related abundance data are often highly variable, due to *S. serrata* behaviour, population biology and dynamics, fishing effort efficiency and magnitude, and environmental conditions. Climate has a strong impact on survival and abundance, such that changes in rainfall and temperature could result in increased or decreased abundance through time. *[Catch and release]*	**25***
Prevalence of rust lesions	Rust spot lesions initially start as an orange discolouration on the crab's carapace, before progressing to perforation and exposure of the soft tissues beneath the carapace. The disease has the potential to damage the mud crab industry in Gladstone Harbour. A method to objectively measure and grade the severity of lesions on crab shell carapace has been developed and described. *[Catch and release]*	**24˜**
Prevalence of other diseases and parasites	*S. serrata* are susceptible to (externally visible) parasites and biofouling organisms such as barnacles, as well as a variety of bacterial, fungal and viral diseases. High prevalence of parasite and disease can be related to environmental stress. [*Catch and release]*	17.5˜
Biomass (CW:BW)	The relationship between carapace width and body weight (CW:BW) can be used as a condition indicator. The body weight to length ratio provides a measure of how well-fed an individual is, with high ratios typically indicating that an ecosystem has high productivity and food density. The wet weight and CW of *S. serrata* are highly correlated with each other (r^2=0.825-0.904) in both sexes, even in recently-moulted 'soft-shelled' crab populations. CW:BW measurements can also be used to calculate biomass and estimate the amount of obtainable edible meat. *[Catch and release]*	**25.3***
Morphometrics	Morphometric indicators such as claw size ratio, dominant and subordinate claws loss, and claw regeneration could provide potentially useful indicators of environmental stressors (previously been used for estuarine crabs in the USA). Morphometric indicators were significantly influenced by site (e.g. reference vs. urbanised locations) and date of sampling. However, there were no clear patterns on the specific influences of such environmental factors to those indicators. *[Catch and release]*	18˜

Mud crab indicator recommendations with discussion

Of the nine potential indicators identified through the literature review and data collection phases of this project, four indicators have been selected and are recommended as mud crab indicators for Gladstone Harbour. The recommendations are based on independent scoring using predetermined selection criteria. The four recommended indicators are:

- Size:Sex ratio;
- Abundance (CPUE);
- Prevalence of rust lesions; and
- Biomass as CW:BW.

Recreational and commercial fishing are major factors driving the management of Queensland's *S. serrata* population, and exert pressure on fished stocks. The indicator "Size:Sex ratio", compares male and female mud crab populations above and below the legal size limit for the species. By comparing the gender structures of mud crabs ≥ 150 mm and mud crabs < 150 mm, the Size:Sex ratio can provide an indication of the level of fishing pressure on male mud crab populations. Since standard crab pots will catch adult crabs that are smaller than 150 mm, the gender structures of the fished and unfished populations can be compared as a measure of specific fishing pressures between zones. Gender proportions can provide an indicator of fishing pressure (Heasman, 1980; Williams and Hill, 1982; Alberts-Hubatsch *et al.*, 2016) and sufficient long term monitoring data are available to determine benchmarks for the Gladstone Harbour region. Identifying the sex of mud crabs is quick and easy, and there are population and ecosystem implications for a shift in sex ratio, for example in terms of bioturbation (Bonine *et al.*, 2008). There is a remaining knowledge gap as to the timing and population effect of the female spawning migration in Gladstone Harbour and associated regions which would benefit from future research, and it would also be valuable to sample an unfished Queensland mud crab population as a direct comparison, for example the population within the Eurimbula mud crab no-take reserve.

Abundance of mud crabs (CPUE) is also recommended as an indicator. This indicator is easily interpretable and will give an accurate estimate of changes in abundance and population size (Dumas *et al.*, 2012; Meynecke *et al.*, 2012; Alberts-Hubatsch *et al.*, 2016) of mud crabs through time and by zone. Consistent methodologies will be employed during each catch period, to ensure opportunity for direct comparison of CPUE and abundance at different zones and between years. Climate has been shown to impact the abundance of mud crabs so there is also potential for this indicator to be used to monitor climate effects on mud crabs in the longer term, as well as in overall fishery management decisions. This indicator could also involve mark-recapture studies to attempt to provide a better estimate of total abundance. Sufficient historical data are available to develop benchmarks.

Within Gladstone Harbour, there have historically been periods during which a proportion of mud crabs caught have presented with rust spot lesions. While the exact cause of these lesions is unknown, it is known that the lesions are not infectious, but may be linked to metal exposure in the environment (Andersen *et al.*, 2000; Andersen and Norton, 2001). 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, with professional fishers and recreational fishers both less likely to keep, or consume, a mud crab with shell lesions such as rust spot. Recording the presence of rust spot is relatively straight forward and non-destructive. A worst case scenario (WCS) can be determined using the results of previous studies in which prevalence was found to be higher than background levels (e.g. Dennis et al. 2016).

The final mud crab indicator that is recommended for inclusion in the Gladstone Harbour Report Card is biomass, using carapace width to body weight comparisons. The carapace width to body weight ratio (CW:BW) is an indicator of health in mud crabs, with high ratios indicating high ecosystem productivity and food densities (Bolger and Connolly, 1989; Ikhwanuddin *et al.*, 2011; Grubert *et al.*, 2012). It is simple to measure and non-destructive. This measure can be compared across zones as an indicator of ecosystem productivity and can be used to estimate the amount of obtainable edible meat from an individual, or a sample of mud crabs. From a fisheries perspective, biomass is likely to give a comparative estimation of condition of mud crabs across zones, and to signal changes to mud crab health and ecosystem productivity through time. There are no historical data with which to determine a benchmark for this indicator, so a relative scoring system has been developed as an interim measure, using current data. It is important to note that biomass will vary significantly between summer (when mud crabs moult more regularly) and winter (when mud crabs moult less often, and tend to be ‘fuller’ and heavier for their size).

In addition to these four recommended indicators, there are two potential indicators which may be useful, but would require additional research and/or monitoring costs. These two indicators are:

- Bioaccumulation of metals; and
- Recruitment to nursery grounds.

Both of these potential indicators would require research to develop confidence in baselines. Bioaccumulation requires lethal sampling of mud crabs to measure metal concentrations in the hepatopancreas, muscle tissue or gills, with additional costs for dissection and analysis. Measuring recruitment to nursery grounds would be non-lethal but would involve extra costs because of the requirement for alternative monitoring techniques and sites.

Mud crab monitoring methodology

Two mud crab surveys were undertaken in 2017 (Table 5). Sites for the capture survey were selected based on historical sampling locations, local knowledge of mud crab populations and accessibility following a reconnaissance of potential sites conducted on 5-6 June 2017.

Sites for the recapture survey were decided based on results of the initial capture survey, and the need to reduce the number of sites sampled from eight to six (as requested by GHHP). A decision to move the Rodds Bay site was made in consultation with the GHHP Science Team and ISP Chair following low capture rates at the first site on 22 June, due to the original site having insufficient mud crab habitat to accommodate the number of pots required (teleconference, 29 June 2017).

Table 5: Zones/sites sampled during June and July 2017.

Zone/site	**Capture date**	**Recapture date**
Zone 1: Narrows	20 June	3 July
Zone 2: Graham Creek	20 June	3 July
Zone 4: Boat Creek	21 June	4 July
Zone 5: Inner Harbour	19 June	5 July
Zone 6: Calliope Estuary	21 June	4 July
Zone 7: Auckland Inlet	23 June	No
Zone 9: South Trees Inlet	19 June	No
Zone 13: Rodds Bay, Site A	22 June	NA
Zone 13: Rodds Bay, Site B	NA	6 July

Sampling dates were determined by tidal cycles. Surveys were conducted on dates where 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 and mortality to any fish caught in the pots). Pots were placed as close as possible to mangrove habitats.

At each site and sampling event, 20 heavy duty 4-entry round collapsible crab pots and two Baited Remote Underwater Video Stations (BRUVS) were set a minimum of 100 m apart (Figure 1). The exception to this was Zone 4 Boat Creek, as only 15 pots could be placed in this smaller system. Round, collapsible, heavy-duty crab pots purchased from a local tackle store were used, as they are easy to transport, assemble on the vessel and replace (Fisheries Queensland, 2009). The BRUVS were composed of a pyramid-shaped stainless steel welded frame with a downward-facing GoPro Hero 5 ® camera attached to the apex. Both a small (450x450x450 mm base x 260 mm high) and a large (550x550x550 mm base x 720 mm high) frame were trialled at each site. The reason for trialling different sized frames for the BRUVS was to examine the trade-off between field of view and visibility of the seabed in turbid conditions. Each pot and each BRUVS was baited with one large sea mullet (*Mugil cephalus*) head, and all floats were attached with 10m 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 or BRUVS to be identified quickly during retrieval. The opening of each pot was secured with a coloured cable tie, so that if crabs were removed by others this could be detected and recorded on retrieval. Figure 1 shows set up of pots and BRUVS.

Figure 1: Sampling equipment used for mud crab monitoring: heavy duty crab pots and steel frames in two sizes for BRUVS (cameras were mounted at the apex, pointed downwards at a bait bag attached to the centre of the base).

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 and BRUVS;
- 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.

For the first 40 mud crabs (*S. serrata*) at each site, the following information was recorded:

- Species;
- Sex;
- Carapace width (notch to notch) (mm);

- Weight (g);
- Abnormalities: type, body location, dimensions of rust spot lesions, grade of rust spot lesions (source Andersen, 2003; Appendix 2); and
- During the capture survey only (19-23 June): mud crabs were marked with nail varnish, with a numerical code according to the zone, pot number and crab number.

For subsequent mud crabs (at sites where more than 40 were caught), the following information was recorded:

- Species; and
- Sex.

All bycatch species (including blue swimmer crabs, fish and other crabs) were also recorded. Blue swimmer crabs were 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, so as to reduce interference with mud crab operators and recreational crabbers in the area.

Site selection methods

Nine sites in eight zones were sampled as GHHP preliminary monitoring sites for 2017 (Table 6). Twenty pots and two BRUVS were set at each of the nine sites from 19-23 June, 2017 (except at Zone 4 Boat Creek, as only 15 pots could be set in this smaller system). Six of the eight sites were then prioritised for the reduced recapture study conducted from 3-6 July, 2017.

After preliminary monitoring was completed, the following site selection criteria were applied to each zone/site to score, rank and recommend GHHP long term monitoring sites (summarised in Box 1):

1. Currently accessible – considering tidal restrictions and vessel travel time (long open-crossings require larger vessels and are more restricted by weather conditions);
2. Likely to remain accessible through time – some historical sites are no longer accessible (e.g. Fisherman's Landing);
3. Site type can be described as a deep-channel mangrove creek – for consistency in habitat types and to reduce habitat-specific variability between zones, muddy-substrate mangrove-dominated creeks are favoured as sampling sites;
4. Mud crab capture rates – based on capture rates during preliminary monitoring in June/July;
5. Proximity to other GHHP monitoring sites – for similar indicators such as fish recruitment; and
6. Historical mud crab monitoring locations – including Fisheries Queensland and various research monitoring programs.

Each site was given a score for each selection criterion based on whether the site was rated by the authors as "High" (score = 3), "Moderate" (score = 2), "Low" (score = 1) or "None/Not suitable" (score = 0). A total score was then calculated by summing the criteria scores and dividing by the total possible score for that site, which was 18 for sites that were sampled in 2017 and 15 for sites that were not sampled in 2017. In order to fully assess the 5 zones that were not sampled in 2017, further testing would be required. However the 8 zones selected for 2017 sampling are considered the most appropriate for ongoing monitoring. A ranking of "Highly suitable", "Moderately suitable" or "Limited suitability" was then applied to each zone/site based on the total score (Highly > 0.7, Moderately < 0.7 and > 0.3, Limited < 0.3). All sites were in proximity to current water quality monitoring sites.

Box 1: Long-term monitoring site selection.

Long-term mud crab monitoring site selection criteria

1. Currently accessible
2. Likely to remain accessible through time
3. Site type (deep-channel mangrove creek preferred)
4. Mud crab capture rates
5. Proximity to other GHHP monitoring sites
6. Historical mud crab monitoring locations

Monitoring site assessment and recommendations

Based on the assessment of sites against selection criteria (Table 6), taking into account the results of sampling conducted in June/July 2017, the following seven zones are recommended as long-term mud crab monitoring sites in Gladstone Harbour (Figure 2):

Zone 1 – Narrows
Zone 2 – Graham Creek
Zone 4 – Boat Creek
Zone 5 – Inner Harbour
Zone 6 – Calliope River
Zone 7 – Auckland Inlet
Zone 13 – either Rodds Bay, Site B or Colosseum Inlet.

