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Fish Health Indicator Scores and Grades for the 2020 Gladstone Harbour Report Card

Final Project Report
Project ISP023-2020

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Prepared for the Gladstone Healthy Harbour Partnership

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

Version history

Version Number	Purpose/Changes	Authors	Date
1.1	Initial draft of interim report – to GHHP	Flint, Irving, Anastasi, De Valck, Jackson	14/08/2020
1.2	Revised following ISP comments	Flint, Irving, Anastasi, De Valck, Jackson	29/09/2020

Contents

Executive Summary

Fish can be used as indicators of environmental contamination as they are frequently the dominant taxa in aquatic ecosystems both in terms of biomass and trophic level, play a variety of important ecological roles, are continuously exposed throughout their life cycle and have high social and economic value.

Fish exposed to contamination can be affected from the population level (abundance and diversity of fish species) down to biochemical impacts on single cells within individual fish. In 2018, Gladstone Healthy Harbour Partnership (GHHP) and the Fisheries Research and Development Corporation (FRDC) commissioned CQUniversity (CQUni) to investigate potential fish health indicators for the Gladstone Harbour Report Card that would be suitable for adaptation across ports and estuaries of Northern Australia. The research identified and tested a range of potential indicators that were of low-to-medium cost and complexity. The indicator that was found to be most suitable for long term application in the Report Card is a version of the Health Assessment Index (HAI), a composite metric that integrates observer evaluations of multiple organs and tissues. The premise of the HAI is that scores will cumulatively reflect the acute and chronic stressors present in the fish's environment, with poorer anatomical condition resulting in higher HAI scores, indicative of a more stressful environment.

In 2019, GHHP commissioned CQUni to continue to monitor fish health in Gladstone Harbour using methods developed in 2018 and provide scores and grades for the Gladstone Harbour Report Card. A single score for fish health in the Gladstone Harbour was required, as fish mobility and data requirements made it impractical to calculate scores by harbour zones. Sampling was conducted across Gladstone Harbour and at two reference sites twice during the 2019 reporting year. For the 2020 Report Card, GHHP reduced the sampling to a single event, which was conducted in October 2019. Sites sampled included a range of estuarine environments in the northern, middle and southern parts of the harbour and a single reference site (Baffle Creek).

The aim of the October 2019 sampling event was to collect and assess the condition of the following target species: barramundi (*Lates calcarifer*), large mullet (sea mullet *Mugil cephalus* and diamondscale mullet *Liza vaigiensis*), barred javelin (*Pomadasys kaakan*) and blue catfish (*Neoarius graffei*). Bream (pikey bream *Acanthopagrus pacificus* and yellowfin bream *Acanthopagrus australis*) are also of interest to GHHP and would have been retained for laboratory analysis if they had been caught.

During the single sampling event, 126 fish from 17 species were caught across Gladstone Harbour and at the reference site. Barred javelin and blue catfish were caught in the highest numbers, and barred javelin were caught at almost all sampled zones. All fish were measured, weighed, checked for abnormalities and released, except for target species which were humanely killed for further analysis. In total, 80 of the target taxa were retained for health assessment (78 fish from Gladstone Harbour and 2 sea mullet from the reference site).

HAI was calculated for each of the fish from Gladstone Harbour that were assessed in October 2019, by scoring and summing gross pathology scores for the following measures: skin, eyes, fins, gills, spleen, kidney, hindgut, liver, and parasite load. HAI is designed to be used as a summed average for a sample population. Using this method, the Gladstone Harbour-wide HAI results (for nine measures) have been determined, by species. A HAI score of zero indicates that no aberrations were present, while positive scores represent increasing amount and severity of aberrations.

Taxa / Measure	Barramundi (n = 11)	Bream (n = 0) No data (ND), not graded	Barred Javelin (n = 31)	Blue Catfish (n = 32)	Mullet (n = 4) HAI was calculated but not graded
Skin	1.82	ND	0	1.25	0
Eyes	0	ND	0	0	0
Fins	2.73	ND	0.32	2.50	0
Gills	2.73	ND	0	0	0
Spleen	0	ND	0	0	0
Kidney	2.73	ND	0.97	5.63	0
Hindgut	0	ND	0	0	0
Liver	13.64	ND	14.52	17.81	7.50
Parasites	13.64	ND	3.55	6.25	0
HAI score	**37.27**	**ND**	**19.35**	**33.44**	**7.50**

Using the previously determined benchmark score of an average HAI of 10, and a pilot worst case scenario (WCS) score of an average HAI of 70, HAI scores and grades were calculated using a distance from the benchmark method. Using GHHP's grading scale, grades for each species group were calculated, and an overall harbour score and grade determined by averaging the scores of the five species groups for 2020.

	Barramundi	Bream	Barred Javelin	Blue Catfish	Mullet
Standardised HAI score/grade	Grade C Score 0.55	ND	Grade B Score 0.84	Grade C Score 0.61	ND
Overall Harbour score	Grade B Score 0.67				

Apart from bream and mullet, of which none or too few were caught during the single sampling event to accurately score and grade, the scores and grades of fish for 2020 are very similar to those from the 2019 Report Card.

The primary considerations when determining confidence in standardised HAI scores are sample size and potential for movement from other areas. Sample sizes of all taxa were lower than in 2019, as only one sampling event was conducted. Samples of barramundi (n = 11) and mullet (n = 4) were relatively low, while higher numbers of blue catfish (n = 32) and barred javelin (n = 31) were captured and retained. Barramundi are also a particularly mobile fish species with tagging evidence of movements across many hundreds of kilometres. This means that a barramundi caught in Gladstone Harbour may have moved from elsewhere and the resulting HAI score may be affected by environmental conditions across a larger spatial scale than the location of capture.

For these reasons, the confidence in scores for barramundi are lower than confidence in the other graded species. As a result of the three years of sampling fish health, six recommendations have been provided for GHHP's consideration.

Recommendation 1: GHHP continues to monitor HAI of fish in Gladstone Harbour.

Recommendation 2: GHHP considers only sampling at reference sites if worse than usual HAI scores are identified in fish caught in Gladstone Harbour during a particular sampling event.

Recommendation 3: GHHP continues to monitor measurements required to calculate other fish condition measures such as Fulton's K, HSI and GSI, to collate a long-term baseline dataset.

Recommendation 4: GHHP considers testing for bioaccumulation of metals and other toxicants in collected fish tissue samples.

Recommendation 5: GHHP considers splitting sampling effort over both spring and autumn to improve the changes of capturing each of the target taxa.

Recommendation 6: GHHP continues to conduct regionally stratified fish sampling to score and grade the whole of Gladstone Harbour.

Introduction

Fish are established biological indicators of aquatic pollution. They are continuously exposed to water-borne contaminants providing a direct measure of ecological consequences, are dominant taxa in terms of biomass, play various important ecological including trophic roles, and are relatively long-lived so the impacts of pollution accumulate over longer periods(Van der Oost, Beyer, & Vermeulen, 2003). Most species can be quickly identified in the field (Pidgeon, 2004), and their high socio-economic importance generates public interest in environmental management. Recognising the importance of fish as components of a healthy marine environment, the Gladstone Healthy Harbour Partnership (GHHP) and the Fisheries Research and Development Corporation (FRDC) commissioned an investigation in 2018 into potential fish health indicators for the Gladstone Harbour Report Card and suitable for adaptation across ports and estuaries of Northern Australia. The research identified and tested a range of potential indicators that were of low-to-medium cost and complexity (Flint et al., 2018).

The indicator that was found to be most suitable for immediate implementation in the Report Card was a modified version of the Health Assessment Index (HAI), first developed by Adams, Brown, and Goede (1993), and subsequently widely used and adapted, including by the Queensland Government during fish health investigations in Gladstone Harbour (Wesche et al., 2013). The HAI is a composite metric that integrates observer evaluations of parasite load as well as the condition of multiple organs and tissues, including skin, eyes, fins, gills, spleen, kidney, hindgut, and liver. The premise of HAI is that scores will cumulatively reflect the acute and chronic stressors present in the fish's environment, with poorer anatomical condition resulting in higher HAI scores, indicative of a more stressful environment. In 2019, GHHP continued the research project to pilot the fish health indicator in the 2019 Gladstone Harbour Report Card.

In 2020, GHHP again commissioned monitoring and assessment of fish health in Gladstone harbour, with reduced sampling effort to lower monitoring costs (one sampling event, to be conducted in October 2019). The objectives of the 2020 research project were to:

- Conduct a fish health survey in October 2019,
- Refine the data collection methods and statistical analytical methods developed in 2018 and 2019 as required, and
- Calculate fish health report card scores and grades for the 2020 Report Card, using scoring methods developed in the 2019 fish health project and data collected in October 2019.

Methods

Permits and approvals

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

- General Fisheries Permit (Queensland Department of Agriculture and Fisheries; Permit Number 196040)
- Animal Ethics Approval (CQUniversity Animal Ethics Committee; Approval Number 20969)
- Authorisation for research in the Great Barrier Reef Marine Park (Approval Number G18/03-029)
- Field Work Risk Assessment (CQUniversity OHS Unit)

Sampling design

Sampling was conducted in the southern, central and northern regions of Gladstone Harbour (Figure 1a) and at a reference site at Baffle Creek (Figure 1b) in October 2019. The sampling strategy in Gladstone Harbour was developed during 2018 and 2019 to achieve an approximately even spread of fish catch and effort between the northern, central and southern areas of the harbour, focusing on inshore and estuarine environments. The primary aim of sampling was to collect the target fish taxa identified by GHHP and during the 2018 research project (Flint et al., 2018) as priorities for further analysis: barramundi (*Lates calcarifer*), large mullet (sea mullet *Mugil cephalus* and diamondscale mullet *Liza vaigiensis*), barred javelin (*Pomadasys kaakan*) and blue catfish (*Neoarius graffei*). Bream (pikey bream *Acanthopagrus pacificus* and yellowfin bream *Acanthopagrus australis*) are also of interest to GHHP, due to their recreational fishing value, and would have been retained for analysis if they had been caught.

