Department of Agriculture, Fisheries and Forestry

Gladstone Harbour
Fish Health Investigation
2011–2012

Great state. Great opportunity.

Queensland Government

Front cover photo supplied by Johnny Mitchell, as featured in his report *Time line Barra*

This publication has been compiled by Dr Stephen Wesche, Dr Tim Lucas, Dr David Mayer, David Waltisbuhl and Dr Ross Quinn of the Department of Agriculture, Fisheries and Forestry.

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Executive summary

The Gladstone Harbour Fish Health Investigation occurred in response to public concerns regarding fish health in Gladstone Harbour, which were raised with the former Department of Employment, Economic Development and Innovation (now Department of Agriculture, Fisheries and Forestry (DAFF)) in August 2011. The majority of concerns were regarding skin redness, lesions and eye damage observed on barramundi caught from the Boyne River or within its vicinity. Further reports included unusual skin redness and occasional lesions observed on a range of fish species from Gladstone Harbour (including sharks), as well as an increase in the incidence of shell erosion on mud crabs. The subsequent investigation aimed to determine the cause of the reported issues and specifically to address public concern regarding the potential impact of industrial activity including dredging in Gladstone Harbour. The investigation, conducted by DAFF, was part of a whole-of-government response, which included sediment and water quality testing; and investigation into human health concerns.

The first reports of abnormalities in fish were received months after a major flood event happened in Gladstone from December 2010 until early 2011. The flood event caused a dramatic influx of large barramundi (estimated 30 000), as well as other species from Lake Awoonga into the Boyne River when the Awoonga Dam spilled over. The dam had not spilled over since the 1990s, well before the dam wall was raised in 2002. Many of these fish were injured (some fatally) from the force of the impact when passing over the spillway. It is believed that a smaller number of fish were washed over during a second flood event in early 2012.

The initial response investigated fish with visible abnormalities (e.g. missing scales and redness), and signs of disease to indicate the cause of the observed issues. This early sampling (Phase 1) between August 2011 and February 2012 was based on observations of commercial fishing activity, contracted fish surveys and submissions by members of the public. Over 5000 fish, crustaceans and molluscs were visually assessed during this sampling.

In January 2012, the Independent Gladstone Fish Health Scientific Advisory Panel released a report recommending a structured sampling program to gauge fish health in Gladstone Harbour sites against comparable unaffected sites (reference sites), through time. It also recommended the development of a conceptual model of possible cause-effect relationships to help guide studies and eliminate potential causal factors.

Structured sampling in Gladstone Harbour (Phase 2) was conducted in two major surveys during April–May (Trip 1) and June–July (Trip 2) 2012. These surveys covered 11 sites including two reference sites and focussed on a subset of 10 species. The selected species represented a range of different life cycles (e.g. catadromous and estuarine) and trophic levels (e.g. predatory, omnivorous detritivores and scavengers), and had been reported with a variety of abnormalities. A third targeted survey was conducted in September 2012 to monitor potential seasonal reoccurrence of parasitic infestations in the barramundi from the Boyne River. A total of 3699 fish and crustaceans were visually assessed in the field, with 452 specimens examined by necropsy, and tissues from 120 specimens subjected to histopathology and chemical residue testing.

Phase 1 (August 2011–February 2012)

Both commercial catch records as well as field observations confirmed there was an abnormally large population of barramundi in the Boyne River, with many of these fish being exceptionally large. Recreational fishers indicated that the increased population of barramundi had been present since the flooding event in early 2011, with fish showing signs of damage but also recovery at first. During winter, when the water temperature was reported to be as low as 18.1^{o}C, anecdotal evidence suggested deteriorating health in the barramundi from the Boyne River including swollen, opaque eyes and ulcerated areas on the skin. The investigation found that the fish were suffering from skin lesions and parasitic infestation (monogeneans on the body surface), conditions not previously documented from fish in a wild environment, but are often recorded from fish held in heavily stocked aquaculture systems.

Other finfish species were observed with a range of generally mild skin conditions, includng localised skin inflammation, scale loss, skin erosion and redness. A fungal infection that causes skin lesions (Epizootic Ulcerative Syndrome (EUS) or 'red-spot' disease) was identified in two fish, both of which were outside of Gladstone Harbour. EUS was excluded as a potential cause of the lesions seen in Gladstone Harbour. No bacterial, parasitic or fungal pathogens were found that could explain the skin conditions.