Using the field sampling methodology described in this report, two Gladstone Harbour sites can be sampled in one day, and the Rodds Bay site requires a full day for sampling due to travel time. This sampling regime allows for seven zones to be sampled in four consecutive days. Adding more zones/sites would require additional sampling days, which would increase the cost. If more than four days are required, sampling should be undertaken over two separate weeks, as low tide time gets later over consecutive days and is too late in the afternoon by the fifth day of sampling.

Table 6: Assessment of potential sites against long-term monitoring site selection criteria. Scores: 3 = High, 2 = Moderate, 1 = Low, 0 = None/Not suitable, NA = not applicable. Ranks: zones are ranked by total score, Highly suitable > 0.7, Moderately suitable < 0.7 and > 0.3, Limited suitability < 0.3

Zone	Criterion	Score	Comments	Total score	Rank
Zone 1: Narrows	1	2	Moderately accessible, long but sheltered vessel crossing	16/18 = 0.89	Highly suitable
	2	3	Too shallow to develop further, not adjacent to shipping channels		
	3	2	Narrows site is like a large estuary, but is not a river and has tidal flow through		
	4	3	High catch		
	5	3	Fish recruitment sampled at mouth of Black Swan Creek (Sawynok and Venables 2016).		
	6	3	Close proximity to the northern Narrows sites monitored by Fisheries Queensland. Monitored by Andersen and Norton (2001), Andersen et al		

Zone	Criterion	Score	Comments	Total score	Rank
			(2005).		
Zone 2: Graham Creek	1	3	Accessible via short harbour crossing	17/18 = 0.94	Highly suitable
	2	3	Not adjacent to shipping channels		
	3	3	Mangrove creek with muddy areas and some rocky areas which can be avoided when setting pots		
	4	2	Moderate-High catch		
	5	3	Fish recruitment sampled at two locations in Graham Creek (Sawynok and Venables 2016).		
	6	3	Historical monitoring sites for Fisheries Queensland. Also monitored by Andersen and Norton (2001; Site 3), Andersen et al. (2005) and Dennis et al. (2016).		
Zone 3: Western Basin	1	2	Only accessible at high tide	8/15 = 0.53	Moderately suitable
	2	1	Adjacent to reclaimed land at Fisherman's Landing, adjacent to coal export terminals		
	3	1	Deep channel with mangroves adjacent, but is not a creek/estuary		
	4	NA	Not sampled in 2017		
	5	2	Fish recruitment sampled offshore in Western Basin zone (Sawynok and Venables 2016).		
	6	2	Close proximity to Andersen et al. (2005) Site 1 and Fisheries Queensland historical monitoring site (exact monitoring site is where Fisherman's Landing is now). Also monitored by Dennis et al. (2016).		
Zone 4: Boat Creek	1	2	Only accessible at high tide	14/18 = 0.77	Highly suitable
	2	2	Likely to remain accessible at high tide only		
	3	3	Narrow, deep channel mangrove creek with muddy substrate		
	4	3	High catch		
	5	3	Fish recruitment sampled at mouth of Boat Creek (Sawynok and Venables 2016).		
	6	3	Monitored by Andersen and Norton (2001; Site 1), Andersen et al. (2005) and Dennis et al. (2016).		
Zone 5: Inner Harbour	1	3	Accessible via short harbour crossing		
	2	3	Likely to remain accessible		

Zone	Criterion	Score	Comments	Total score	Rank
	3	3	Deep channel mangrove creek	17/18 = 0.94	Highly suitable
	4	3	High catch		
	5	3	Fish recruitment sampled at mouth of Enfield Creek (Sawynok and Venables 2016).		
	6	2	Monitored by Andersen and Norton (2001; Site 4) and Dennis et al. (2016).		
Zone 6: Calliope Estuary	1	3	Easily accessible	17/18 = 0.94	Highly suitable
	2	3	Likely to remain accessible		
	3	3	Large mangrove creek, muddy substrate with some rocky areas which can be avoided when setting pots		
	4	2	Moderate catch		
	5	3	Fish recruitment sampled at several sites along Calliope Estuary (Sawynok and Venables 2016).		
	6	3	Historical monitoring sites for Fisheries Queensland.		
Zone 7: Auckland Inlet	1	3	Easily accessible	13/18 = 0.72	Highly suitable
	2	3	Likely to remain accessible		
	3	2	Mangrove creek, but shorelines are developed		
	4	2	Moderate catch		
	5	3	Fish recruitment sampled at several sites along Auckland Inlet (Sawynok and Venables 2016).		
	6	0	Not a historical sampling site.		
Zone 8: Mid Harbour	1	2	Moderately accessible, long but sheltered harbour crossing	9/15 = 0.60	Moderately suitable
	2	2	Likely to remain moderately accessible		
	3	2	Some small mangrove creeks on Facing Island		
	4	NA	Not sampled in 2017		
	5	2	Fish recruitment sampled offshore in Mid Harbour zone (Sawynok and Venables 2016).		
	6	1	Sampled initially by Andersen and Norton (2001; Site 6) but was later disregarded.		
Zone 9: South Trees Inlet	1	2	Moderately accessible, long but sheltered harbour crossing		
	2	2	Likely to remain moderately accessible		
	3	3	Large mangrove creek with some sandy areas which can be avoided by site		

Zone	Criterion	Score	Comments	Total score	Rank
			selection	12/18 = 0.67	Moderately suitable
	4	1	Low catch		
	5	3	Fish recruitment sampled at several sites along South Trees Inlet (Sawynok and Venables 2016).		
	6	1	Only historical sampling of mud crabs was one study at nearby Spillway Creek (Andersen et al. 2003).		
Zone 10: Boyne Estuary	1	2	Moderately accessible, via alternative launch site (requires whole day sampling)	8/15 = 0.53	Moderately suitable
	2	2	Likely to remain moderately accessible		
	3	1	Predominantly sandy substrate with few mangroves		
	4	NA	Not sampled in 2017		
	5	3	Fish recruitment sampled at several sites along Auckland Inlet (Sawynok and Venables 2016).		
	6	0	Not a historical sampling site for mud crabs.		
Zone 11: Outer Harbour	1	1	Not easily accessible. Long open water crossing – requires larger vessel (higher cost).	4/15 = 0.27	Limited suitability
	2	1	Likely to remain lower accessibility		
	3	0	Open water – not suitable habitat		
	4	NA	Not sampled in 2017		
	5	2	Fish recruitment sampling at western side of Hummock Hill Island (Sawynok and Venables 2016).		
	6	0	Not a historical sampling site for mud crabs.		
Zone 12: Colosseum Inlet	1	1	Not easily accessible. Long open water crossing – requires larger boat (higher cost) and requires whole day sampling.	10/15 = 0.67	Moderately suitable
	2	1	Likely to remain lower accessibility		
	3	3	Large mangrove creek system		
	4	NA	Not sampled in 2017		
	5	3	Fish recruitment sampled at several sites in Colosseum Inlet (Sawynok and Venables 2016).		
	6	2	Sampled by Dennis et al. (2016).		
Zone 13: Rodds Bay, Site B	1	2	Moderately accessible, via alternative launch site (requires whole day sampling)		

Zone	Criterion	Score	Comments	Total score	Rank
	2	2	Likely to remain moderately accessible	12/18 = 0.67	Moderately suitable
	3	3	Large mangrove creek system		
	4	1	Low catch		
	5	2	Fish recruitment sampled at Turkey Beach (Sawynok and Venables 2016).		
	6	2	Seven Mile Creek and Turkey Beach sampled by Dennis et al. (2016)		

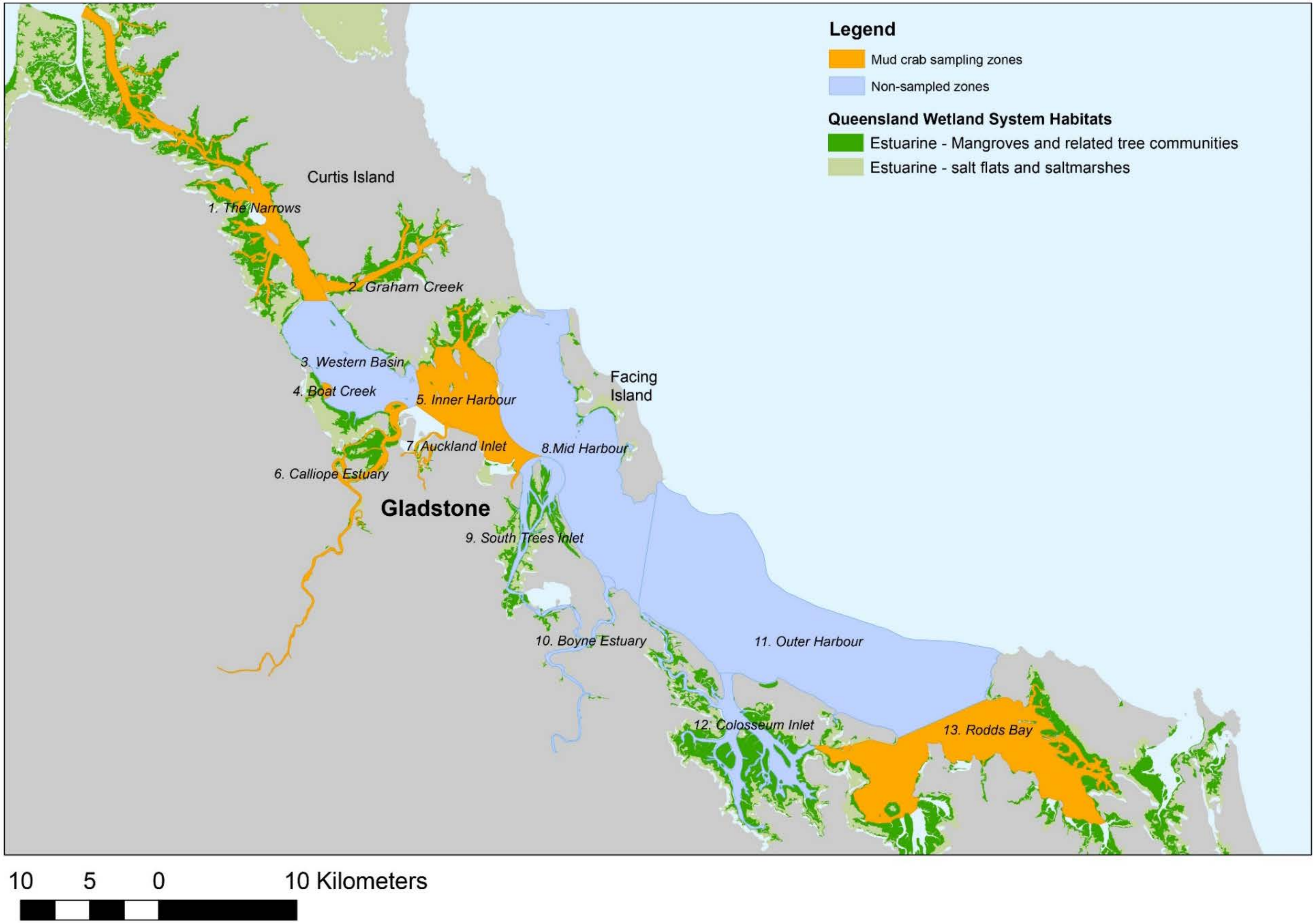


Figure 2: Map of the Gladstone Harbour zones showing recommended long-term mud crab monitoring sites.

Data analysis

Data from the first (19-23 June 2017) and second (3-6 July 2017) field sampling events were analysed separately. 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.

Historical data provided by Fisheries Queensland from the LTMP for the Mud Crab Fishery were analysed for comparison to 2017 results and to aid in developing baselines for some indicators. The LTMP dataset included carapace width and sex of mud crabs caught at six sampling sites from 2001 to 2009. A total of 1932 mud crabs are included in the dataset (728 males and 1204 females).

Of the six LTMP sites, two are still available for sampling in 2017 – Calliope River (only sampled by LTMP from 2006-2008 following an oil spill) and Graham Creek (sampled continuously from 2001 to 2009). The Little Oakie Creek, Deception Creek and Conner Creek sites are located outside of GHHP Zones. The Gladstone Harbour site sampled by the LTMP is now inaccessible.

All analyses were conducted in R version 3.4.1 (https://www.r-project.org/).

Results of mud crab sampling in Gladstone Harbour, June and July 2017

First sampling event (19-23 June)

A total of 305 mud crabs were caught and checked for gender across the eight zones sampled. Of these, 205 mud crabs were measured, weighed and checked for lesions (the remaining 100 crabs being in excess of the maximum of 40 crabs processed at each site). Of these 205 'checked' mud crabs, 122 were male and 83 were female.