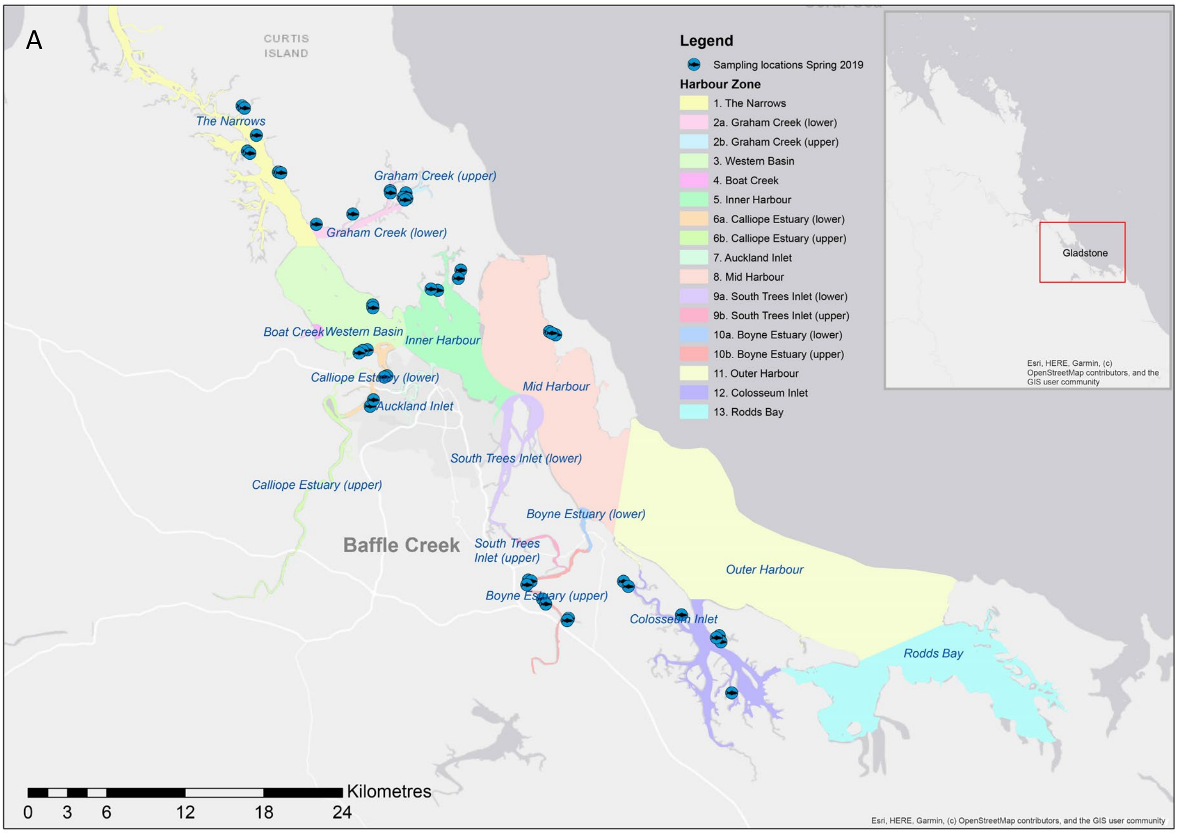
Understanding the mobility of fish in Gladstone Harbour is an important consideration that was taken into account when confirming target species (Flint et al., 2019).

Field sampling methods

Field collections of fish were undertaken using 3 x 50 m long gill nets with stretched mesh sizes 4.5”, 6” and 8”. A fourth gill / ring net of 110 m length, 2.13” stretched mesh size was used at some sites to supplement catch. Gears were deployed in areas and at times when the chances of catching these target species were maximised, and bycatch minimised.

Field sampling was undertaken during Spring 2019 (October) using the sampling procedures described in Flint et al. (2018). In summary, at each sampling location nets were deployed, details of deployment (including time and location) were recorded as well as physicochemical measurements (Appendix 1). Nets were soaked for approximately 30 minutes during each deployment, and several deployments of nets occurred at each site throughout each approximately 10-hour long sampling day.

Captured fish were assigned a unique identifier code and either processed immediately or placed into an aerated swim tank to be kept alive until on-board processing. Teleost fish were photographed, measured and weighed, and the skin, fins and eyes were examined for abnormalities, parasites, lesions or erosion. Cartilaginous fishes (sharks and rays) were recorded and photographed but were not handled except to ensure their safe removal from the net and live release. Non-target fish were released alive, while target species were retained at each site and euthanased for laboratory analysis. Immediately following euthanasia, gill arch samples were collected and fixed in 10% formalin. All retained fish were individually bagged with their unique identifier tag and placed in an ice slurry for return to the laboratory as soon as possible on the same day.



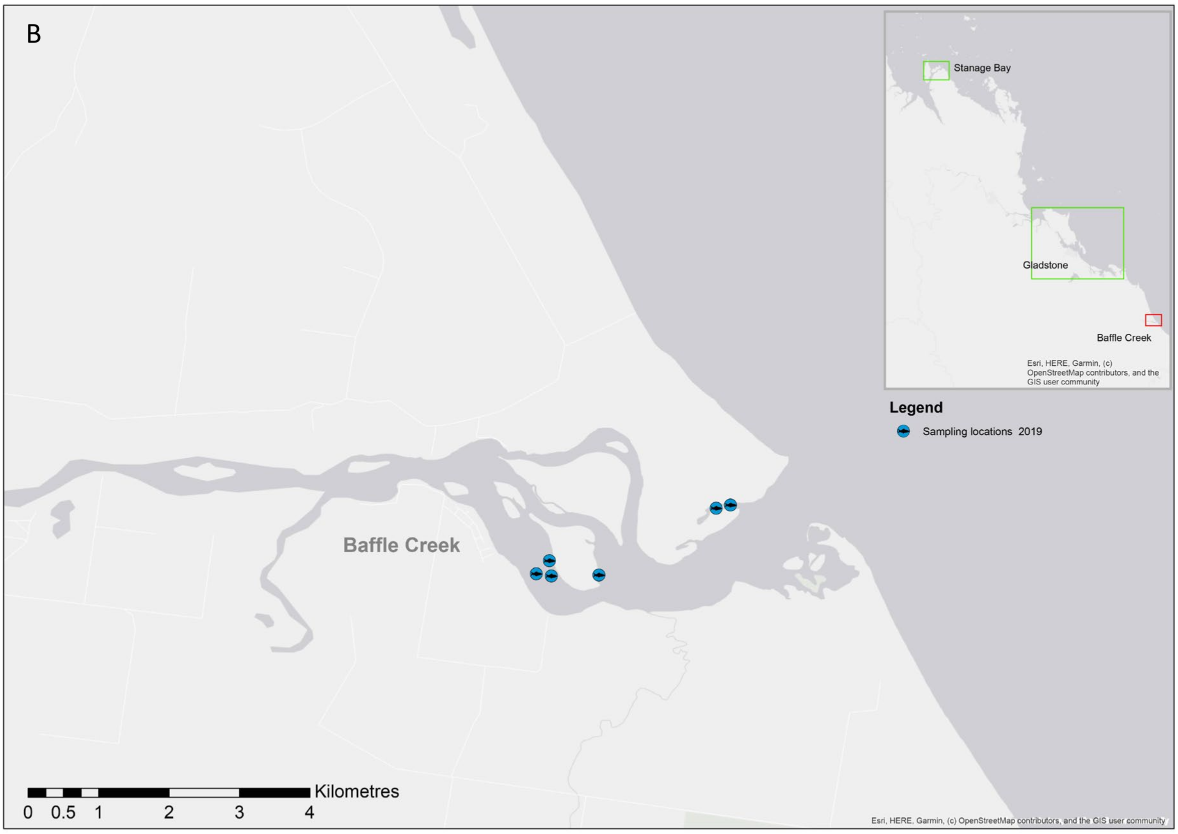


Figure 1: October 2019 sampling sites in A – Gladstone Harbour and B – Baffle Creek.

Laboratory methods

Retained fish from all sites were returned to the lab at CQUniversity's Gladstone Marina Campus for same day mid-level pathological examination as described by Cowled (2016). Pathological examination also included the dissection of organs and fixation in 10% formalin for later histopathological analysis, if required.

Each fish was dissected by a team of two researchers, using the step by step protocol detailed in Flint et al. (2019). Fins, spleen, hindgut, kidney, skin, liver, eyes, gills and parasites were scored following the protocol originally developed by Adams et al. (1993) and modified by Wesche et al. (2013) (Table 1).

Calculating fish condition measures

Scores for the organs of each fish were recorded based on the gross pathological data collected during fish dissections. Total HAI score for each individual fish was calculated (sum of all organ scores) and then the average of the total HAI scores was calculated for each fish taxon. These total scores at taxa level were averaged to give the overall harbour score. Barramundi, blue catfish and barred javelin are reported as individual species. The species groups bream and mullet both include two species, which are pooled due to their similar ecological characteristics and to allow for higher sample sizes.

Other fish condition measures including Fulton's condition factor (K), Hepatosomatic index (HSI), and Gonadosomatic index (GSI), were opportunistically calculated for each fish. Calculations used were as follows:

Fulton's condition factor (K):

$$K = 100*(W/L^3)$$

where: W = wet body weight (g); L = total length (cm)

Hepatosomatic index (HSI):

$$HSI = 100*(H/W)$$

where: H = wet liver weight (g); W = wet body weight (g)

Gonadosomatic index (GSI):

$$GSI = 100*(G/W)$$

Where: G = wet gonad weight (g); W = wet body weight (g)

Table 1: Variables and substituted values used in the Health Assessment Index for this project (source: Wesche et al., 2013).