Phase 2 (April 2012–September 2012)

Barramundi

The barramundi from the Boyne River caught in April 2012 were observed to have high prevalence of skin lesions thought to be caused by physical trauma, while fish captured in September 2012 were observed with lesions that were healing. Infestations of the mongenean parasite identified in Phase 1 were not observed during the Phase 2 structured sampling.

Based on weight-length relationships, mesentery fat assessment and liver melanomacrophage centres, barramundi sampled from Gladstone Harbour sites were in poor nutritional condition compared with those from the reference sites. There were some barramundi caught during sampling with relatively high levels of mesentery fat, indicating excessive intake of food. These fish tended to be found in the Boyne River and had lesions typical of physical trauma caused by fish passing over a spillway, suggesting that they probably originated from Lake Awoonga.

Chemical residue analysis of barramundi livers showed that fish collected from Gladstone Harbour had significantly higher concentrations of iron and cadmium during April 2012, and higher arsenic and zinc levels than the reference sites in June/July 2012 ($P \leq 0.05$). Comparison of concentrations of metals in barramundi livers with toxicological effects data indicated that the concentrations in the barramundi liver were of no concern. In addition, there were no significant differences in these metal concentrations between fish with and without visible abnormalities.

Other species

Species other than barramundi were generally observed to be in good condition. Some abnormalities were observed in other species (e.g. shell lesions in mud crabs), however analysis of the data indicated there was no evidence that these conditions were more prevalent in fish or crustaceans from Gladstone Harbour when compared to those from the reference sites, or when compared with historical data.

Areas of detached scales associated with parasitic infection were observed on a number of shark species caught during the sampling program from Gladstone Harbour and the reference sites. It is unknown whether the numbers of parasites observed on sharks during this program are abnormal, however it is evident that these parasites and associated pathology are not unique to Gladstone. Severe skin redness was observed during necropsy, however this was not apparent in freshly caught sharks; redness developed in the hours after death. This post-mortem redness was not unique to Gladstone.

Large numbers of prawns and fish were caught on trawlers both in Gladstone Harbour and the reference sites, with no significant redness or other abnormalities observed.

No significant abnormalities were identified from the sampling and necropsy examination of pelagic species.

Histopathology investigation indicated that gill, liver and skin tissue of grinner, and the gill, hepatopancreas and muscle tissue of mud crabs was similar in Gladstone samples to reference sites. Some bleeding and swelling was observed in shark skin from both Gladstone and reference sites.

Chemical residue testing of crabs from both Gladstone and reference sites found a significant association between aluminium and selenium concentrations, and shell lesions. Given that concentrations of metals in mud crab hepatopancreas were similar to historical data from sites across the east coast of Queensland, any effect of these metals on shell lesions is unlikely to be unique to Gladstone. The available data also indicated that the suite of organic chemicals tested did not contribute to crab disease.

Chemical residue testing of muscle tissue from grinner showed no significant difference between Gladstone and reference sites.

Conclusions

It is obvious from all the reports that something happened to aquatic life in Gladstone Harbour in 2011. The findings provided in this study indicate that the fish health in Gladstone Harbour had returned to a more normal situation in 2012. Other than the physical damage or recovery from physical damage, this study found the sampled fish in a health status similar to that observed in the Bundaberg and Fitzroy reference sites.

All industrial activities in Gladstone that were operating in Gladstone in 2011 have continued to operate, including dredging. The only factor different in 2011 compared to previous years was the significant rainfall, the subsequent flooding and the introduction of a significant biomass of fish from Lake Awoonga. Flooding has also occurred in 2012 and 2013. However, other than continuing reports of barramundi showing physical damage as the result of being washed over the Awoonga spillway in these flooding events, reports of any reoccurence of sick fish have not been received by DAFF.

The most likely cause of the abnormalities observed during this investigation is the sudden introduction of barramundi and other fish from Lake Awoonga during December 2010 to early 2011. Although barramundi are naturally able to move between fresh and salt water environments, it appears that these fish were overcrowded and unable to feed normally after they were suddenly re-located from Lake Awoonga into the Boyne River. The stress preceded parasitic infestation during the colder winter water temperatures in 2011. The

Neobenedenia infestation subsided as the water temperature increased and fish density reduced, as shown by improving fish health in late 2011 and 2012.

The results in this study support that it was flooding combined with the introduction of a significant biomass of fish (including barramundi, mullet, catfish and bony bream) that stressed the ecosystem in Gladstone Harbour and adjacent waterways. This study cannot rule out the possibility that the activity of dredging and associated turbidity provided additional stress to the ecosystem, but it was not the primary stressor.