The average size of mud crabs caught in June 2017 was 149.16 mm carapace notch width, and crabs were, on average, larger in samples from June than from the LTMP which were collected during summer months (Table 7, Figure 3). Results of a Welch Two sample t-test found that females caught in June were significantly larger than males caught in June ($t = -8.3318$, $df = 167.54$, $p < 0.001$).

The largest maximum mud crab size was recorded from Graham Creek (mean notch width 155.97 mm) and the minimum from Boat Creek (135.54 mm) (Table 8, Figure 4).

Table 7. Notch width (in mm) of mud crabs caught in June and July 2017, and by the LTMP in the period 2001-2009.

	FULL SAMPLE			MALES			FEMALES		
	June 2017 data	July 2017 data	Historical data (2001-2009)	June 2017 data	July 2017 Data	Historical data (2001-2009)	June 2017 data	July 2017 data	Historical data (2001-2009)
Mean	149.16	155.00	145.45	141.36	148.11	135.12	160.63	161.27	151.67
Standard deviation	18.59	18.93	20.74	15.54	17.95	18.65	16.72	17.67	19.43

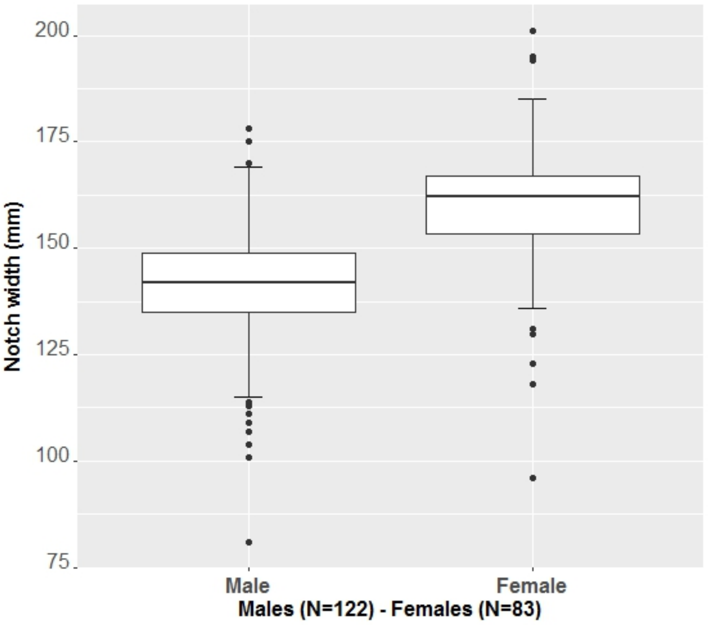


Figure 3: Notch width distribution of ‘checked’ male and female mud crabs caught in June 2017.

Table 8: Notch width (mm) of mud crabs caught in each zone in June 2017.

ZONE	ZONE NAME	MEAN NOTCH WIDTH	SD NOTCH WIDTH
1	Narrows	153.85	15.13
2	Graham Creek	155.97	14.95
4	Boat Creek	135.54	18.34
5	Inner Harbour	151.83	13.15
6	Calliope Estuary	147.21	14.74
7	Auckland Inlet	154.46	18.08
9	South Trees Inlet	147.22	36.50
13 - A	Rodds Bay	148.09	22.27

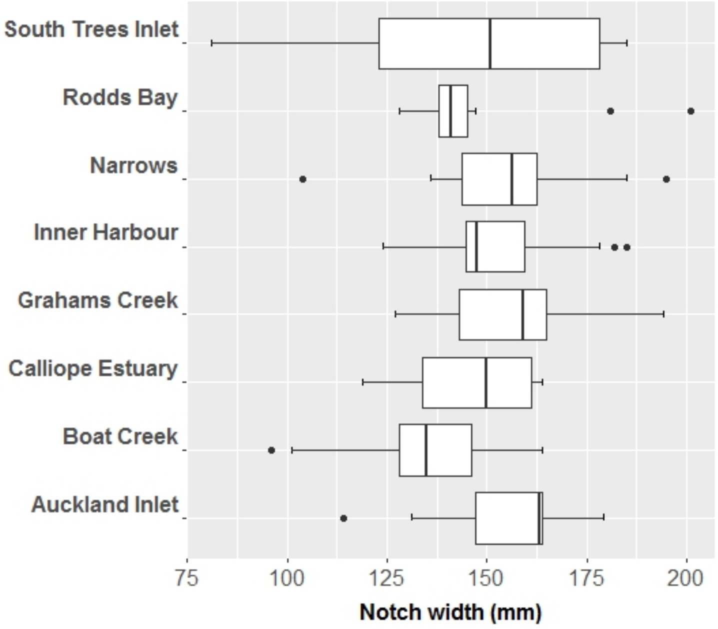


Figure 4: Notch width (mm) of ‘checked’ mud crabs caught in June 2017, by zone.

The average weight of mud crabs caught in June was 718.44 g (Table 9, Figure 5). Results of a Welch Two sample t-test found that females caught in June were significantly heavier than males caught in June (t = -3.6316, df = 194.81, p < 0.001).

The highest average mud crab weight was recorded at Graham Creek (mean weight 868.86 g) and the lowest at Boat Creek (522.13 g) (Table 10, Figure 6).

Table 9: Weight (g) of mud crabs caught in June and July 2017.

	FULL SAMPLE		**MALES**		**FEMALES**	
	June 2017 data	**July 2017 data**	**June 2017 data**	**July 2017 data**	**June 2017 data**	**July 2017 data**
Mean	718.44	741.28	667.93	746.79	794.83	736.27
Standard deviation	262.77	282.33	280.14	335.58	213.96	225.45

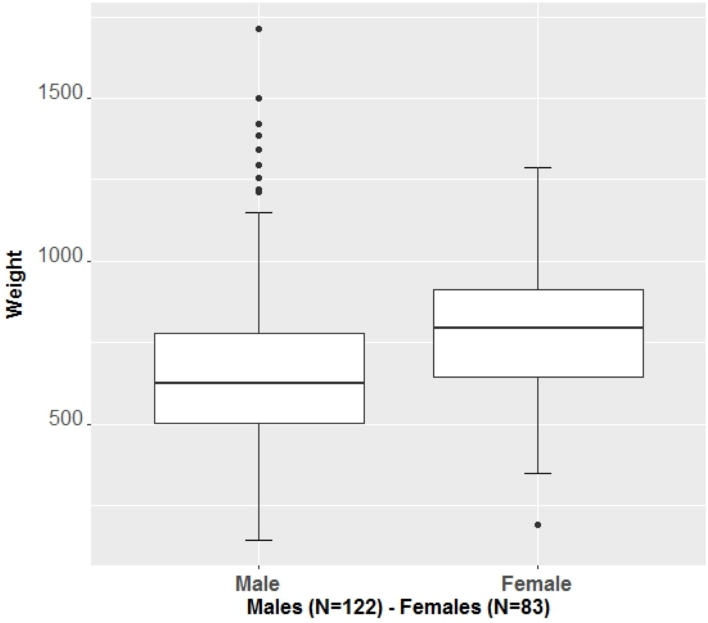


Figure 5: Weight (g) of checked male and female mud crabs caught in June 2017.

Table 10: Weight (g) of 'checked' mud crabs caught in June 2017, by zone.

ZONE	**ZONE NAME**	**MEAN WEIGHT**	**SD WEIGHT**
1	Narrows	702.10	184.26
2	Grahams Creek	868.86	284.89
4	Boat Creek	522.13	199.85
5	Inner Harbour	765.33	203.47
6	Calliope Estuary	689.14	232.91
7	Auckland Inlet	797.83	354.60
9	South Trees Inlet	790.44	415.36
13 - A	Rodds Bay	719.18	211.89

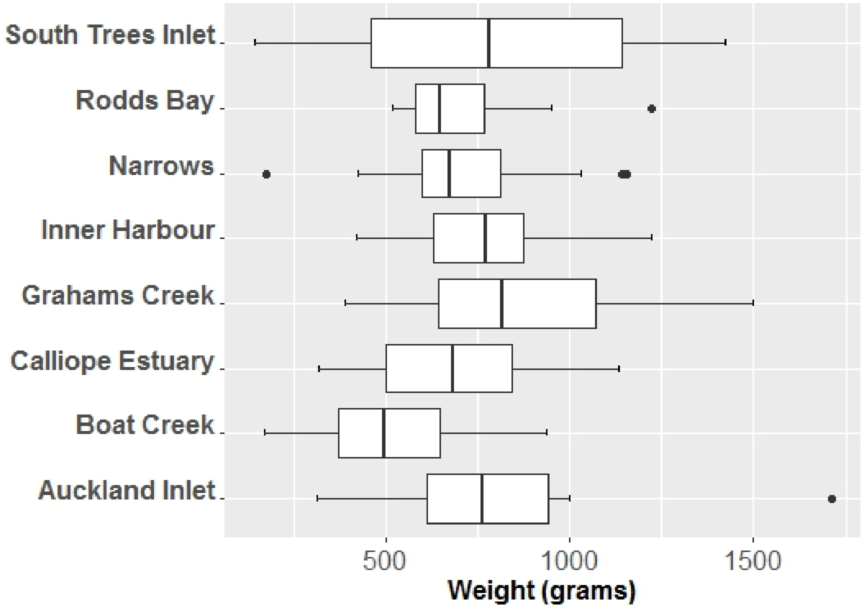


Figure 6: Weight (g) of checked mud crabs caught in June 2017, by zone.

Total catch per unit effort (CPUE) was highest at the Narrows and lowest at South Trees Inlet (Table 11, Figure 7). Of the 205 crabs measured, weighed and checked for lesions in June 2017, 12 had rust lesions and 193 did not. The percentage of crabs with lesions was highest at Auckland Inlet (15.4%) and lowest at the Narrows (2.5%) (Table 12).

Table 11: Catch per unit effort in June 2017, by zone.

ZONE	ZONE NAME	CPUE
1	Narrows	4.62
2	Grahams Creek	1.95
4	Boat Creek	3.60
5	Inner Harbour	3.50
6	Calliope Estuary	0.70
7	Auckland Inlet	0.65
9	South Trees Inlet	0.45
13 - A	Rodds Bay	0.55

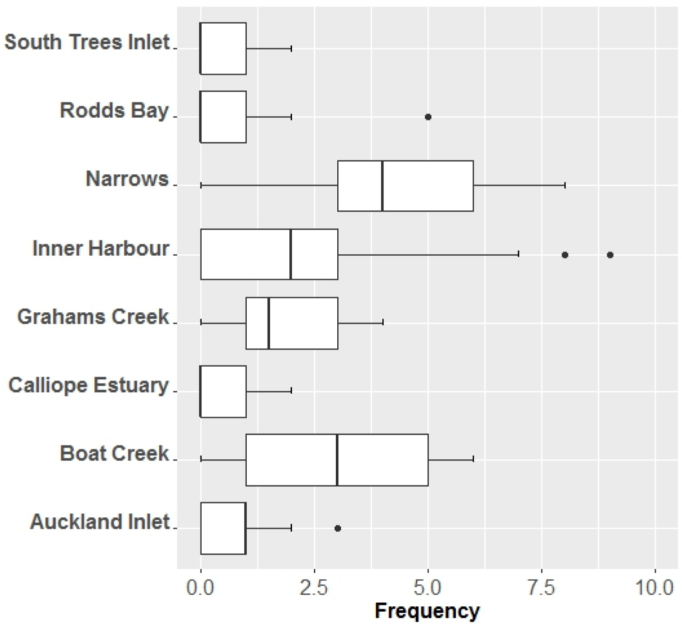


Figure 7: Number of mud crabs in each pot set in June 2017, by zone.

Table 12: Percentage of mud crabs caught in June 2017 with and without rust spot lesions, by zone.

ZONE	ZONE NAME	MUD CRABS WITH LESIONS	MUD CRABS WITHOUT LESIONS	% WITHOUT LESIONS	% WITH LESIONS
1	Narrows	1	39	97.5	2.5
2	Grahams Creek	2	35	94.6	5.4
4	Boat Creek	1	40	97.6	2.4
5	Inner Harbour	3	37	92.5	7.5
6	Calliope Estuary	1	13	92.9	7.1
7	Auckland Inlet	2	11	84.6	15.4
9	South Trees Inlet	1	8	88.9	11.1
13 - A	Rodds Bay	1	10	90.9	9.1

Second sampling event (3-6 July)

A total of 165 mud crabs were caught and checked for gender across the six zones sampled in July 2017. Of these, 147 mud crabs were measured, weighed and checked for lesions (the remaining 18 crabs being in excess of the maximum of 40 crabs processed at each site). Of the 147 'checked' mud crabs, 70 were male and 77 were female. Only 9 of the 165 crabs caught were recaptures that had been 'marked' with nail varnish. Two of the recaptures were from Graham Creek and 7 were from Boat Creek. Due to the small recapture numbers, it was not possible to calculate estimates of total abundance using mark-recapture methodologies.