Variable	Variable condition	Field Designation	Substituted Value
Fins	No active erosion	F0	0
	Light active erosion	F1	10
	Severe active erosion	F2	20
Spleen	Normal: black, very dark red or red	B	0
	Normal: granular, rough appearance	G	0
	Nodular, containing fistulas or nodules	D	30
	Enlarged	E	30
	Other: aberration not fitting any above	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal: firm, dark, flat	N	0
	Swollen: enlarged or swollen	S	30
	Mottled: gray discolouration	M	30
	Granular in appearance and texture	G	30
	Urolithiasis or nephrocalcinosis	U	30
	Other: aberration not fitting any above	OT	30
Skin	Normal: no aberration	SK0	0
	Mild skin aberrations	SK1	10
	Moderate skin aberrations	SK2	20
	Severe skin aberrations	SK3	30
	Extensive redness as a rash. Scales intact	SK4	40
Liver	Normal: solid red or light red color	A,B	0
	'Fatty' liver, 'coffee with cream' colour	C	30
	Nodules or cysts in liver	D	30
	Focal discolouration	E	30
	General discolouration	F	30
	Other: deviation not fitting any above	OT	30
Eyes	No aberration, good, clear eyes	E0	0
	Fresh haemorrhage (eg net damage)	EOa	0
	Opaque eye (one or both)	E1	30
	Cloudy and swollen, red or haemorrhaging	E2	30
	Ruptured (one or both)	E3	30
Gills	Normal: no apparent aberrations	N	0
	Frayed, ragged appearance	F	30
	Clubbed, swelling of tips	C	30
	Marginate: light discoloured margin	M	30
	Pale, very light colour	P	30
	Other	OT	30
Parasites	No observed parasites	P0	0
	Few observed parasites	P1	10
	Moderate parasite infestation	P2	20
	Numerous parasites	P3	30

Statistical analytical methods

Results for each fish health measure were graphed to visually compare differences between sampling zones and species. To determine whether there was a statistically significant difference in fish health between zones, a formal analysis of each measure of fish health was done using PERMANOVA (Permutational Analysis of Variance, conducted using the PRIMER 7 + PERMANOVA software package, version 7.0.13; Anderson et al., 2008; Anderson, 2017), which is a non-parametric approach that closely approximates standard parametric analysis of variance when considering univariate data (as used herein), but is a statistical method that accommodates uneven replication common to fish capture data, and is robust to departures from non-normality of data. Zones were included for statistical analysis if three or more replicate fish were sampled. Note that even though this excluded several zones from formal statistical analysis, all the available data have been plotted in the graphs below for a full comparison.

Results

Fish catches

During a single 7-day sampling event conducted in October 2019, a total of 126 fish from 17 species were caught over six days across Gladstone Harbour zones and one day at the reference site (Table 2). Barred javelin and blue catfish were caught in the highest numbers (Figure 2), and barred javelin were also caught at the most zones (Table 2). In total, 80 fish from four of the five target species groups were retained for health assessment, from all sampling zones, including two sea mullet from the reference site Baffle Creek. No bream were captured in October 2019.

Table 1: Fish species (listed by common name) and abundance at Gladstone Harbour (divided by GHHP zones) and the reference sites. Note that Zones 7, 9 and 13 were not sampled. White = 0 or not sampled; blue = 1-5; orange = 6-10; green = 10+ specimens. Common names of target species retained for further analysis are shaded grey. BC = Baffle Creek

Fish species	**GHHP Zone**											**Reference site**
	1	**2**	**3**	**5**	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**BC**
Barramundi	7		1	1					2			
Barred Javelin	6	12	6	3			4					
Beach Salmon	1											
Blubber lip bream	3				1				1			
Blue catfish		1		26					4	1		
Blue threadfin	11	5		1								
Bull shark				1								
Diamondscale mullet			1	1					2			
Flathead	1											2
Golden trevally									1			
Lemon shark												2
Queenfish									3	1		1
Rabbit fish									1			
Sea mullet												2
Shovel nose shark												2
Sickle fish		1					1		4			
Sole				1						1		

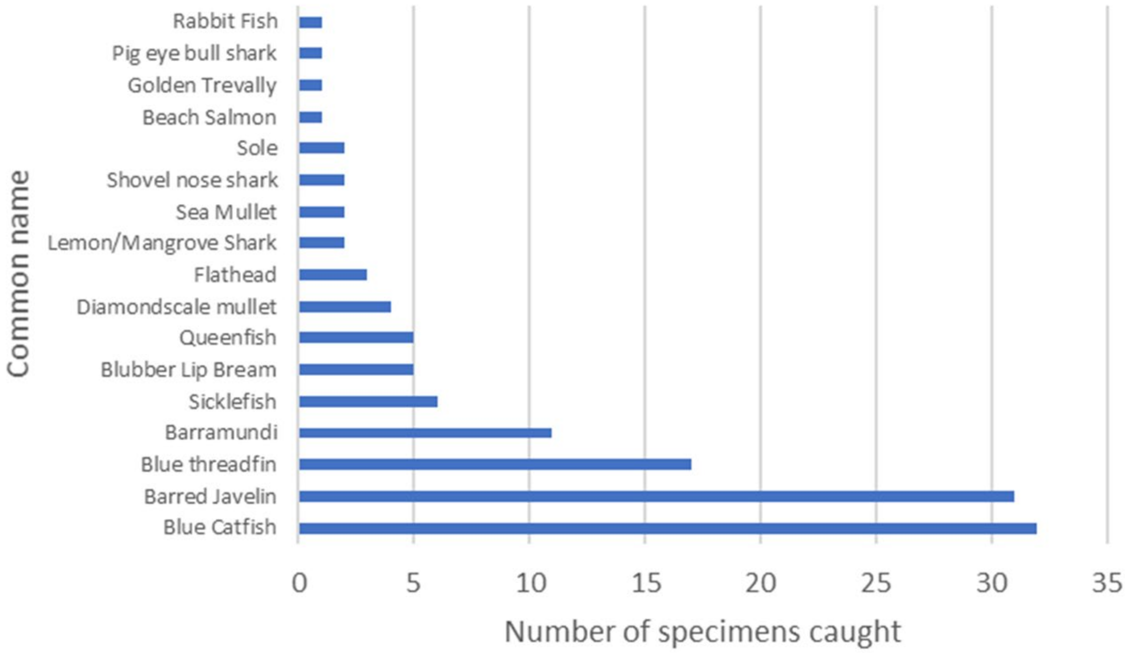


Figure 2: Total number of each fish species caught in October 2019.

Fish condition measures

The Health Assessment Index (HAI) scores of each of the four taxa captured ranged from 37 (barramundi) to 19 (barred javelin) (Figure 3). The HAI score is scored as a subtractive measure, such that a score of 0 is ideal (all assessed organs appear normal) and higher scores equate to more abnormalities (up to a maximum theoretical score of 270) (as detailed in Flint et al., 2019).

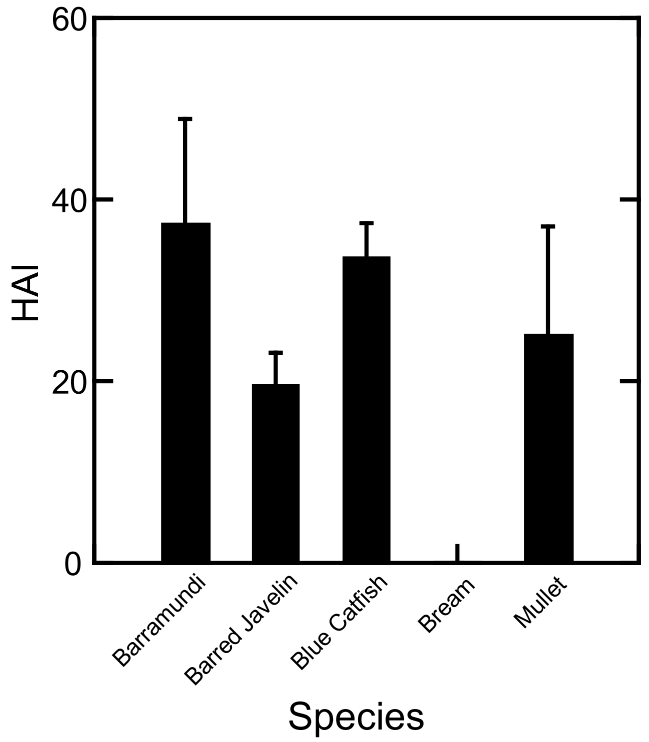


Figure 3: Average Health Assessment Index (HAI) ± SEM scores by species. No bream were captured during the sampling event. "Mullet" includes four diamondscale mullet caught in Gladstone Harbour and two sea mullet caught at the reference site, Baffle Creek.

Fulton's condition factor (K) has been graphed by Zone (Figure 4) and a difference in Barred Javelin Fulton's K among zones was detected (Figure 4B). Post-hoc analyses failed to reveal an unambiguous outcome among all zone comparisons, but nonetheless showed that Fulton's K for Barred Javelin in zone 2 was less than that in zones 1 and 5, and that Fulton's K in zone 8 was less than that in zone 5.