Background

In August 2011, the Queensland Government received reports of barramundi and subsequently other species being caught with obvious signs of ill health, including bulging/red eyes, blindness, severe skin lesions and skin discolouration.

On 16 September 2011, Fisheries Queensland, in the former Department of Employment, Economic Development and Innovation (now Department of Agriculture, Fisheries and Forestry (DAFF)), closed Gladstone Harbour and the surrounding area to fishing for a period of 21 days, under section 96 of the *Fisheries Act 1994* in response to concerns about human health and to allow further testing to be carried out on the conditions affecting locally-caught fish.

From the initial testing of nine ill barramundi, two conditions were identified that were affecting barramundi in the Gladstone area:

- Red-spot disease (Epizootic Ulcerative Syndrome (EUS) or 'red-spot' disease), which is caused by a fungus endemic to finfish species of mainland Australia. This condition was only confirmed in one barramundi from Port Alma, near the Fitzroy River;
- An external parasite *Neobenedenia sp.*, which was affecting the eye and skin particularly on some of the barramundi in Gladstone Harbour.

Barramundi and other finfish species displayed a range of generally mild skin conditions from localised skin inflammation, skin erosion and redness. No bacterial, parasitic or fungal pathogens were found that could explain the skin conditions.

Reports were also received from the Gladstone Area Water Board that an estimated 30 000[1] large barramundi entered the Boyne River in early 2011 when the Awoonga Dam spilled over for the first time since 1996. Commercial catches of barramundi in 2011 were in excess of 20 times the average annual Gladstone Harbour (Grid S30) catch from 2005 to 2010, providing support for this observation.

In response to the fish health issues, the Queensland Government set up an investigation program that included fish sampling and testing, water quality sampling and testing, and investigation into human health concerns.

Fisheries Queensland commenced monitoring fish health in Gladstone Harbour after the closure of the Harbour was declared, and continued to monitor fish health in Gladstone and adjacent areas in conjunction with commercial fishers until September 2012. Biosecurity Queensland conducted examination of animals of particular concern, which were submitted by either Fisheries Queensland or members of the public.

In October 2011, an Independent Scientific Advisory Panel was established to provide independent scientific advice to the Queensland Government on the fish health investigation in Gladstone Harbour. The membership of the panel comprised eminent scientists with

1 Fisheries Queensland was advised verbally that an estimated 30 000 barramundi were washed over the spillway in 2010/11 and has used this figure in all its publications. However, in the GAWB Annual report 2011, 20 000 barramundi are reported as being washed over the spillway. Given that the commercial fishers caught almost 250 t of barramundi in Gladstone in 2011and the average weight of barramundi in October 2011 was 9 kg; it is estimated that the commercial fishers caught more than 27 700 barramundi. In the last quarter of 2011, catch rates were still more than two and a half times the pre-flood rate for this quarter, therefore Fisheries Queensland believes that the figure of 30 000 barramundi being washed out of Awoonga Dam is an underestimate.

recognised expertise and research publications concerning aquatic environmental science including water quality; fish health and toxicology; and human health especially in relation to the potential for transmission of diseases from marine species to humans.

The Panel reviewed the Queensland Government's monitoring regimes, results collected in 2011 and analysis primarily focusing on fish health in Gladstone Harbour and surrounds, but also considered water quality monitoring and human health issues where relevant and appropriate.

The Panel acknowledged that this was a complex issue and supported the Government's ongoing investigation of the issue, noting that good progress had been made. The Panel made specific comments and recommendations in relation to the issues of fish health, water quality and human health with a view to identifying a possible cause(s) of the fish health issues being observed in Gladstone Harbour.

In particular, the Panel emphasised the need for comparative information from similar unaffected systems to determine the scale of the problem being observed in barramundi and other fish species in the Gladstone area. It was recommended that a more sophisticated study design be conducted, including baselines and trends during 'normal' periods, and appropriate areas outside the Harbour to act as a form of control for comparative analysis. The Panel also recommended constructing a conceptual model to illustrate possible cause-effect relationship(s) to help guide studies and eliminate potential causal factors.