The average size of mud crabs caught in July was 155.00 mm carapace notch width, and crabs were, on average, larger in samples from July than from June 2017 or the LTMP (Table 7, Figure 8). Results of a Welch Two sample t-test found that females caught in July were significantly larger than males caught in July ($t = -4.4696$, $df = 143.2$, $p < 0.001$).

The largest average mud crab size in July 2017 sampling was recorded at the Narrows (mean notch width 167.71 mm) and the smallest at Boat Creek (139.85 mm) (Table 13, Figure 9).

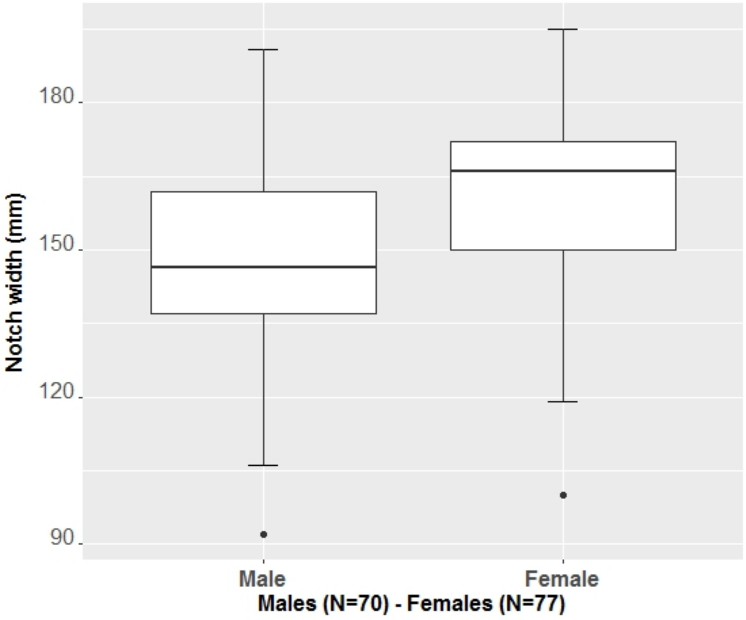


Figure 8: Notch width of ‘checked’ male and female mud crabs caught in July 2017.

Table 13: Notch width (mm) of mud crabs caught in each zone in July 2017.

ZONE	ZONE NAME	MEAN NOTCH WIDTH	SD NOTCH WIDTH
1	Narrows	167.71	11.49
2	Grahams Creek	157.64	16.13
4	Boat Creek	139.85	14.11
5	Inner Harbour	153.00	16.46
6	Calliope Estuary	147.86	36.03
13 - B	Rodds Bay	166.00	12.77

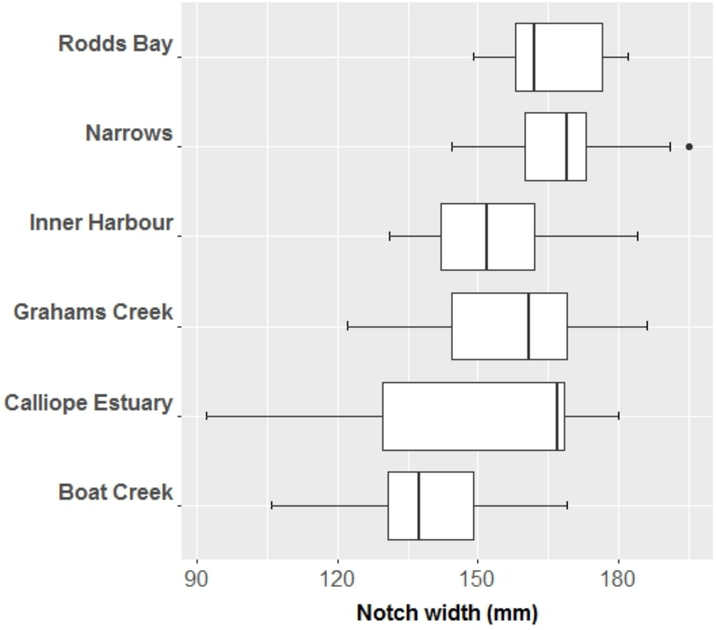


Figure 9: Notch width (mm) of ‘checked’ mud crabs caught in July 2017, by zone.

The average weight of mud crabs caught in July was 741.28 g, higher than the average weight in June 2017 (Table 9, Figure 10). In contrast to June sampling event, the results of a Welch Two sample t-test found that females caught in July were not significantly heavier than males caught in July (t = 0.22071, df = 119.05, p = 0.826).

The highest average mud crab weight was recorded at Rodds Bay Site B (mean weight 917.14 g) and the lowest at Boat Creek (532.43 g) (Table 14, Figure 11).

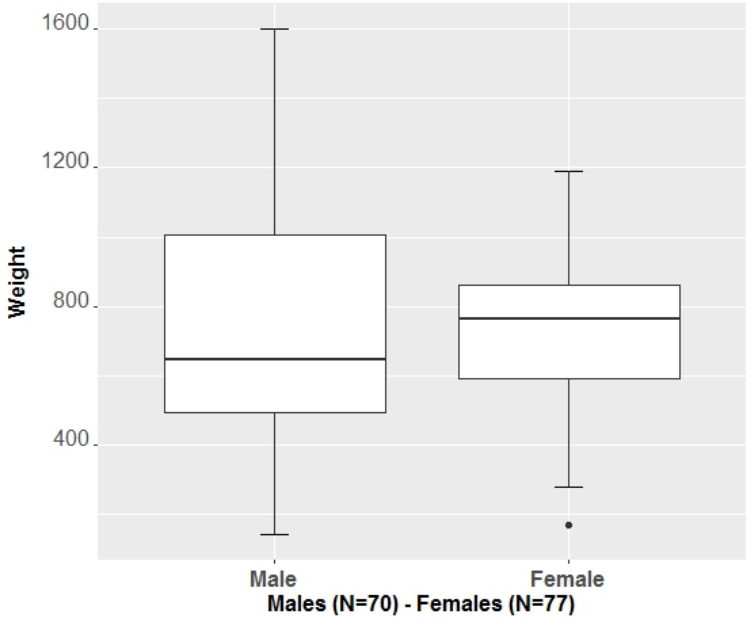


Figure 10: Weight (g) of 'checked' male and female mud crabs caught in July 2017.

Table 14: Weight (g) of 'checked' mud crabs caught in July 2017, by zone.

ZONE	ZONE NAME	MEAN WEIGHT	SD WEIGHT
1	Narrows	861.71	231.50
2	Grahams Creek	770.06	244.89
4	Boat Creek	532.43	195.14
5	Inner Harbour	778.76	250.34
6	Calliope Estuary	818.57	503.92
13 - B	Rodds Bay	917.14	363.42

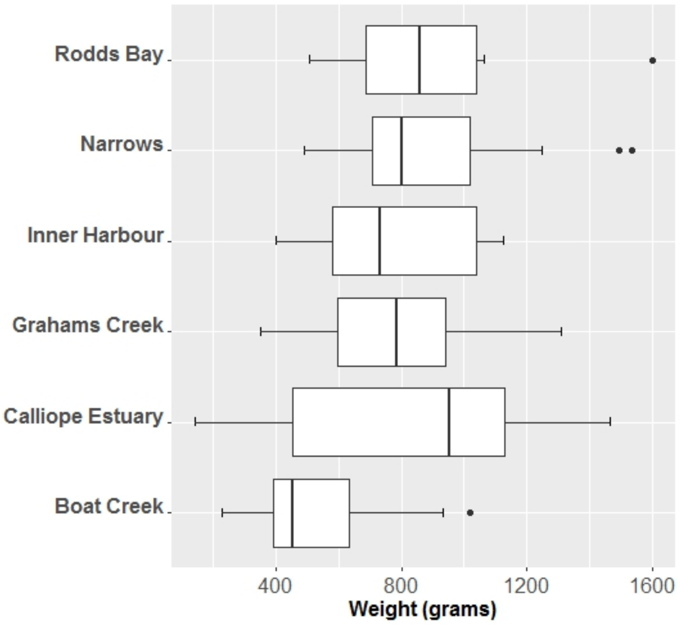


Figure 11: Weight (g) of 'checked' mud crabs caught in July 2017, by zone.

Total CPUE was highest at Boat Creek and lowest at Calliope Estuary and Rodds Bay (Table 15, Figure 12). Of the 147 crabs measured, weighed and checked for lesions in July 2017, 6 had rust lesions and 141 did not. The percentage of crabs with lesions was highest at Inner Harbour (17.65%) and was zero at the Narrows, Boat Creek and Calliope Estuary (Table 16).

Table 15: Catch per unit effort in July 2017, by zone.

ZONE	ZONE NAME	CPUE
1	Narrows	2.75
2	Grahams Creek	1.75
4	Boat Creek	2.93
5	Inner Harbour	0.85
6	Calliope Estuary	0.35
13	Rodds Bay	0.35

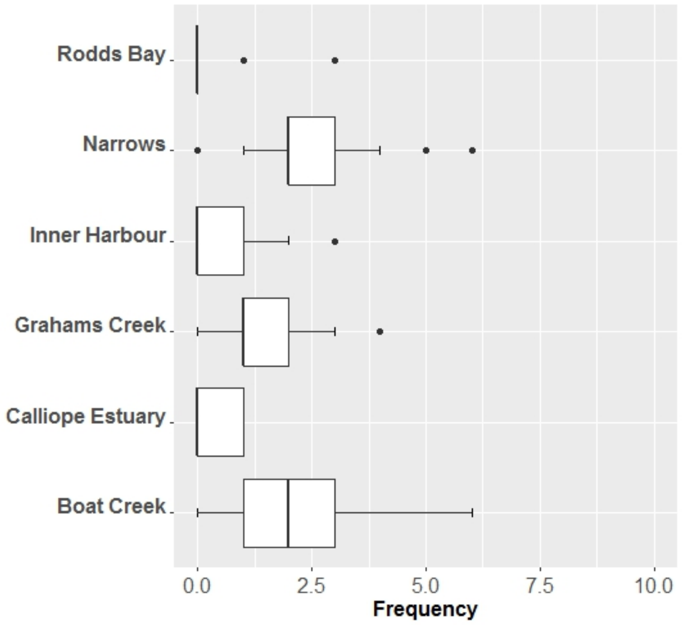


Figure 12: Number of mud crabs caught in each pot set in July 2017, by zone.

Table 16: Percentage of mud crabs caught in June 2017 with and without rust spot lesions, by zone.

ZONE	ZONE NAME	MUD CRABS WITH LESIONS	MUD CRABS WITHOUT LESIONS	% WITHOUT LESIONS	% WITH LESIONS
1	Narrows	0	41	100	0
2	Grahams Creek	2	33	94.29	5.71
4	Boat Creek	0	40	100	0
5	Inner Harbour	3	14	82.35	17.65
6	Calliope Estuary	0	7	100	0
13 – B	Rodds Bay	1	6	85.71	14.29

Baited Remote Underwater Video Stations (BRUVS)

Scylla serrata were observed at half (nine of a total 18) of the BRUVS that were set throughout the zones during the initial BRUVS deployment period (19-23 June 2017; Table 17). Other species of crabs (*Decapoda* sp.), along with fish (*Acanthopagrus australis* and *Terapon jarbua*) and polychaetes were also observed. Time to first observation varied (and is useful as an indication of minimum soak times for pots) from nine minutes at one BRUVS set in Rodds Bay, to two hours forty minutes at a BRUVS in Enfield Creek. No observations were recorded at Calliope River, Auckland Creek, and from one BRUVS each at South Trees and Rodds Bay. Lack of observations was due to particularly turbid water obscuring visibility of the bait bag, or due to obstruction of the camera by macroalgae.

Similar results were observed from BRUVS during the second deployment period (3-6 July 2017; Table 18), with little variation in species detected. The main exceptions were the observation of a *Portunus* sp. during the second deployment period in Enfield Creek (5 July 2017) and observation of *Favonigobius* sp. at Graham Creek and Calliope River (3 and 4 July 2017, respectively).

Table 17: Species sightings from BRUVS deployed during June 2017.