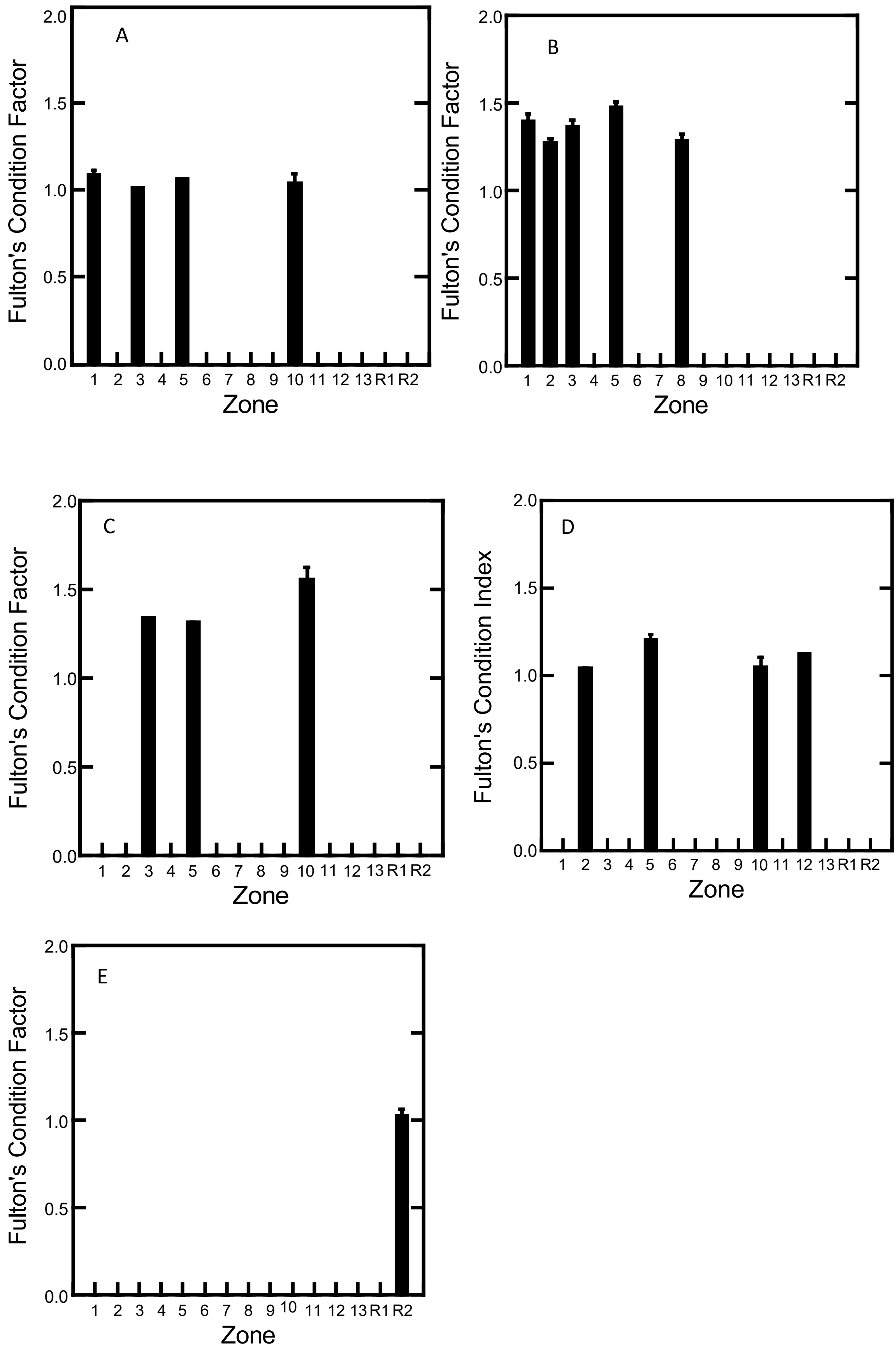


Figure 4: Average Fulton's Condition Factor (K) ± SEM scores by zone for A barramundi, B barred javelin, C diamondscale mullet, D blue catfish and E sea mullet (reference site only).

Hepatosomatic index (HSI) varied among zones (Figure 5), but the differences, where possible to test, were not significant (Appendix 2).

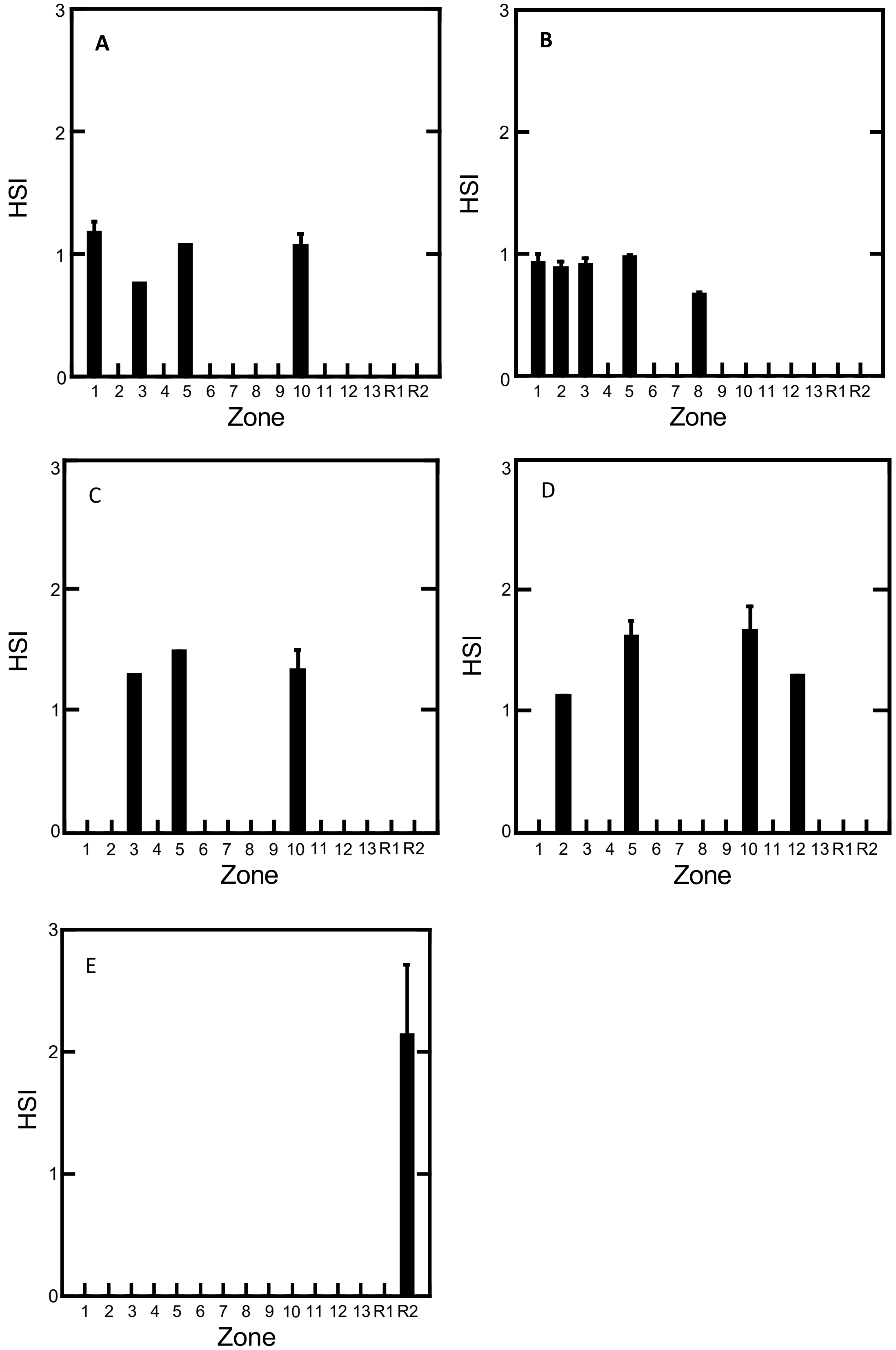


Figure 5: Average Hepatosomatic index (HSI) ± SEM scores by zone for A barramundi, B barred javelin, C diamondscale mullet, D blue catfish and E sea mullet (reference site only).

Gonadosomatic index (GSI) was calculated separately for male and female fish of each species, but low replication precluded meaningful statistical analysis, except for female barred javelin (for which there were no significant differences detected between zones) and female blue catfish (no differences detected) (Figure 6).

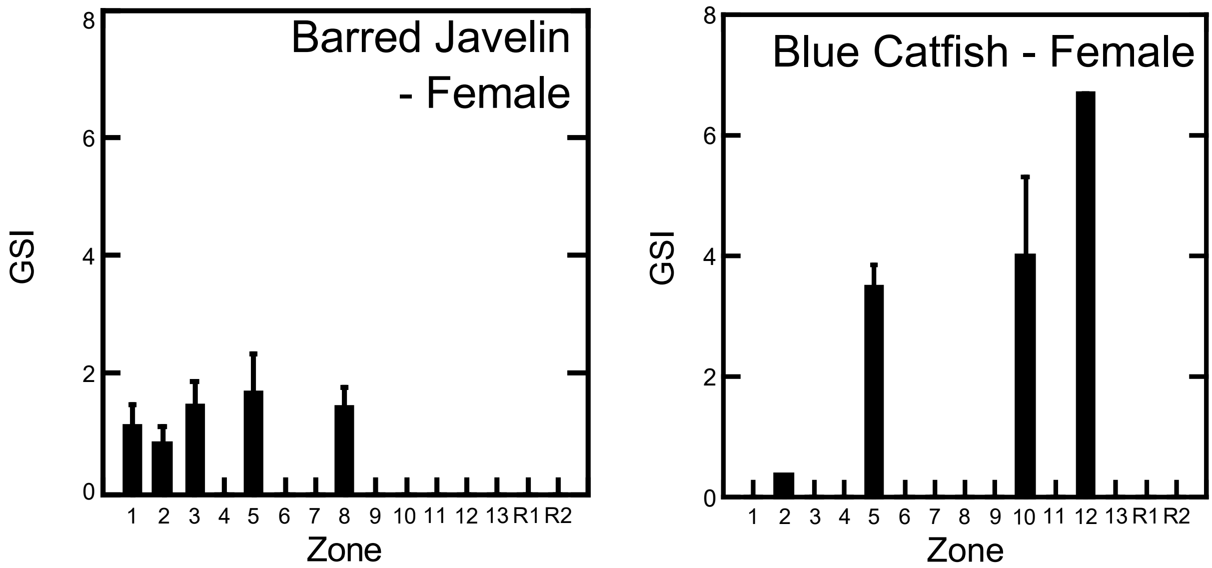


Figure 6: Average Gonadosomatic Index (GSI) ± SEM scores of female barred javelin and blue catfish by zone.

2020 Fish Health Indicator Results for Gladstone Harbour

In 2018, HAI was identified as the most appropriate fish health indicator for immediate implementation in the Gladstone Harbour Report Card (Flint et al., 2018; 2019). The metric requires gross pathological analysis during dissection and produces a composite metric that integrates evaluations of the condition of multiple organs and tissues. The premise of the index is that scores will cumulatively reflect the acute and chronic stressors present in the fish's environment, with poorer anatomical condition resulting in higher HAI scores and thus indicative of a more stressful environment. The version of the HAI used in this study was also used by Wesche et al. (2013) during the fish health investigation in Gladstone Harbour in 2011-2012.

Measures and baselines

In 2020, HAI was calculated for each of the 78 target fish captured from Gladstone Harbour by scoring and summing gross pathology scores for the following measures: skin, eyes, fins, gills, spleen, kidney, hindgut, liver, and parasite load. The best possible score for each measure, and in total, is 0. Any increase from a score of 0 indicates the identification of gross pathologies visible during a routine necropsy dissection. The highest (worst) score for each individual measure is 30 and in total is 270.

The HAI is designed to be used as a summed average for a sample population (Adams et al., 1993). Using this method, the Gladstone Harbour-wide HAI results (over nine measures) were determined, by taxa (Table 3). Reference site data were excluded from these calculations. Average HAI ranged from 37 (barramundi) to 19 (barred javelin).