Stocking in Lake Awoonga

Barramundi have been stocked in Lake Awoonga since 1996. According to the Gladstone Area Water Board website, over 4.54 million fish had been released into Lake Awoonga including 3.69 million barramundi, 465 718 sea mullet and 70 942 mangrove jack. Since 2006, an additional 1.3 million barramundi have been stocked in Lake Awoonga. Until water flowed over the spillway on 12 December 2010, the stocked fish had not been able to migrate downstream because the dam had not overflowed for the previous 14 years. In 2002, the height of Awoonga Dam was raised.

Calliope and Boyne Rivers flooding event

Above average rainfall occurred in November and December 2010. Totals of 93 mm and 587.4 mm respectively were recorded at the Calliope weather station, compared with the median level of 69.4 mm and 95.9 mm respectively. The total annual rainfall in 2010 was 1702 mm, the second highest level since records started in 1906 up until 2010 (the highest level was 1801 mm, recorded in 1956).

For comparison, 1973 was a wet year across Queensland, which resulted in the Brisbane floods in January 1974. The total annual rainfall at the Calliope weather station in 1973 was 1326 mm, which was the eighth highest on record. The mean total annual rainfall is 854 mm and the median total annual rainfall is 811 mm.

The rainfall in 2010/11 resulted in an annual stream flow in the Boyne River of 1 194 848 ML (measured between October 2010 to September 2011), the highest stream flow since records started in 1984. This is greater than the full supply capacity of Wivenhoe Dam, which is 1,165,238 ML. The monthly stream flow for December 2010 was 634 999 ML, also the highest since records started.

On 12 December 2010, Awoonga Dam overflowed and did not cease spilling until June 2011. On Tuesday 28 December 2010, the water level in the dam had reached its highest point ever at 44 m; 4 m above the spillway level. In 2013, a higher flood peak in the Boyne River was reached at 48.3 m, more than twice the height above the spillway in 2010/11.

It was estimated that 30 000 large barramundi were washed out of Awoonga Dam into the Boyne River between December 2010 and March 2011, as the result of water flow over the spillway. If the average weight of these fish was ten kilograms, this represents an additional 300 tonnes of barramundi introduced into the Boyne River and Gladstone Harbour.

Recreational catch

A charter operator in the Gladstone region, Johnny Mitchell, provided a report to Fisheries Queensland of his observations of the recreational catch of barramundi in the Boyne River system in 2011 (Appendix E).

His report documented the movement of large schools of large barramundi (Figure 2), with each school exceeding an estimated one thousand barramundi moving from the freshwater section of the Upper Boyne through the tidal sections to the mouth of the Boyne River and into the Harbour. Within a week of the initial overflow at Awoonga Dam, he “witnessed many new fresh and lively arrivals (escapee lake fish), detected with the use of high definition depth sounder in areas within the harbour up to 43 km from the dam wall” and reported high catches of barramundi in the Harbour up to 50 km north and south of the Boyne River in late January 2011. Anglers reported catching 12 to 15 barramundi averaging around 100 cm in just one to two hours of fishing. Many of the fish showed signs of physical damage to the jaw or head; some wounds were severe, although advanced healing was obvious.