Zone Number	Zone Name	Date	BRUVS number	Species sighted	Time to sighting (hours:minutes)
1	The Narrows	20/06/2017	1	*Scylla serrata* *Terapon jarbua*	0:21 1:02
1	The Narrows	20/06/2017	2	*Scylla serrata*	1:09
2	Graham Creek	20/06/2017	1	*Scylla serrata*	1:11
2	Graham Creek	20/06/2017	2	*Scylla serrata*	0:27
4	Boat Creek	21/06/2017	1	*Scylla serrata*	0:27
4	Boat Creek	21/06/2017	2	*Acanthopagrus australis* *Scylla serrata*	0:51 1:05
5	Enfield Creek	19/06/2017	1	*Decapoda* sp.	1:29
5	Enfield Creek	19/06/2017	2	*Acanthopagrus australis*	2:40
6	Calliope River	21/06/2017	1	-	-
6	Calliope River	21/06/2017	2	-	-
7	Auckland Creek	23/06/2017	1	-	-
7	Auckland Creek	23/06/2017	2	-	-
9	South Trees	19/06/2017	1	*Acanthopagrus australis*	1:21
9	South Trees	19/06/2017	2	-	-
13	Rodds Bay	22/06/2017	1	-	-
13	Rodds Bay	22/06/2017	2	Polychaeta *Decapoda* sp. *Scylla serrata*	0:16 0:26 0:32
13	Rodds Bay	22/06/2017	3	*Scylla serrata*	0:09
13	Rodds Bay	22/06/2017	4	*Scylla serrata*	1:35

Table 18: Species sightings from BRUVS deployed during July 2017.

Zone Number	Zone Name	Date	BRUVS number	Species sighted	Time to sighting (hours:minutes)
1	The Narrows	3/07/2017	1	*Scylla serrata*	0:25
1	The Narrows	3/07/2017	2	*Scylla serrata* *Acanthopagrus australis*	0:11 1:51
2	Graham Creek	3/07/2017	1	*Favonigobius* sp.	2:07
4	Boat Creek	4/07/2017	1	*Scylla serrata* *Acanthopagrus australis*	0:35 1:15
4	Boat Creek	4/07/2017	2	-	-
5	Enfield Creek	5/07/2017	1	*Decapoda* sp. *Portunus* sp.	0:09 0:16
5	Enfield Creek	5/07/2017	2	*Portunus* sp. *Acanthopagrus australis*	0:06 0:20
5	Enfield Creek	5/07/2017	3	*Acanthopagrus australis*	0:57
5	Enfield Creek	5/07/2017	4	-	-
6	Calliope River	4/07/2017	1	-	-
6	Calliope River	4/07/2017	2	*Favonigobius* sp.	0:31
13	Rodds Bay	6/07/2017	1	*Acanthopagrus australis*	0:53
13	Rodds Bay	6/07/2017	2	-	-

Long Term Monitoring Program (LTMP) data

Data from the LTMP were provided by Fisheries Queensland. From 2001 to 2009, 1934 mud crabs were caught across six locations in Gladstone Harbour and the northern creeks of the Narrows (slightly to the north of GHHP Zone 1). Mud crabs were measured and gender recorded. This valuable dataset provides a comparison to the new 2017 data. Of the 1934 crabs, 728 were male, 1204 were female and two were unrecorded.

The mean size of mud crabs caught in the LTMP was 149.16 mm carapace notch width (Table 7, Figure 13). Results of a Welch Two sample t-test found that females caught in the LTMP were significantly larger than males (t = -18.595, df = 1576.9, p < 0.001). The largest average mud crab size was recorded in 2005 (200 mm, Figure 14), and trends in size by year are provided in Figure 15.

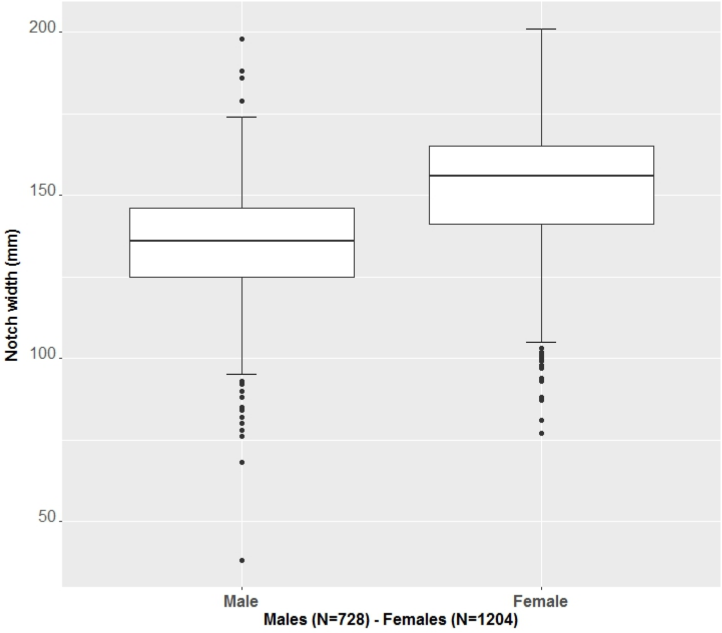


Figure 13: Notch width distribution of male and female mud crabs caught in the LTMP.

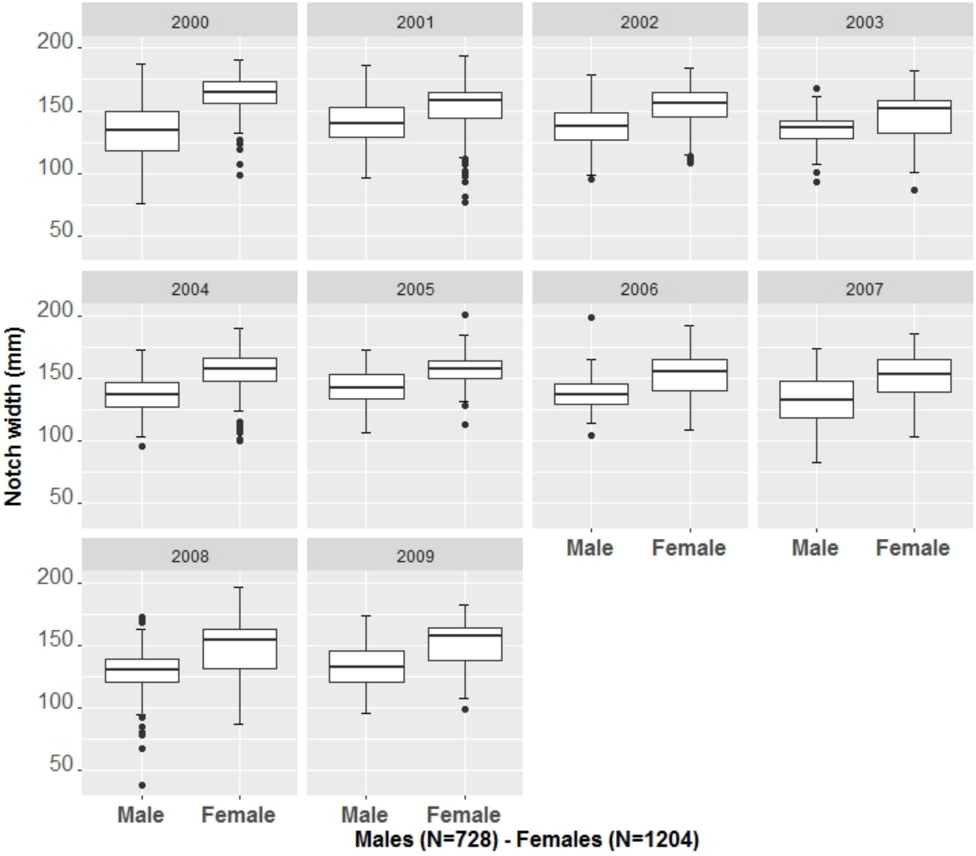


Figure 14: Notch width distribution of male and female mud crabs caught in the LTMP, by year.

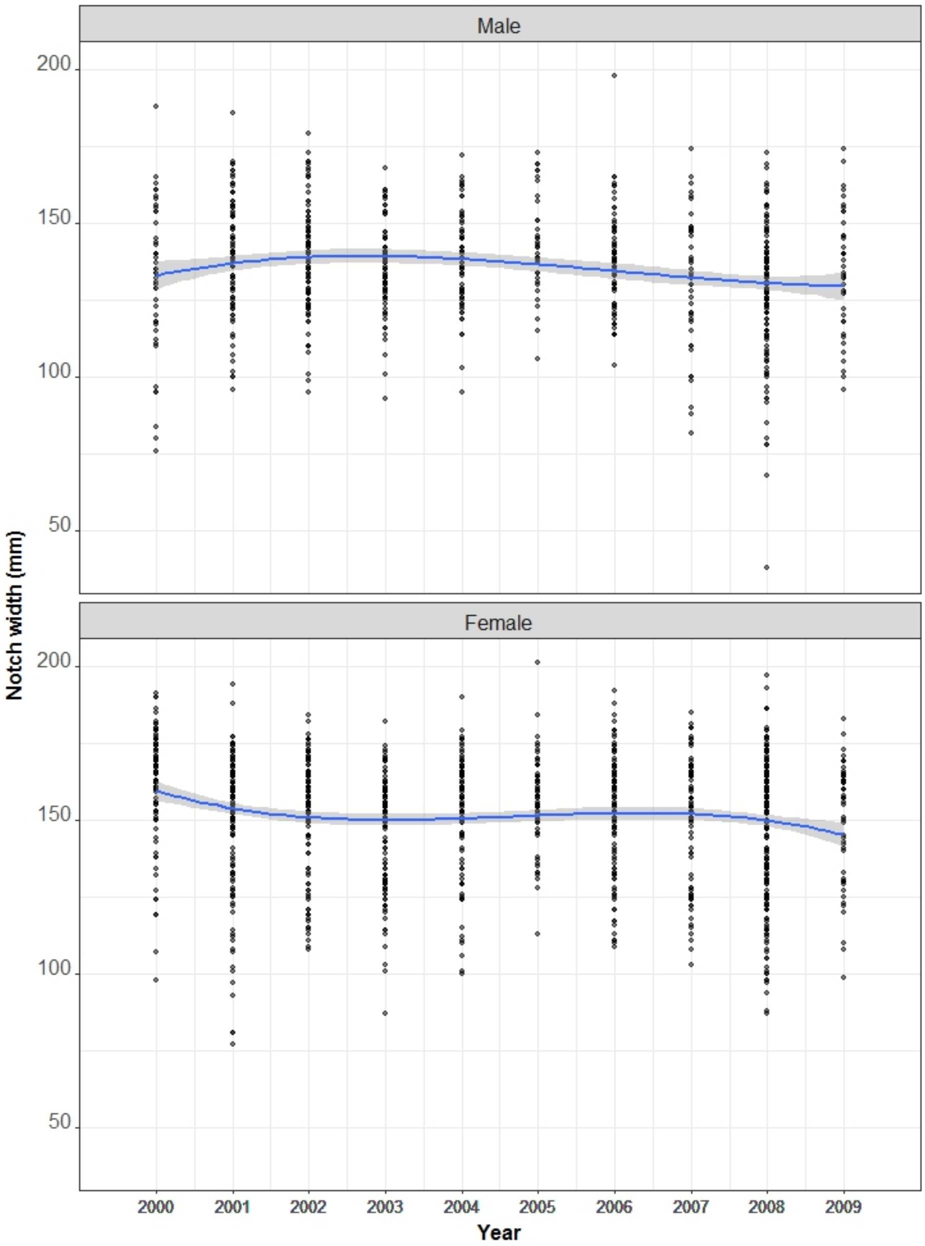


Figure 15: Trend in notch width for mud crabs caught in the LTMP from 2000 to 2009.

Total CPUE in the LTMP was 0.06-1.47 (Table 19, Figures 16 and 17).

Table 19: Catch per unit effort (CPUE) of mud crabs in the LTMP by year and location.