Table 3: Average measure and HAI total scores, calculated for fish caught in Gladstone Harbour in October 2019. Individual scores for each organ range from 0-30. Total individual HAI scores range from 0-270. The category "Bream" includes pikey bream and yellowfin bream. The category "Mullet" includes sea mullet and diamondscale mullet. No bream and only four mullet were caught in Gladstone Harbour during the single sampling event, so these two species are not included in the fish health indicator scores and grades.

Taxa / Measure	Barramundi (n = 11)	Bream (n = 0) No data (ND), not graded	Barred Javelin (n = 31)	Blue Catfish (n = 32)	Mullet (n = 4) HAI was calculated but not graded
Skin	1.82	ND	0	1.25	0
Eyes	0	ND	0	0	0
Fins	2.73	ND	0.32	2.50	0
Gills	2.73	ND	0	0	0
Spleen	0	ND	0	0	0
Kidney	2.73	ND	0.97	5.63	0
Hindgut	0	ND	0	0	0
Liver	13.64	ND	14.52	17.81	7.50
Parasites	13.64	ND	3.55	6.25	0
HAI score	**37.27**	**ND**	**19.35**	**33.44**	**7.50**

Scoring the HAI

Using the benchmark of 10 and the Worst Case Scenario (WCS) of 70, standardised HAI scores and grades were calculated using a distance from the benchmark method (Flint et al., 2019). The standardised scores and grades calculated using collected in October 2019 are provided in Table 4.

The distance from the benchmark function used is as follows:

Calculated score = 1-((x-B)/(WCS-B))

Where:

x = average HAI

B = benchmark

WCS = worst case scenario

Table 4: Calculation of standardised HAI scores for Gladstone Harbour using data collected in October 2019. ND – not enough data, as no bream and only four mullet were caught.

Species	Average HAI	Benchmark	WCS	Calculated score
Barramundi	37.27	10	70	0.55
Bream	ND	10	70	ND
Barred javelin	19.35	10	70	0.84
Blue catfish	33.44	10	70	0.61
Mullet	ND	10	70	ND

Using GHHP's grading scale, grades for each species group were calculated (Table 5), and an overall harbour score and grade determined by averaging the scores of the five species groups.

Table 5: Fish Health Indicator scores and grades for the 2019 Gladstone Harbour Report Card. ND – not enough data, as no bream and only four mullet were caught.

	Barramundi	Bream	Barred Javelin	Blue Catfish	Mullet
Standardised HAI score/grade	Grade C Score 0.55	ND	Grade B Score 0.84	Grade C Score 0.61	ND
Overall Harbour score	Grade B Score 0.67				

Confidence in scores

The primary considerations when determining confidence in HAI scores for are sample size and potential for interference by ecological characteristics of each species group.

Sample size of barramundi (n = 11) was relatively low compared to blue catfish and barred javelin. Also, as discussed in detail in Flint et al. (2018), barramundi are a particularly mobile fish species with tagging evidence of movements across many hundreds of kilometres. This means that a barramundi caught in Gladstone Harbour may have moved from elsewhere, and thus their condition at the time of capture may represent conditions across a broader spatial scale than the capture zone.

Higher numbers of barred javelin (n = 31) and blue catfish (n = 32) should result in lower variability of their measures and therefore provide greater confidence in the results for these taxa.

Discussion of 2020 results and recommendations

Recommendation 1: GHHP continues to monitor HAI of fish in Gladstone Harbour.

In 2018 and 2019, HAI was identified and trialled as a suitable indicator for immediate implementation in the Gladstone Harbour Report Card. In 2020, HAI scores and grades have been calculated for the 2020 Gladstone Harbour Report Card using data collected in October 2019. This year's calculated scores show satisfactory (C) to good (B) fish health in Gladstone Harbour across three species groups, and an overall harbour grade of B. No bream and only four mullet were caught in the single sampling event conducted in October 2019, so these species couldn't be included in the scores and grades. The 2020 results are similar to the 2019 Report Card, in which barramundi scored 0.58 (C), bream scored 0.78 (B), barred javelin scored 0.77 (B), blue catfish scored 0.60 (C) and mullet scored 0.73 (B). the Overall Harbour score in 2019 was 0.69 (B).

Recommendation 2: GHHP considers only sampling at reference sites if worse than usual HAI scores are identified in fish caught in Gladstone Harbour during a particular sampling event.

As in previous years, fish were sampled at Baffle Creek as a reference site. While regular collection at a reference site provides a useful comparison, of the five species groups scored and graded for the Report Card, only sea mullet were captured at Baffle Creek in October 2019. This represents a large time and cost investment for minimal data. An alternative option is to sample only in Gladstone Harbour, but if laboratory assessment of HAI suggests poor condition, add sampling days at Baffle Creek and potentially other reference sites (e.g. Stanage Bay, or tributaries draining to the northern section of the Narrows) to gather fish samples for comparison. As the dataset grows through time, a baseline for identification of future fish health issues will be established.

Recommendation 3: GHHP continues to monitor measurements required to calculate other fish condition measures such as Fulton's K, HSI and GSI, to collate a long-term baseline dataset.

Several other condition measures can be calculated using the data collected during GHHP's fish health monitoring in Gladstone Harbour. The condition measures Fulton's K, HSI and GSI are biologically variable which makes establishment of scientifically defensible baselines difficult in the short term. For example, all three measures are affected by the reproductive status of the fish.

All three of these condition metrics can be rapidly measured during dissections, so while they may not yet be useful indicators for the Report Card, it is worthwhile continuing to collect data from future samples to establish a long time series. Following the Gladstone fish health investigation in 2011-2012, Wesche et al. (2013) reported significantly lower condition factors of barramundi from Gladstone harbour than from reference sites (at the $p < 0.05$ level), and barramundi from Gladstone also had significantly higher proportions of sunken abdomens and lower levels of mesentery fat. During events such as that experienced in 2011-2012, noticeable changes in condition measures are more likely.

Recommendation 4: GHHP considers testing for bioaccumulation of metals and other toxicants in collected fish tissue samples.

In 2018 and 2019, eye diameter was measured to gather data on fluctuating asymmetry. A CQUniversity Masters student is now conducting a research project on the utility of fluctuating asymmetry as an indicator of estuarine fish condition, and is measuring a range of bilateral structures.

As discussed in the 2018 fish health research project (Flint et al., 2018), bioaccumulation of toxicants in fish tissues may also be a useful indicator for future consideration. While bioaccumulation only becomes an indicator of fish health at levels that cause the initiation of detoxification mechanisms and tissue damage (Whitfield & Elliott, 2002), it also provides information on the bioavailability of toxicants in the environment and is an important consideration for fish that are consumed by people. Bioaccumulation is regarded as an integrative measure and an indicator of exposure of organisms to toxicants in polluted ecosystems. Metals are not metabolised by organisms, and therefore, bioaccumulation of metals and metalloids is of particular value (Luoma & Rainbow, 2005). Therefore, tissue samples were collected from dissected fish and stored to allow future testing for bioaccumulated metals and other toxicants.

Target species in 2020 included barramundi, bream (including pikey bream and yellowfin bream), large bodied mullet (including diamondscale mullet and sea mullet), barred javelin and blue catfish. Smaller samples of all species were caught in this sampling year, as only 7 days of sampling were undertaken, compared with 16 days in the previous year. In previous years, higher mullet catches were achieved in April than in September/October. For continuity of data in future years, and to gain a broader indication of fish health, it would be ideal for GHHP to divide sampling effort across spring and autumn.

Recommendation 5: GHHP considers splitting sampling effort over both spring and autumn to improve the changes of capturing each of the target taxa.

In October 2020, no bream and few mullet were captured, so these taxa could not be scored and graded for the Report Card. As discussed in the 2018 research report and with the GHHP ISP, bream are not frequently caught in gill nets, and the total number of bream caught in 2018-19 was 9. Other options that could be considered if GHHP wishes to increase the sample size for bream, are to include targeted hook and line fishing for bream in the monitoring program, or to link with other GHHP projects to provide bream from another source. The most likely reason for the low mullet catches was because only one sampling event was conducted, in Spring (October). In future years it would be advisable to split the sampling effort over spring and autumn (e.g. October and April) to have a better chance of capturing more of the target taxa. This would not only have the benefit of a likely increase in sample size to facilitate meaningful statistical analyses, but would also allow analysis of seasonal differences in fish health, against which spot sampling events, such as during acute disturbances to the coastal environment, could be more reliably interpreted and understood.

Similar to previous years, a range of other inshore and estuarine fish species were captured incidentally in October 2019. While some other species may be captured more frequently, the target species of barramundi, blue catfish, bream, barred javelin and mullet are demersal or benthic species that are likely to be in closer contact with pollutants accumulated in sediments, making them useful indicator species (Cowled, 2016).

Recommendation 6: GHHP continues to conduct regionally stratified fish sampling to score and grade the whole of Gladstone Harbour.

A fish movement analysis was conducted as part of the 2018 research project (Appendix 1 in Flint et al., 2018), and the range and the average movements of a variety of recreationally caught inshore and estuarine fish species were compared from tag-recapture data provided by the SunTag recreational fishing tagging program. Barramundi are a wide-ranging fish species and can move many hundreds of kilometres between tagging and recapture. There are some issues with the interpretation of any identified health issues for this species, as it may be difficult to determine how long the fish has been resident in the area of capture. Some other inshore species, including bream and barred javelin, are all more resident, so continued monitoring of these taxa is recommended. Large mullet and blue catfish can also travel long distances, but no local tagging records are available for these species because they are not normally targeted by recreational fishers.

The fish movement analysis conducted during the 2018 research project also detected high transience of fish between different areas within Gladstone Harbour. As such, the ISP elected to report scores of fish health at the harbour-wide scale. Because fish health scores are reported on a harbour-wide scale, the ISP also decided for 2019 to amend the sampling design to allow for higher catches in a shorter time (i.e. spend time fishing in zones with a high probability of target species catch). After this change in sampling strategy the catch per day of target species increased.

References

Adams, S. M., Brown, A. M., & Goede, R. W. (1993). A Quantitative Health Assessment Index for Rapid Evaluation of Fish Condition in the Field. *Transactions of the American Fisheries Society, 122*, 63-73.

Anderson, M.J., Gorley, R.N., & Clarke, K.R. (2008). PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth, UK, 214pp.

Anderson, M.J. (2017). Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online, DOI: 10.1002/9781118445112.stat07841

Cowled, B. (2016). *Final review of the use of fish health methods worldwide and their potential use in Gladstone Harbour* (Project ISP016a). Canberra, Australia. Retrieved from: www.ghhp.org.au

Flint, N., Anastasi, A., Irving, A., De Valck, J., Chua, E., Rose, A., French, K. & Jackson, E.L. (2018). Fish Health Indicators for the Gladstone Harbour Report Card, Final Report to the Gladstone Healthy Harbour Partnership. CQUniversity Australia, Queensland.

Flint, N., Irving, A., Anastasi, A., De Valck, J. & Jackson, E.L. (2019). A Fish Health Indicator for the 2019 Gladstone Harbour Report Card, Final Report to the Gladstone Healthy Harbour Partnership. CQUniversity Australia, Queensland.

Luoma, S. N., & Rainbow, P. S. (2005). Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environmental Science and Technology, 39*(7), 1921-1931. doi:10.1021/es048947e

Pidgeon, B. (2004). *A review of options for monitoring freshwater fish biodiversity in the Darwin Harbour catchment*. Darwin, Australia. Retrieved from http://www.environment.gov.au/system/files/resources/ba440664-fd8f-4947-a702-e0caab030d51/files/ir470.pdf

Van der Oost, R., Beyer, J., & Vermeulen, N. P. E. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology, 13*(2), 57-149. doi:10.1016/S1382-6689(02)00126-6

Wesche, S., Lucas, T., Mayer, D., Waltisbuhl, D., & Quinn, R. (2013). *Gladstone Harbour Fish Health Investigation 2011–2012*. Queensland Department of Agriculture and Fisheires, Brisbane, Australia.

Whitfield, A. K., & Elliott, M. (2002). Fishes as indicators of environmental and ecological changes within estuaries: A review of progress and some suggestions for the future. *Journal of Fish Biology, 61*(SUPPL. A), 229-250. doi:10.1006/jfbi.2002.2079

Appendix 1: Site physicochemical data

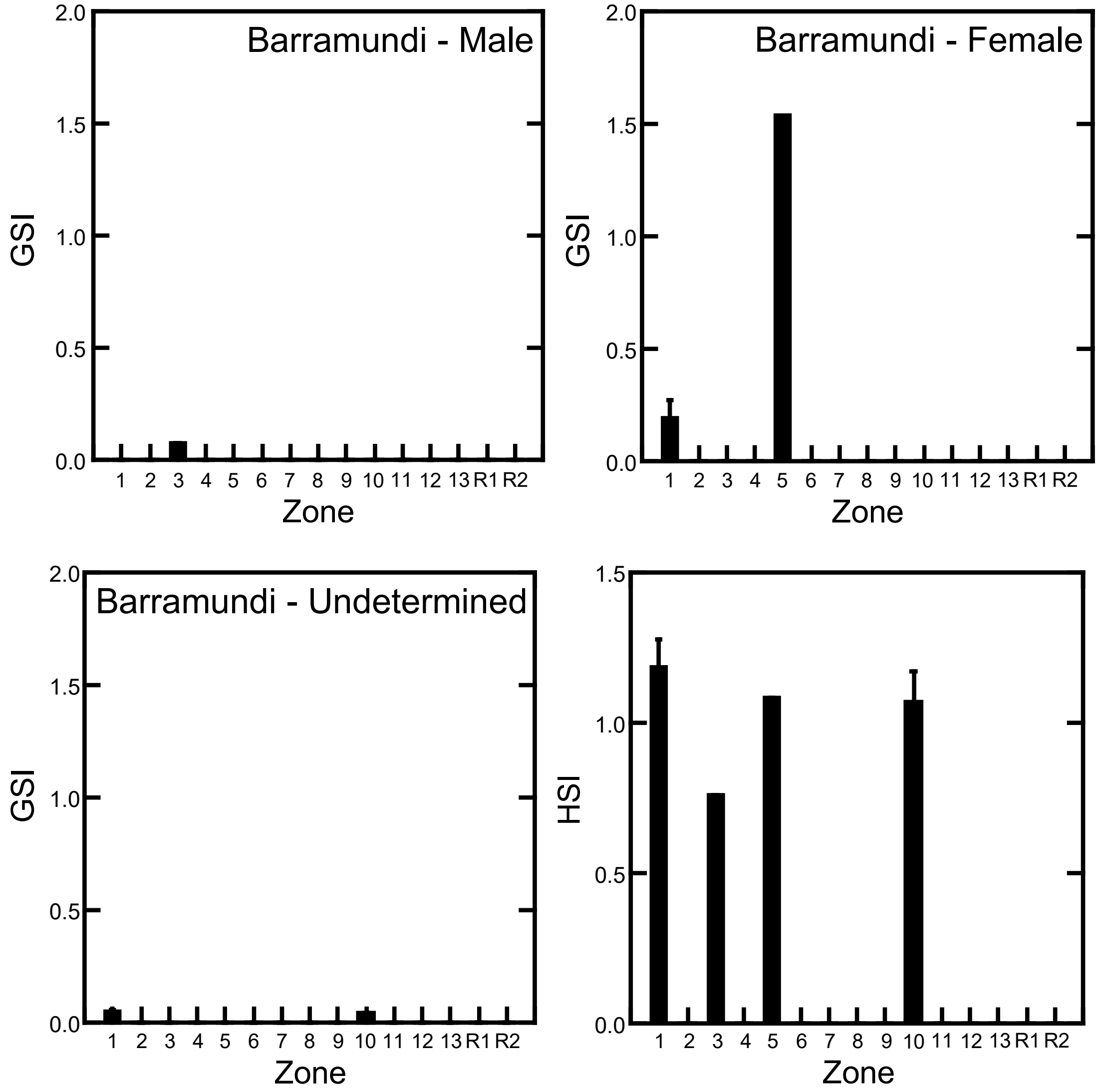
Zone	GHHP Zone Number	Date/Time	Temp (°C)	DO (%)	DO (mg/L)	EC (µs/cm)	pH	Turbidity (NTU)	TDS	ORP	Salinity (ppt)
Colosseum	12	10/11/2019 9:39	23	75.4	5.13	60080	8.45	3	39045	92.4	40.9
Colosseum	12	10/11/2019 9:43	23	77.2	5.24	60175	8.39	3.6	39104	67.1	40.38
Middle Harbour	5	10/10/2019 14:02	25	95.2	6.22	59182	8.41	15.4	38470	102	39.59
Narrows	1	10/4/2019 12:09	23.3	109.8	7.53	57336	8.81	5.7	37272	108.1	38.23
Boyne River	10	10/3/2019 12:34	24.5	88.2	6	53609	8.56	4.8	34847	112.5	35.42
Western Basin	3	10/2/2019 10:34	24.4	98.2	6.54	58733	8.2	7.6	381777	118.2	39.26
Grahams Creek	2	10/8/2019 12:40	25.4	93.4	6.11	59338	8.64	3.5	38568	101.5	39.69
Baffle Creek	Reference	10/9/2019 12:24	26.5	133.1	8.63	57848	9.06	2.9	37604	70.5	38.55

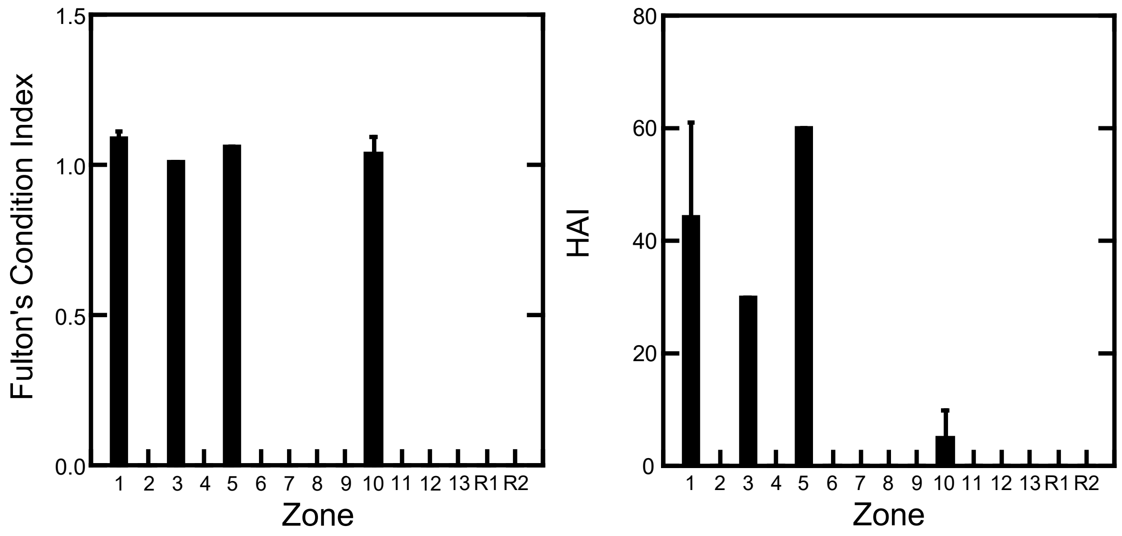
Appendix 2: Statistical analysis results

***Formal statistical analyses done where n = 3 or greater per zone**

Barramundi

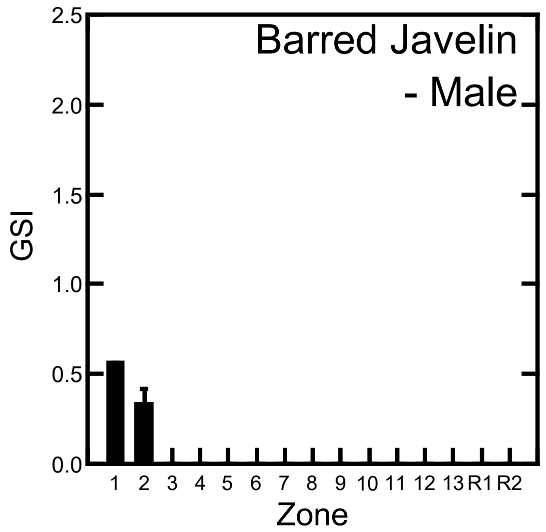
Formal statistical analyses of Barramundi variables among zone was not possible due to inadequate replication (more than 3 replicates were sampled only at zone 1).