YEAR	ZONE NAME	MEAN	SD
2006	Calliope River	0.45	0.68
2007	Calliope River	0.51	0.92
2008	Calliope River	0.98	1.03
2001	Conner Creek	1.14	1.33
2002	Conner Creek	1.06	1.14
2003	Conner Creek	1.26	1.40
2004	Conner Creek	0.81	1.40
2005	Conner Creek	0.46	0.73
2006	Conner Creek	0.55	0.88

2008	Conner Creek	0.85	1.15
2009	Conner Creek	0.56	0.91
2000	Deception Creek	0.49	0.66
2001	Deception Creek	0.95	1.15
2002	Deception Creek	0.51	0.66
2004	Deception Creek	0.40	0.67
2005	Deception Creek	0.13	0.40
2006	Deception Creek	0.06	0.24
2008	Deception Creek	0.38	0.51
2009	Deception Creek	0.11	0.32
2006	Gladstone Harbour	0.90	1.00
2007	Gladstone Harbour	1.24	1.19
2008	Gladstone Harbour	1.46	1.10
2000	Graham Creek	0.81	0.90
2001	Graham Creek	1.23	1.19
2002	Graham Creek	1.29	1.31
2003	Graham Creek	1.47	1.46
2004	Graham Creek	1.11	1.05
2005	Graham Creek	0.75	0.93
2006	Graham Creek	0.71	1.10
2008	Graham Creek	1.16	1.29
2009	Graham Creek	0.56	0.82
2000	Little Oakie Creek	0.56	0.69

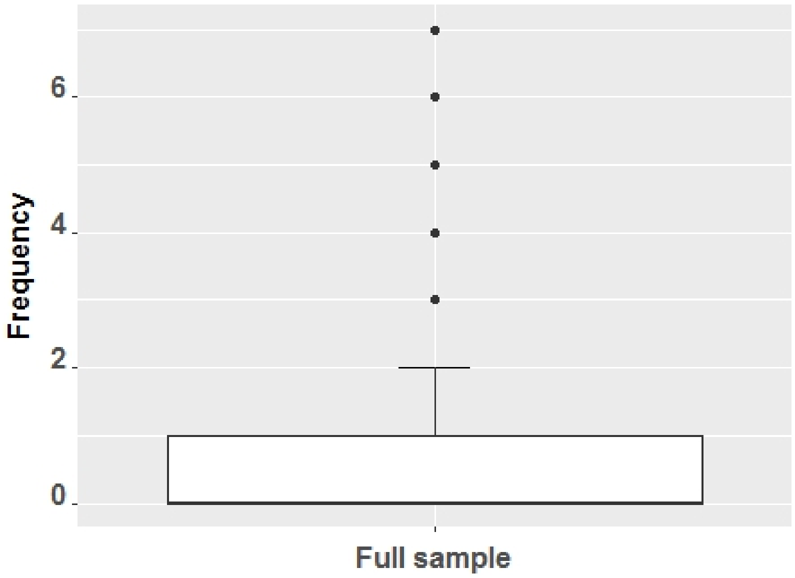


Figure 16: Catch per unit effort (CPUE) across all sampling dates and locations in the LTMP.

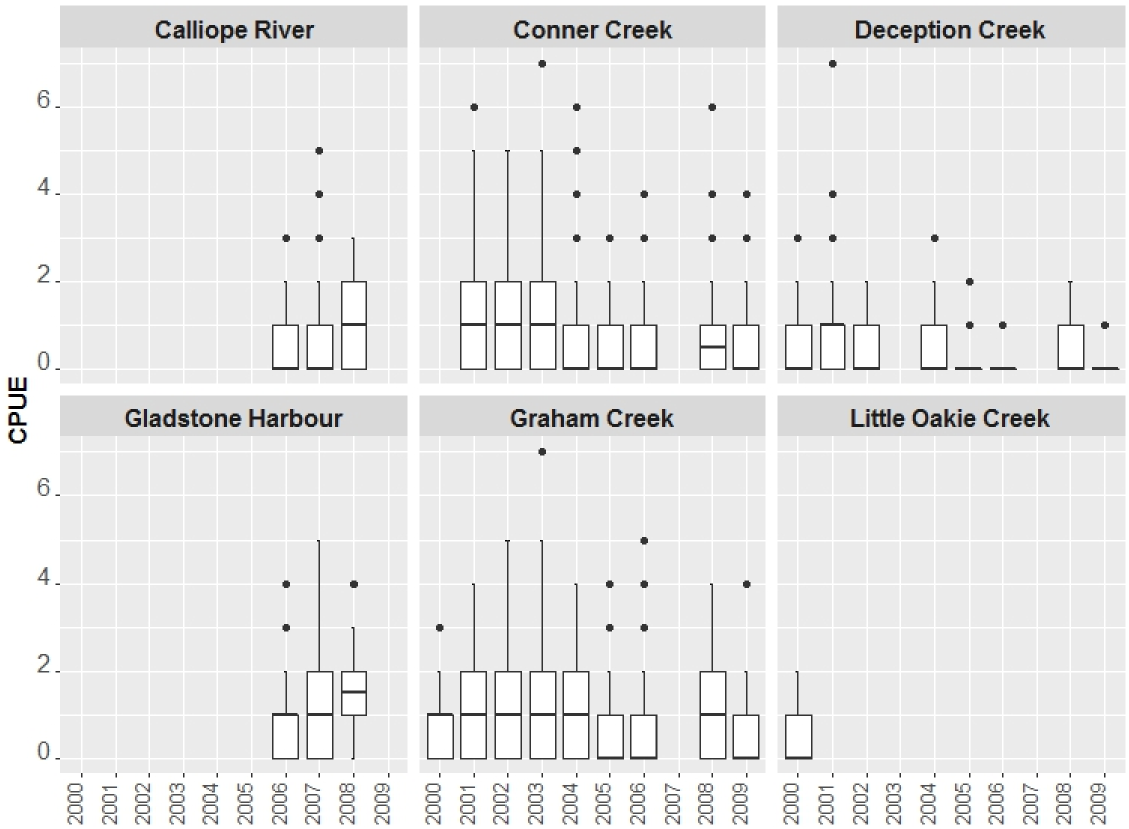


Figure 17: Catch per unit effort (CPUE), by year and location of sampling, in the LTMP.

Scores by zone

The following mud crab data sets were used to score each selected zone for the Gladstone Harbour Report Card:

Zone 1: Narrows – data from June 2017 sampling event

Zone 2: Graham Creek – data from June 2017 sampling event

Zone 4: Boat Creek – data from June 2017 sampling event

Zone 5: Inner Harbour – data from June 2017 sampling event

Zone 6: Calliope River – data from June 2017 sampling event

Zone 7: Auckland Inlet – data from June 2017 sampling event

Zone 13: Rodds Bay Site B – data from July 2017 sampling event**

** For Zone 13, Rodds Bay, July 2017 sampling data is used because the site sampled in June (Site A) was determined to be less suitable mud crab habitat. An alternative site (Site B) was sampled in July, and was more suited to ongoing monitoring. The Rodds Bay score should not be considered directly comparable to other Zones, because of the different sampling time. However it was determined in discussion with ISP members that the site most suitable for ongoing monitoring should be scored. Another alternative is trial replacing Rodds Bay with Colosseum Inlet in future years.

The set of seven sites/dates listed above is henceforth referred to as "the 2017 data". Results for other zone/sampling event combination were also calculated but will not be included in the Report Card.

Scoring, grading and aggregation method

The mud crab measures were calculated in 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

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

- **Biomass** as a condition measure of individual male and female mud crabs, calculated for each mud crab and then averaged for each zone (where width is measured in mm and weight in g)

  $$= \frac{\text{carapace (notch) width}}{\text{body weight}}$$

- **Size:sex ratio** based on legal size limits (150 mm spine width; equates to 143 mm notch width), for each zone

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

  When size:sex ratio was calculated, a limitation of the indicator was identified. Four of the scored zones recorded no small female mud crabs, meaning the denominator of the function used to calculate Size:Sex ratio can't be calculated (as it is not possible to divide by zero). Instead, a sex ratio was calculated:

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

  = (male mud crabs > 150 mm / female mud crabs > 150 mm)

Results for each Zone are provided in Table 20.

Table 20: Calculated index values for 2017, for each of the four recommended measures in each of the seven recommended long term monitoring sites.

Zone	Abundance (CPUE)	Prevalence of rust lesions	Sex ratio	Biomass	
				Male	Female
1 Narrows	4.62	0.025	0.24	0.23	0.21
2 Graham Creek	1.95	0.054	1.23	0.17	0.19
4 Boat Creek	3.60	0.024	0.56	0.28	0.22
5 Inner Harbour	3.50	0.075	2.20	0.20	0.19
6 Calliope Estuary	0.70	0.071	1.25	0.21	0.21
7 Auckland Inlet	0.65	0.154	0.11	0.17	0.20
13-B Rodds Bay	0.35	0.143	1.33	0.17	0.20

The recommended method for scoring the mud crab measures is to use the formulae provided in Table 21 to compare each index value against a 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. A similar approach is taken for environmental indicators in the South East

Queensland Report Card (Healthy Waterways, 2007) and the Fitzroy Basin Report Card (Flint et al., 2017). The Gladstone Harbour Report Card grading system is provided in Table 22.

For one of the four recommended measures, biomass (CW:BW), calculated separately for male and female mud crabs, the only available data on which to base scoring was collected by this project during 2017. For this reason, it is recommended that the measure is retained, but not scored, until at least three years of mud crab monitoring data are available to calculate baselines.

Abundance (as CPUE) can be calculated from the 2017 data as well as from the LTMP data collected between 2000 and 2009 at six locations in and near Gladstone Harbour. The 75th percentile, median and mean values for both the 2017 data and LTMP are possible benchmarks. As LTMP data were collected in summer using a different soak time and smaller crab pots, CPUE is not directly comparable to 2017 results. For this reason, it is recommended that 2017 data are used to select the benchmark for the abundance measure. As a benchmark should represent a 'minimally-disturbed' condition, the 75th percentile of 2017 scores is recommended. In contrast, the WCS should represent a population in poor condition. While the minimum and 25th percentile values of a range of samples are possible WCS values, the minimum and 25th percentile values for 2017 data and the LTMP data were all 0, which if used as a WCS would indicate a value of no mud crabs caught, regardless of the number of pots set. As an alternative to this 'no catch' value, a catch rate of 0.25 is suggested. The maximum number of pots that can be set by a recreational crabber in Queensland is four, and a catch of < 1 mud crab from this four-pot limit (assuming pots have been deployed in a way that is conducive to catching mud crabs, as is the case in the monitoring program) would be undesirable. This suggested WCS value of 0.25 is, in effect, a social WCS based on assumed fisher preferences.

Prevalence of rust lesions can be calculated from the 2017 data and is recorded in previous literature (Andersen et al., 2000; Andersen, 2003; Dennis et al., 2016). A background level of 5% prevalence has been previously reported for other portunid crab populations and is a possible benchmark value, but the 25th percentile of 2017 data was slightly less than this, at 4% of crabs sampled. A precautionary approach has been taken in recommending a benchmark of the lower value of 4% (0.04). Possible WCS values include the maximum prevalence of lesions in 2017 (15.4%), and values extracted from the literature: 14.3% mean prevalence in Gladstone Harbour reported by Andersen (2003); 21.7% prevalence in Gladstone Harbour reported by Andersen et al. (2000); 37% mean prevalence in Gladstone Harbour reported by Dennis et al. (2016). As the study by Dennis et al. (2016) was conducted during a period of unusually high prevalence of fish and mud crab disease following the 2012 flood event, the prevalence of rust lesions reported by that study can be considered representative of a population in poor condition. The recommended WCS is based on the prevalence recorded by Dennis et al. (2016), rounded down to 35% (0.35).

Sex ratio of legal-sized crabs (> 150 mm carapace width) can be calculated from the 2017 data and from LTMP data. The 75th percentile of either of these samples is a possible benchmark. However, all of the data collected in 2017 and in the LTMP are from fished populations of mud crabs. Because of the strong influence of the sex-based fishery on sex ratios of mud crab populations, a 'minimally disturbed' benchmark would require sampling from a non-fished population of mud crabs. A benchmark such as this is available from the international literature based on a study in Micronesia, where male mud crabs are three times as prevalent as female mud crabs (Hubatsch et al., 2016), and a study from South East Queensland which reported mature male mud crabs were twice as prevalent as mature females (Pillans *et al.*, 2005). Until data are available for an unfished region in central Queensland (e.g. Eurimbula Creek, which is located approximately 20 km south of Rodds Bay) the more conservative ratio of 3:1 (*sec.* Hubatsch et al., 2016) is recommended as the benchmark for sex ratio. A range of possible values for the WCS can be calculated from 2017 and LTMP data (from fished/disturbed populations), including 25th percentile, median and mean. As the LTMP data is a longer time series with more data points, the 25th percentile of that dataset is suggested as the WCS (0.25).

Table 21: Benchmarks and scoring method for each of the four recommended measures. NC = not calculable. LTMP = Fisheries Queensland Long Term Monitoring Program for the Mud Crab Fishery.