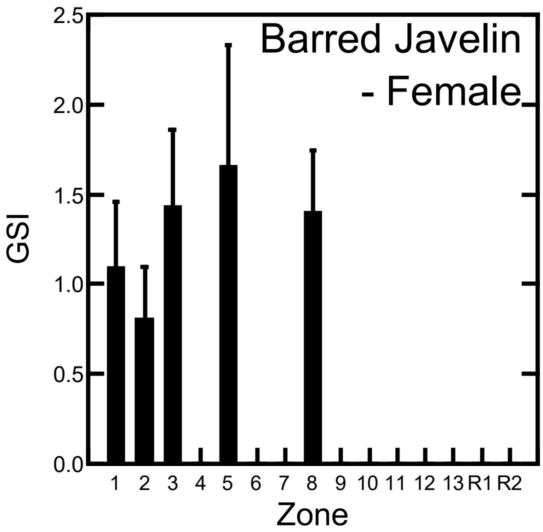



Fulton's Condition Index
1.5
1.0
0.5
0.0
1 2 3 4 5 6 7 8 9 10 11 12 13 R1 R2
Zone
HAI
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 R1 R2
Zone

Barred Javelin

Formal statistical analyses of Barred Javelin were possible for most variables, with adequate replication sampled across zones 1, 2 3, 5 and 8.

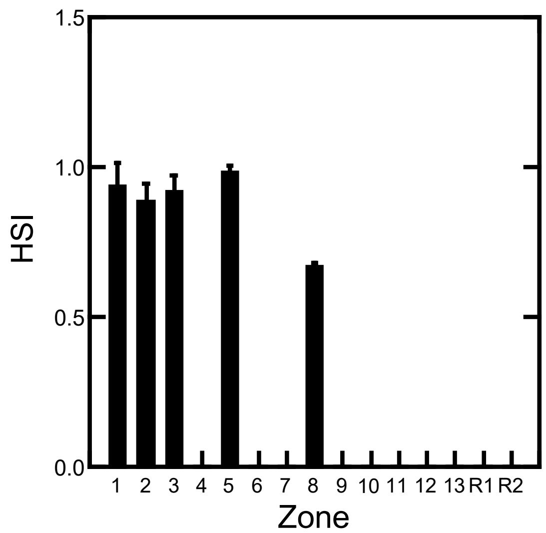




PERMANOVA analysis of Barred Javelin Female GSI among Zones

Source	df	MS	F	P
Zone	4	0.418	0.627	0.645
Residual	26	0.667		

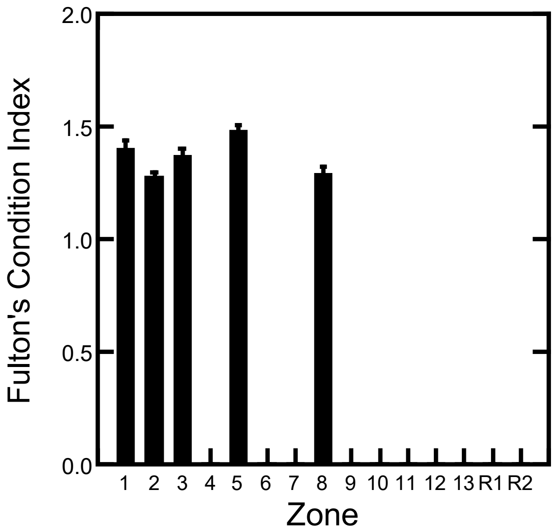
No differences detected for female Barred Javelin GSI among zones.



PERMANOVA analysis of Barred Javelin HSI among zones

Source	df	MS	F	P
Zone	4	0.060	0.201	0.126
Residual	26	0.299		

No differences detected for Barred Javelin HSI among zones.



PERMANOVA analysis of Barred Javelin Fulton's Condition Index among zones

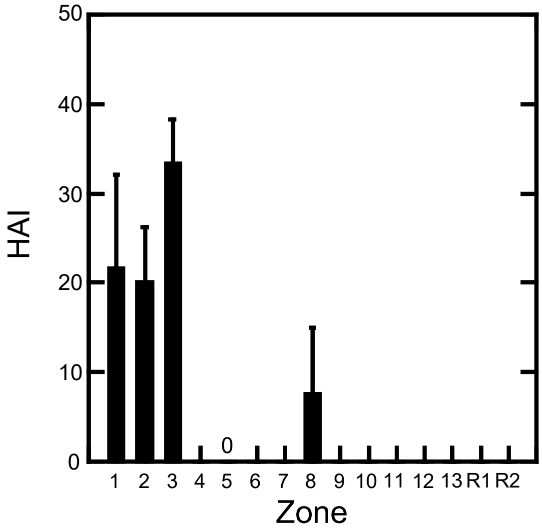
Source	df	MS	F	P
Zone	4	0.036	4.892	**0.005**
Residual	26	0.007		

A difference in Barred Javelin Fulton's Condition Index among zones was detected.

Post hoc analyses

Groups	t	P(perm)	Unique perms
1, 2	2.6935	**0.02**	973
1, 3	0.54186	0.583	417
1, 5	1.2575	0.229	84
1, 8	1.8293	0.089	209
2, 3	2.1976	0.051	970
2, 5	3.8674	**0.001**	410
2, 8	0.25386	0.821	780
3, 5	2.0429	0.088	84
3, 8	1.5457	0.177	208
5, 8	3.6711	**0.048**	35

Post-hoc analyses failed to reveal an unambiguous outcome among all zone comparisons, but nonetheless showed that Fulton's Condition Index of Barred Javelin in zone 2 was less than in zones 1 and 5, and zone 8 was less than zone 5.



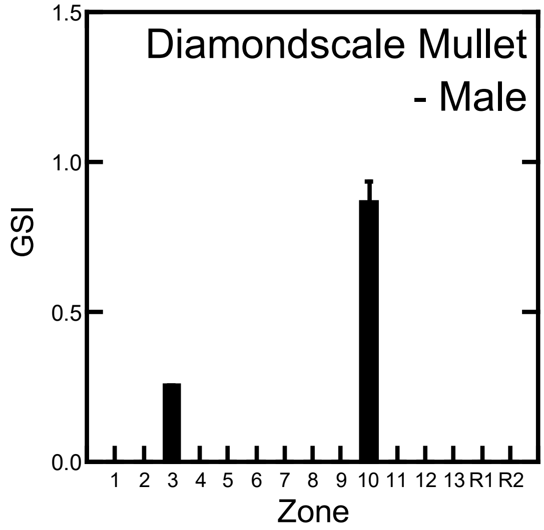
PERMANOVA analysis of Barred Javelin HAI among zones

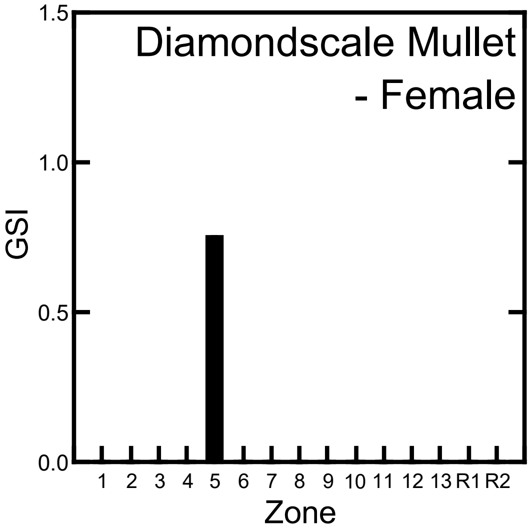
Source	df	MS	F	P
Zone	4	723.860	1.903	0.131
Residual	26	380.450		

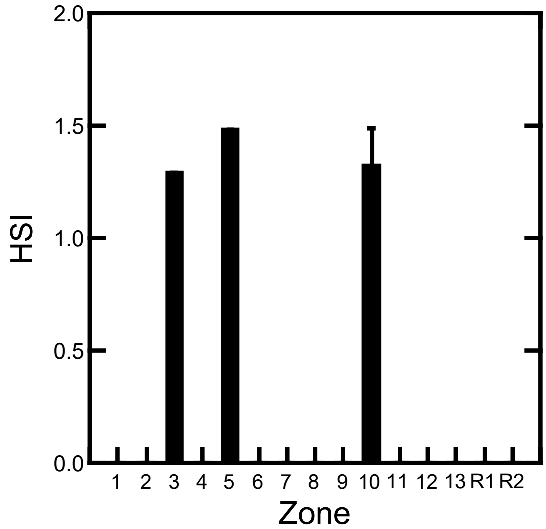
No differences in Barred Javelin HAI was detected among zones.

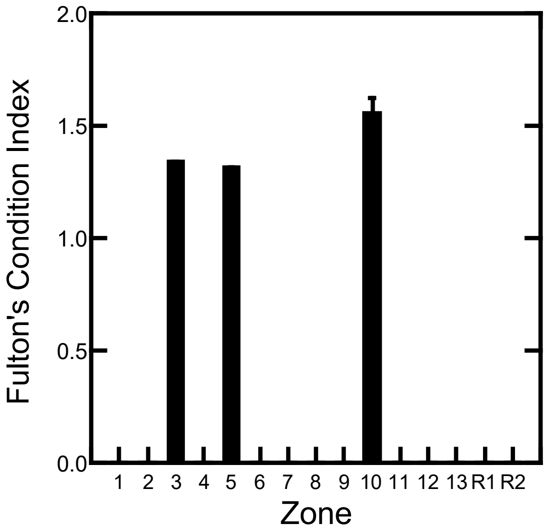
Diamondscale Mullet

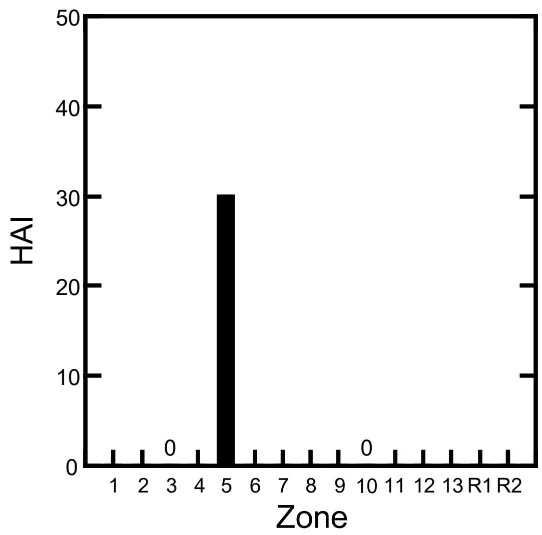
No formal analyses were possible for diamondscale mullet due to inadequate replication.





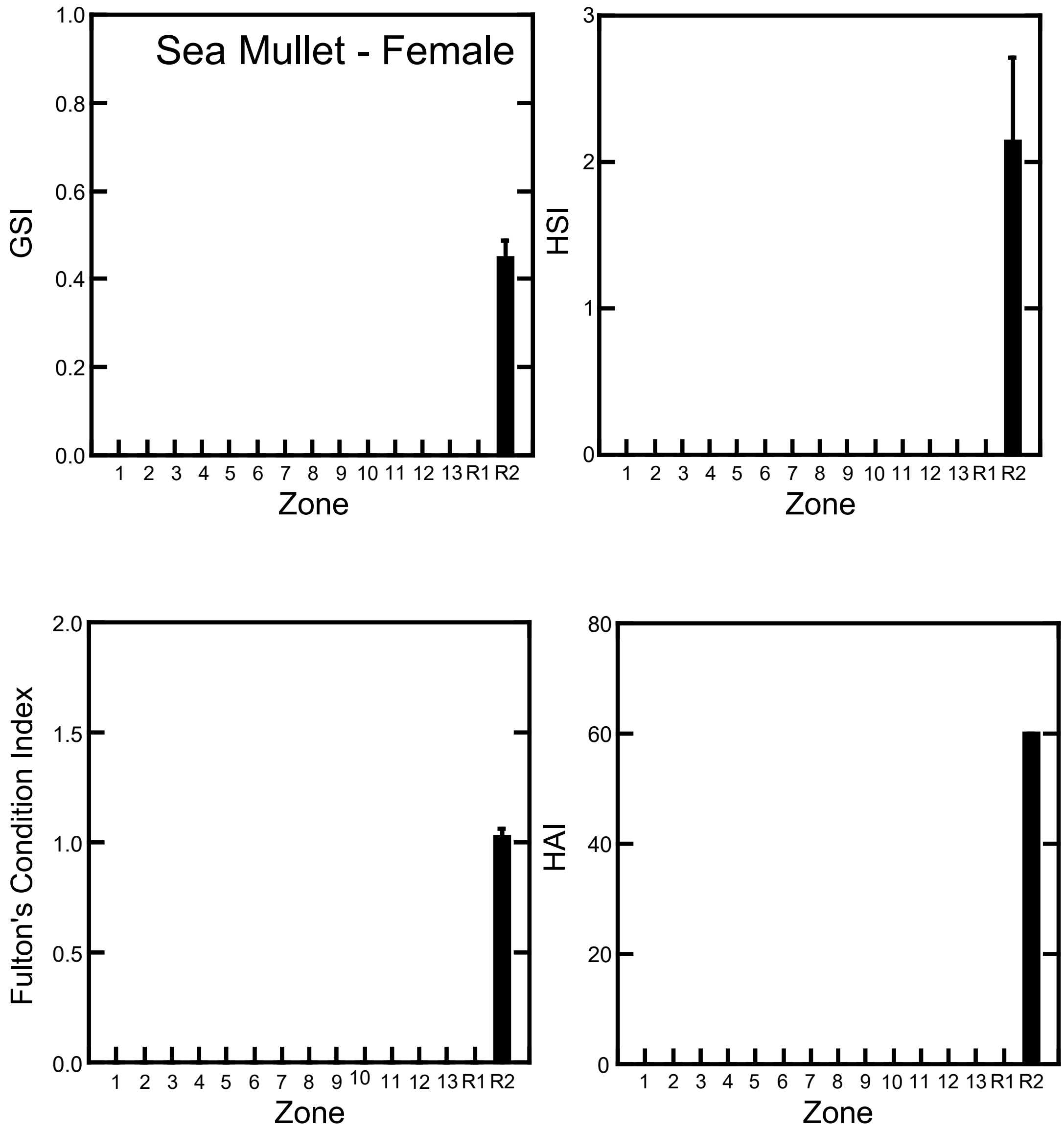






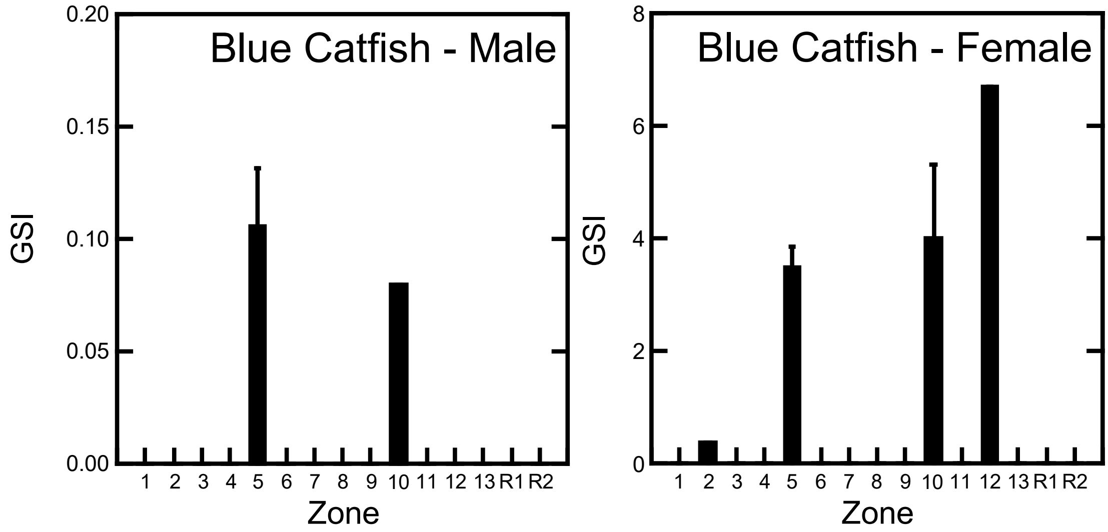
Sea Mullet

No formal analyses were possible for Sea Mullet due to inadequate replication.



Blue Catfish

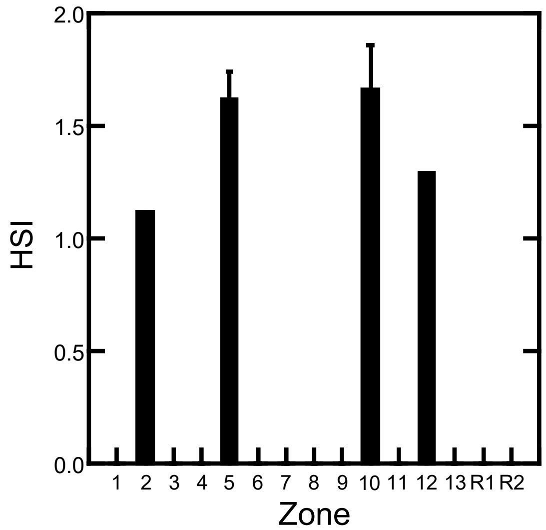
Formal statistical analyses were possible between zones 5 and 10 for Blue Catfish variables (for GSI, only adequate replication was available for females).



PERMANOVA analysis of Blue Catfish Female GSI between zones 5 and 10

Source	df	MS	F	P
Zone	1	0.621	0.287	0.610
Residual	28	2.160		

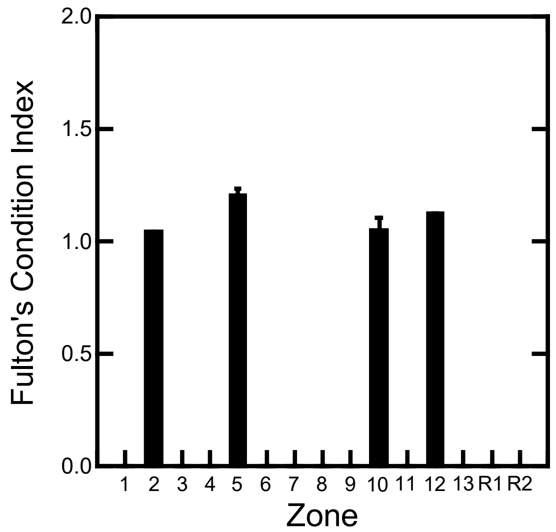
No differences detected for female Blue Catfish GSI between zones 5 and 10.



PERMANOVA analysis of Blue Catfish HSI between zones 5 and 10

Source	df	MS	F	P
Zone	1	0.008	0.019	0.843
Residual	28	0.393		

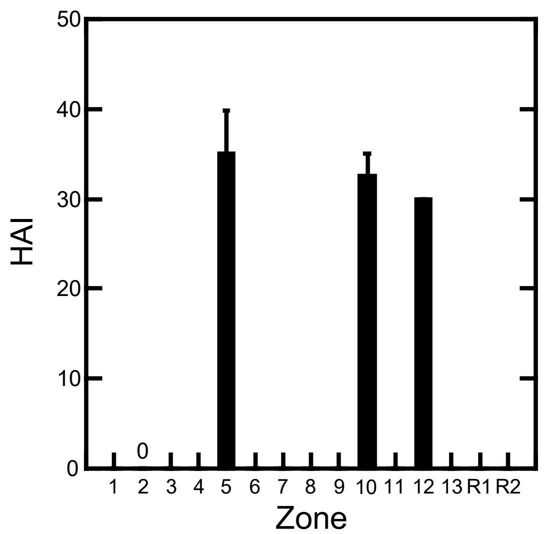
No differences detected for Blue Catfish HSI between zones 5 and 10.



PERMANOVA analysis of Blue Catfish Fulton's Condition Index between zones 5 and 10

Source	df	MS	F	P
Zone	1	0.082	4.029	0.062
Residual	28	0.020		

No differences detected for Blue Catfish Fulton's Condition Index between zones 5 and 10.



PERMANOVA analysis of Blue Catfish HAI between zones 5 and 10

Source	df	MS	F	P
Zone	1	21.667	0.041	0.914
Residual	28	525.890		

No differences detected for Blue Catfish HAI between zones 5 and 10.