Measure	**Benchmark**		**Worst case scenario**		**Method / comments**
	Options	**Selected**	**Options**	**Selected**	
Abundance (CPUE)	Max of 2017 data (9) 75th percentile of 2017 scores (3.5) 75th percentile of 2017 data (3) Median of 2017 data (1) Mean of 2017 data (2.8) Max of LTMP (7)* 75th percentile of LTMP (1)* Median of LTMP (0)* Mean of LTMP (0.8)*	3.5	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)	0.25	The function used to calculate scores for abundance is: 1-((x-B)/(WCS-B)) Where: x = recorded CPUE B = benchmark (3.5) WCS = worst case scenario (0.25)
Prevalence of rust lesions	Background level proposed by Sindermann, 1989 (5%) Dennis et al. 2016 mean prevalence in Stanage Bay (14%) Dennis et al. 2016 mean presence in Rodds Bay (7%) Max prevalence from June 2017 data (15%) Median prevalence from June 2017 data (7.1%) Mean prevalence from June 2017 data (7.8%) 25th percentile of 2017 data (4%)	4% = 0.04	Dennis et al. 2016 mean prevalence in Gladstone Harbour (37%) Max of 2017 data (15.4%) Mean prevalence from Andersen, 2003 (14.3%) Reported prevalence from Andersen et al. 2000 (21.7%)	35% = 0.35	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)
Biomass	NC	NC	NC	NC	No previous data are available for weight of mud crabs in Gladstone Harbour. This measure is recommended for inclusion in the Report Card, but should not be scored until at least three years of mud crab weight data are available to calculate benchmarks. Data from an unfished area would also provide a good comparison.

Sex ratio	Male:female sex ratio of 3:1 from an unfished mud crab population reported in Hubatsch et al. 2016 (3) Max of 2017 data (2.2) 75th percentile of 2017 data (1.29) Max of LTMP data (0.28) 75th percentile of LTMP data (0.27) Male:female ratio from an unfished Queensland population *(not currently available)*	2017: 3 2018+: ratio from an unfished Queensland population	Min of 2017 data (0.1) 25th percentile of 2017 data (0.4) Median of 2017 data (1.23) Mean of 2017 data (1) Min of LTMP data (0.07) 25th percentile of LTMP data (0.25) Median of LTMP data (0.26) Mean of LTMP data (0.25)	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 (3) WCS = worst case scenario (0.25)

Table 22: 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

Indicator grades

Scores and grades for the three calculable mud crab measures are provided in Table 23. 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.

Table 23: Scores and grades for mud crab measures and the mud crab indicator by Zone. NC = Not calculable.

Zone	Abundance (CPUE)	Prevalence of rust lesions	Sex ratio*	Biomass	Zone score 2017
1. The Narrows	1.00	1.00	0.00	NC	0.67
2. Graham Creek	0.52	0.95	0.36	NC	0.61
4. Boat Creek	1.00	1.00	0.11	NC	0.70
5. Inner Harbour	1.00	0.89	0.71	NC	0.87
6. Calliope Estuary	0.14	0.90	0.36	NC	0.47
7. Auckland Inlet	0.12	0.63	0.00	NC	0.25
13. Rodds Bay	0.03	0.67	0.39	NC	0.36
Harbour Average					0.56

* Sex ratio based on legal size limits.

Discussion of results

The overall grades for the Mud Crab Indicator for Gladstone Harbour in 2017 are as follows:

A: Zone 5 – Inner Harbour;

B: Zone 1 – Narrows and Zone 4 – Boat Creek;

C: Zone 2 – Graham Creek;

D: Zone 6 – Calliope Estuary, Zone 7 – Auckland Inlet and Zone 13 – Rodds Bay; and

E: No zones.

The grades reflect the variety of pressures on mud crabs in Gladstone Harbour, including commercial fishing, recreational fishing and environmental/habitat condition. Low overall scores (D) for Calliope Estuary, Auckland Inlet and Rodds Bay were driven by lower grades for abundance of mud crabs (E) in comparison to other zones.

The prevalence of rust lesions measure was scored, with moderately-high confidence in the benchmark and WCS based on research data published by Andersen and Norton (2001) and Dennis et al. (2016), and new data collected in June 2017. The 2017 grades were highest (A) in Zone 1 – Narrows, Zone 2 – Graham Creek, Zone 4 – Boat Creek, Zone 5 – Inner Harbour and Zone 6 – Calliope Estuary. The lowest grade (C) was recorded in Zone 7 – Auckland Inlet.

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 thought to be related to inhibition of calcium uptake following sublethal copper and zinc exposure although this theory has not been proven (Andersen *et al.*, 2000; Andersen and Norton, 2001). An increased incidence of disease in finfish and crabs in Gladstone Harbour in 2011-12 was analysed by Dennis *et al.* (2016), and rust spot lesions were recorded on 37% of *S. serrata* caught in Gladstone Harbour. Prevalence of rust spot lesions measured during the Dennis *et al.* (2016) study was greater than previously recorded prevalence in the same region, and higher than the prevalence recorded during 2017 sampling.

The abundance measure was scored, with only moderate confidence in benchmarks and worst case scenarios, based on June 2017 data. Historical data provided the Fisheries Queensland LTMP for the Mud Crab Fishery was collected in summer, including sites from north of Gladstone Harbour, using different soak times and smaller crab pots, all of these factors may have affected CPUE more than the other measures, so cautious interpretation was required. The 75th percentile of June 2017 data is proposed as the benchmark, and a catch rate of less than one crab in four pots as a WCS. The 2017 grades were highest (A) at Zone 1 – Narrows, Zone 4 – Boat Creek and Zone 5 – Inner Harbour. The lowest grade (E) was recorded at Zone 6 – Calliope Estuary, Zone 7 – Auckland Inlet and Zone 13 – Rodds Bay.

Discrepancies in abundance can be caused by capture technique, sampling areas and sampling times, or by differences in crab distribution, growth or survival induced by habitat and environmental conditions (Alberts-Hubatsch *et al.*, 2016). During 2017 sampling, possible variations due to seasonality, catch and sampling techniques were controlled as much as possible. When these factors are controlled, abundance is a simple indicator of pressures such as extraction (fishing) and recruitment limitation. In a study of the abundance and distribution of *Scylla* spp. in the Philippines, variation in CPUE was detected between years, and was associated with variations in recruitment and increased fishing pressures (Walton *et al.*, 2006). A decrease in CPUE of *S. olivacea* was observed, while abundance of other crab species in the study area increased, suggesting that the area was still a suitable mud crab habitat, but that fishing pressures and recruitment limitations had impacted on the abundance of *S. olivacea* (Walton *et al.*, 2007). Meynecke *et al.* (2012) analysed catch and effort data for *S. serrata* from the Northern Territory (NT; 1990 to 2008) in comparison to climatic data and the Southern Oscillation Index. Between 30 and 40% of variation in CPUE was explained by periods of high rainfall and the Southern Oscillation Index, with greater freshwater inputs into the estuarine habitats of *S. serrata*, along with warmer water temperatures (Meynecke *et al.*, 2012). Temperatures were also found to affect CPUE rates, with *S. serrata* less active in high (>40 °C) and low (<20 °C) temperatures (Meynecke *et al.*, 2012). Similarly, Chandrasekaran and Natarajan (1994) found that high rainfall and associated lower water salinity (as low as 1.52 ppt) in an Indian estuary resulted in lower densities of juvenile

S. serrata. Mud crabs tend to be dominant in mangrove habitats which typically have salinities around 34 ppt but are vulnerable to reduced salinity following flood events (Meynecke *et al.*, 2012). Global warming is predicted to increase the occurrence of extreme La Nina events (Cai *et al.*, 2015), and the associated higher rainfall and lower water salinity could potentially be detrimental to *S. serrata* populations.

Due to zero catch of female crabs <150 mm spine width in some zones, it was not possible to calculate size:sex ratio in 2017. As an alternative, a sex ratio measure comparing the number of male crabs over the legal size limit to female crabs over the legal size limit was used. It is recommended that the option of a size:sex ratio is reconsidered for future years as it is possible that 2017 was an unusual catch year for small female crabs. A benchmark for size:sex ratio was established using scientific literature about unfished mud crab populations elsewhere. This benchmark is considered to be of low reliability and it is recommended that an unfished population from Queensland should sampled in 2018 to provide a reference guideline. The proposed WCS for the size:sex ratio measure is the 25th percentile of LTMP data. For 2017, all zones except Zone 5 – Inner Harbour (B) scored poorly on the sex ratio measure. The lowest grades (E) were recorded at Zone 1 – Narrows, Zone 4 – Boat Creek and Zone 7 – Auckland Inlet.

In areas such as Queensland, where a sex-based fishery is enforced, changes in the ratio of males to females are likely to be indicative of a change in fishing pressure. It is also worth noting 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. For example, male, rather than female mud crabs dig burrows (Bonine *et al.*, 2008) which may aid the process of bioturbation in mangrove systems.

As the 2017 dataset contains the only available mud crab weight data from Gladstone Harbour, scores have not been calculated for biomass (CW:BW) this year. Once three years of data are available, a scoring system can be developed. The relationship between carapace width and body weight can be used as a general indicator of condition, providing a measure of how well-fed an individual is, with high ratios typically indicating that an ecosystem has high productivity and food density.

The highest overall mud crab grade was recorded for Zone 5 – Inner Harbour (A), while the lowest overall grades (D) were recorded for Zone 6 – Calliope Estuary, Zone 7 – Auckland Creek and Zone 13 – Rodds Bay. The Harbour Average for 2017 is a C. As 2017 is the first year this study has been conducted (Pilot Year) the accuracy and reliability of the mud crab grades may improve as more data are collected and all measures, including biomass, are able to be calculated.

Recommendations

The following additional recommendations are provided for consideration in future monitoring years:

1. **VALIDATION OF HISTORICAL METHODS:** To allow historical data to be standardised for direct comparison to new data, we recommend trials of the previous overnight soaks vs daytime soaks, and trials of new pots to the pots used in Fisheries Queensland's LTMP. The LTMP crab pots were similar to the pots used for this project, but were custom made to be smaller and with gutter guard crab entrances. Fisheries Queensland still has the LTMP pots and has offered their use for catch trials. This will be particularly useful for confirming the benchmark and WCS for abundance (CPUE).
2. **BIOMASS MEASURE:** As the 2017 data represents the only available mud crab weight data from Gladstone Harbour, it was not possible to determine a benchmark and WCS this year. We recommend the benchmark and WCS for this measure are calculated once three years of mud crab monitoring data are available (in 2019).
3. **SAMPLING UNFISHED POPULATIONS:** Gender ratios have been scored in 2017 against a benchmark which is based on a 3:1 male to female ratio in unfished mud crab populations internationally (Alberts-Hubatsch

et al., 2016). This value could be confirmed for local mud crab populations by sampling a nearby unfished population, for example at Eurimbula Creek near Round Hill. Data from an unfished population would also be useful for increased confidence in the abundance measure and to calculate the biomass benchmark.

4. **ALTERNATIVE USE OF BRUVS:** BRUVS were used in 2017 as a cross-check against crab pot data. As catch rates were generally high, in future years it may be more beneficial to instead install cameras on selected crab pots and collect data on mud crab behaviour, for example the order of entry of the crabs into the pots, bait consumption behaviour, bait defence behaviour and pot saturation rates.
5. **REPLACING MARK: RECAPTURE:** Two sampling events were conducted in 2017 – a 'mark' sampling event in June when crabs were marked with nail varnish, and a 'recapture' sampling event in July when captured crabs were checked for traces of 'marks'. Very few of the mud crabs that were caught in July displayed evidence of a 'mark' (9 of 165 mud crabs). It is unclear whether some of the marks rubbed off, in which case a more invasive tagging method would be required for future mark-recapture studies, or if the recapture rates were low. The latter seems likely, as 7 of the recaptures were in a smaller, more enclosed system (Boat Creek) and the closer proximity of mud crabs to baited pots may have contributed to the higher recapture rates. Instead of conducting a mark:recapture study, in future years it may be beneficial to conduct two discrete sampling events, one in late summer (Jan/Feb) and one in late autumn (Apr/May). This would provide a better index of variability of catch between seasons moving forward.
6. **OTHER MEASURES:** Bioaccumulation and nursery value both scored lower than the four selected measures based on higher costs associated with field sampling, but would potentially be useful to include if future budgets allowed. The bioaccumulation results would be of interest as an additional indication of water and sediment quality, while recruitment surveys to determine nursery value would tie in well with GHHP's fish recruitment indicator.

Appendix 1: Indicator scoring results

Table A1: Scoring of potential mud crab indicators for the Gladstone Harbour Report Card. Key: Total scores represent either a consensus (˜) or an averaged (*) score. + = individual indicators within this category score differently and have been averaged.

Potential Indicator	Description	Criterion	Scorer #1	Scorer #2	Scorer #3	Consensus or Average (*) Score	Total score /30
Biomarkers, e.g. • GST induction and ChE inhibition • RNA/DNA ratios • Glutathione peroxidase activity and lipid peroxidases • Antioxidant enzymes and oxidative stress parameters	• Three biomarkers (glutathione-S-transferase (GST) activity, cholinesterase (ChE) activity and benzo-a-pyrene (BaP) urinary metabolites) indicate possible exposure to PAHs and organophosphate contamination. • RNA/DNA ratios are known to be a useful estimator of physiological condition in fish and invertebrates, with the ratio expected to increase when environmental conditions are favourable • Glutathione peroxidase activity is suggested as a biomarker of metal exposure with lipid peroxidases as a secondary marker when bioaccumulated metal concentrations are high. Increases in these biomarkers suggest exposure to metal contaminated sites, with resultant oxidative stress in the individual. • Higher oxidative stress with lower antioxidant concentrations are observed during periods of poor water quality conditions. [Sometimes requires collection and dissection of crabs for analysis.]	SC1	1	3	3	2	14˜
		SC2	0	2	2	1	
		SC3	2	3	2	2	
		SC4	0	2	0	1	
		SC5	0	1	1	0	
		SC6	0	1	0	0	
		SC7	1	2	3	1.5+	
		SC8	1	2	1	1.5+	
		SC9	1	3	3	3	
		SC10	2	2	2	2	
	INDICATOR SUBTOTAL		8 /30	21/30	17/30	14	
Bioaccumulation of toxicants, e.g. • Bioaccumulation of metals in tissues • Structural deformities of organs (associated with metal bioaccumulation) • POPs bioaccumulation • Pesticides bioaccumulation	Several studies have been conducted using crabs as bioindicators of heavy metal and toxicant bioaccumulation. Structural deformities of the gills, muscles and hepatopancreas have been observed in *S. serrata* due to metal toxicity, with the degree of damage correlated with elevated bioaccumulated metal concentration. Tissue and metal-specific bioaccumulation was observed, with Cu, Pb, Cd and Mn highest in the hepatopancreas, lower in the gills and significantly lower in the muscle samples analysed, whereas Zn and Fe were highest in the gills, lower in the hepatopancreas and significantly lower in the muscles (Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014)(Arockia Vasanthi *et al.*, 2014). In addition to metals, *S. serrata* can accumulate a range of other contaminants including persistent organic pollutants (POPs) and pesticides. *S. serrata* were sampled in six estuaries in Qld, for analysis of metals, metalloids and pesticides in muscle and hepatopancreas tissue. Most metals and metalloids were found to be preferentially	SC1	3	3	3	3*	21.3*
		SC2	2	3	2	2.3*	
		SC3	3	3	2	2.7*	
		SC4	1	2	1	1.3*	
		SC5	1	0	1	0.7*	
		SC6	1	1	1	1*	

	sequestered to the hepatopancreas, and industrialised areas showed higher concentrations of Pb, Se and Sn than less impacted locations. DDT and dieldrin were detected in tissues along with other banned organochlorines. Dieldrin concentrations suggested that ambient exposures of dieldrin exceeded national water quality guidelines at all sampling locations, with one of the 77 samples of crab body muscle concentrations of dieldrin exceeding Australian Food Standards. Contemporary pesticides were also analysed in the hepatopancreas, endosulfan and chlorpyrifos were detected, while diuron, atrazine, simazine were not detected. [Require collection and dissection of crabs for analysis.]	SC7	3	3	3	3*	
		SC8	3	2	1	2*	
		SC9	2	2	3	2.3*	
		SC10	3	3	3	3*	
	INDICATOR SUBTOTAL		22/30	22/30	20/30	21.3*	
Size: Sex ratio	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. For example, the fishery for *S. serrata* in Queensland is limited to the collection of males, and it has been found that populations are female dominated. However, in unfished populations of mud crabs, the male: female ratio can be as high as 3:1. Seasonal changes in the sex ratio of crabs have been demonstrated in other portunid species. While not previously suggested in the literature, a size:sex ratio differentiating between both fished (male) and unfished (female) and fished (>150mm) and unfished (<150mm) *S. serrata* populations, would provide an indicator of fishing pressure. There is not conclusive evidence to determine times when spawning females are offshore in this region. [Catch and release]	SC1	2	3	2	2.5	26.5˜
		SC2	3	3	3	3	
		SC3	3	3	3	3	
		SC4	3	2	2	3	
		SC5	3	3	3	3	
		SC6	3	2	3	2.5	
		SC7	2	1	2	2	
		SC8	3	3	3	3	
		SC9	3	1	3	2	
		SC10	3	2	3	2.5	
	INDICATOR SUBTOTAL		28/30	23/30	27/30	26.5	
Nursery value (juvenile crabs; CPUE)	Mature female *S. serrata* range from 138 to 204 ,m and mature males range from 108 to 187 mm. Measurements of a crab's body size, such as CW thus provides a means of assessing if a crab has reached maturity. This could potentially be useful in estimating the population and ratio of juveniles to adults, providing a potential indicator of overfishing and also of important nursery habitats. The different life stages of *S. serrata* tend to reside separately; juveniles are reported to reside mostly in the upper inter-tidal zone, sub-adults mostly reside in the sub-tidal zone, though move into the intertidal zone to feed during flood tides, and adults mostly reside and feed within the sub-tidal zone. As adult mud crabs face the most fishing pressure due to legislated catch size limits in Queensland, monitoring and sampling that is more focused on the subtidal zone could better target and monitor the adult *S. serrata* populations, and provide a potential indicator of overfishing. Monitoring	SC1	1	2	3	2	18˜
		SC2	2	3	3	3	
		SC3	2	3	3	2	
		SC4	1	2	2	1	
		SC5	2	2	3	1	
		SC6	2	2	3	2	
		SC7	2	3	2	2	

	that targets juvenile crab populations on the other hand could provide an indicator of recruitment rates, and this is in turn sensitive to pollution and other environmental stressors such as climate. [Catch and release using different methods/locations]	SC8	3	3	3	3	
		SC9	3	1	2	2	
		SC10	0	2	2	0	
	INDICATOR SUBTOTAL		18/30	23/30	26/30	18	
Total abundance (number of crabs; CPUE)	Estimating total harbour abundance (population size) requires repeated mark:recapture experiments. Trends in relative abundance between areas or through time are more straightforward, and can be measured using consistent monitoring methodologies to reduce variability. Discrepancies in abundance can be due to catch method, sampling areas and sampling times, or due to differences induced by habitat and environmental conditions. CPUE and related abundance data are often highly variable, due to *S. serrata* behaviour, population biology and dynamics, fishing effort efficiency and magnitude, and environmental conditions. Climate has a strong impact on survival and abundance, such that changes in rainfall and temperature could result in increased or decreased abundance through time. [Catch and release]	SC1	2	3	2	2.3*	25*
		SC2	3	3	3	3*	
		SC3	3	3	2	2.7*	
		SC4	3	2	2	2.3*	
		SC5	3	2	3	2.7*	
		SC6	3	3	3	3*	
		SC7	3	2	2	2.3*	
		SC8	3	3	3	3*	
		SC9	3	3	2	2.7*	
		SC10	0	1	2	1*	
	INDICATOR SUBTOTAL		26/30	25/30	24/30	25*	
Prevalence of rust lesions	Rust spot lesions initially start as an orange discolouration on the crab's carapace, before progressing to perforation and exposure of the soft tissues beneath the carapace. The disease has the potential to damage the mud crab industry in Gladstone Harbour. A method to objectively measure and grade the severity of lesions on crab shell carapace has been developed and described. [Catch and release]	SC1	3	2	3	3	24˜
		SC2	3	3	3	3	
		SC3	3	3	3	3	
		SC4	3	2	3	2.5	
		SC5	3	3	3	3	
		SC6	3	3	3	2.5	
		SC7	1	1	1	1	
		SC8	3	1	3	3	
		SC9	2	2	3	2	
		SC10	2	1	1	1	
	INDICATOR SUBTOTAL		26/30	21/30	26/30	24	
Prevalence of other diseases and parasites	*S. serrata* are susceptible to (externally visible) parasites and biofouling organisms such as barnacles, as well as a variety of bacterial, fungal and viral diseases. High prevalence of parasite and disease can be related to environmental stress. [Catch and release]	SC1	1	2	2	1	17.5˜
		SC2	3	3	3	3	
		SC3	1	2	3	2	
		SC4	1	1	1	1	
		SC5	2	3	3	3	
		SC6	2	2	2	2	
		SC7	1	2	2	2	
		SC8	2	1	2	1.5	
		SC9	2	3	2	2	

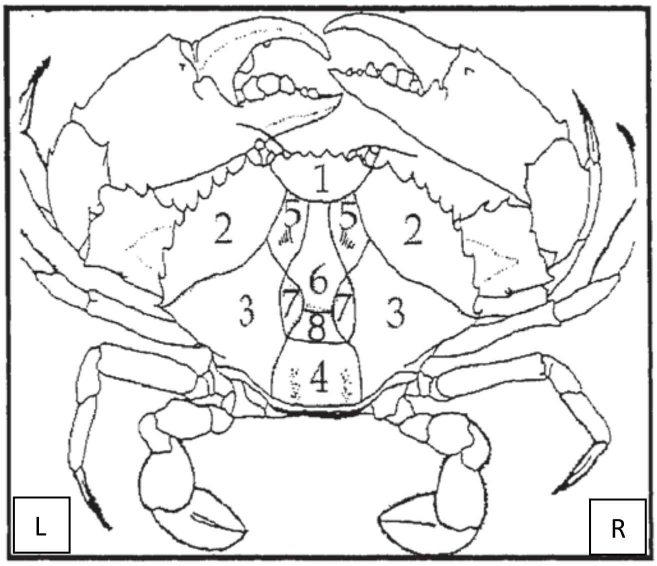
		SC10	0	1	2	0	
	INDICATOR SUBTOTAL		15/30	20/30	22/30	17.5	
Biomass / CW:BW	The relationship between carapace width and body weight (CW:BW) can be used as a condition indicator. The body weight to length ratio provides a measure of how well-fed an individual is, with high ratios typically indicating that an ecosystem has high productivity and food density. The wet weight and CW of *S. serrata* are highly correlated with each other (r^2=0.825-0.904) in both sexes, even in recently-moulted 'soft-shelled' crab populations. CW:BW measurements can also be used to calculate biomass and estimate the amount of obtainable edible meat. [Catch and release]	SC1	2	3	3	2.7*	25.3*
		SC2	2	3	2	2.3*	
		SC3	2	3	3	2.7*	
		SC4	2	2	3	2.3*	
		SC5	3	3	3	3*	
		SC6	3	3	3	3*	
		SC7	3	2	3	2.7*	
		SC8	3	3	3	3*	
		SC9	3	2	2	2.3*	
		SC10	1	1	2	1.3*	
	INDICATOR SUBTOTAL		24/30	25/30	27/30	25.3*	
Morphometrics	Morphometric indicators such as claw size ratio, dominant and subordinate claws loss, and claw regeneration could provide potentially useful indicators of environmental stressors (previously been used for estuarine crabs in the USA). Morphometric indicators were significantly influenced by site (e.g. reference vs. urbanised locations) and date of sampling. However, there were no clear patterns on the specific influences of such environmental factors to those indicators. [Catch and release]	SC1	2	3	2	2	18˜
		SC2	2	3	2	2	
		SC3	2	3	3	3	
		SC4	2	2	1	1	
		SC5	3	3	2	3	
		SC6	3	3	2	2	
		SC7	1	1	1	1	
		SC8	2	3	2	2	
		SC9	3	2	2	1	
		SC10	0	1	1	1	
	INDICATOR SUBTOTAL		20/30	24/30	18/30	18	

Appendix 2: Mud crab rust spot lesion grading system (Source: Andersen, 2003)

Gross grading system for rust spot lesions in mud crabs.

Grade	Description
Grade 1	Non Perforated; < 5mm diameter
Grade 2	Non Perforated; ≥ 5mm diameter
Grade 3	Perforated cuticle (either partially or fully); < 5mm diameter
Grade 4	Perforated cuticle (either partially or fully); ≥ 5 mm diameter & < 20mm diameter
Grade 5	Perforated cuticle (either partially or fully); ≥ 20mm diameter

Numbered areas (locations) of the mud crab carapace to which lesions are allocated.



Legend

1. FrontaVorbital
2. Hepatic/epibranchial
3. Mesobranchial/metabranchial
4. Intestinal
5. Progastric
6. Mesogastriclmetagastric/ uro- gastric
7. Internal cuticular partition
8. Cardiac

(Adapted from McLaughlin, 1980).

Also note Left (L) or Right (R) side – when looking down on carapace.

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