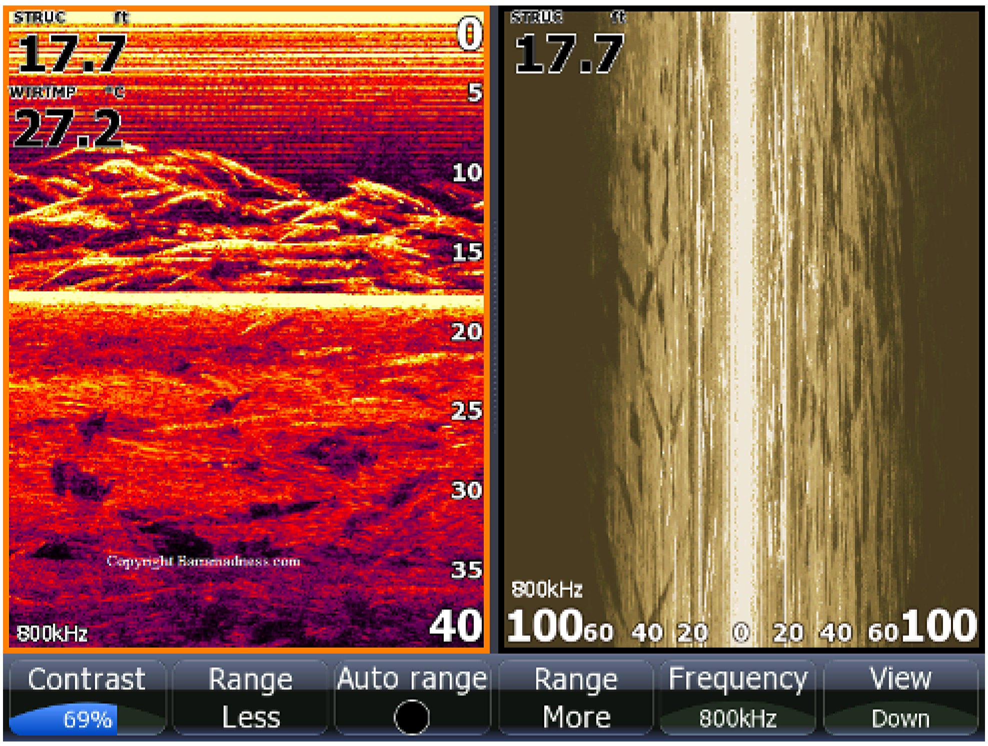

Figure 1. A school of large fish in the Boyne River detected on a fish finder during recreational fishing in 2011.

Mr Mitchell reported high catches until late April 2011 in the freshwater, tidal reaches of the Boyne River, Gladstone Harbour and extending into adjoining areas. It is evident that many of these fish migrated out of the Harbour as tagged Awoonga fish were reported in the Burnett River (Bundaberg) and Ross River (Townsville) (Tagged 9/2/2011 at Awoonga Spillway, recaptured 31/8/2011 at Ross River).

By April/May 2011, Mr Mitchell reported that the anal and tail fins were eroded and the fish were in thinner condition compared with the barramundi caught shortly after being washed over the spillway. In late July 2011, Mr Mitchell observed the first signs of eye problems and the “skinny fish, tattered fins and the loss of that glossy gleaming iconic barra glint” became more common and obvious. “The fish were duller and the body slime seemed more whitish rather than opaque.”

Sawynok, et al (2013) also document the change in recreational fishing in Gladstone after the 2010/11 flood. Information about fishing trips was obtained from boat ramp surveys and directly from recreational fishers and summarised seasonally for the period Autumn 2006 to Spring 2012. The influx of fish from Lake Awoonga changed the catch composition of fish reported by recreational fishers. Barramundi for the period 2006–10 represented only 3.1% of the catch. This changed to 28.4% for the period 2010–12. In the year 2010/11, barramundi represented more than 50% of the fish caught.

Phase 1: Fish Health Survey – Gladstone Harbour (August 2011–February 2012)

Overview

The first fish samples were provided to Biosecurity Queensland from August 2011. In late September 2011, Fisheries Queensland commenced monitoring fish health in Gladstone Harbour. The monitoring was based on a visual examination of fish and crustaceans. The initial monitoring focused on understanding the distribution of fish within the Harbour with external symptoms and the severity of those symptoms.

A number of specific species were monitored in more detail after reports of high levels of abnormalities were received from recreational and commercial fishers. These included trawl species (banana prawns and trawled fish), mud crabs, sharks, scallops and barramundi.

Field staff were given training on the collection of biological samples (skin swabs, tissue samples from various organs, collection of observed parasites), and photographed abnormalities as reference material. After the initial monitoring trips in September and October 2011, the definitions of skill discolouration, eye condition and lesion categories were refined and used in the rest of Phase 1 and in Phase 2 (Appendix A). After the initial monitoring, Fisheries Queensland established a routine monthly fish health monitoring program.

Study area

The principal study area was Gladstone Harbour and adjacent waterways, encompassing the areas closed to fishing during September 2011. Sampling took place at a range of sites within the principal study area to allow an understanding of fish presenting with signs of ill health and their potential movement within the principal study area. The sites sampled were the Narrows, Hamilton Point, Calliope River, Gladstone Harbour (trawl), spoil ground, Upper Boyne River, Lower Boyne River and Rodds Bay (Figure 1).

The main reference sites (i.e. for comparison with the principal study area) for the structured monitoring program included the nearby Fitzroy River to the north and waters adjacent to Bundaberg with its nearby coastal waters to the south. Both of these sites have various land use activities conducted adjacent to the waterways. These sites are useful as reference sites given their environments, fish communities and geographic location. It must be noted that not all species of finfish and crustaceans were sampled at each site because of differences in their distribution and the suitability of sampling methods in different areas. In Phase 1 only, occasional samples were also collected from within the principal study area but outside of the locations described in Figure 2.

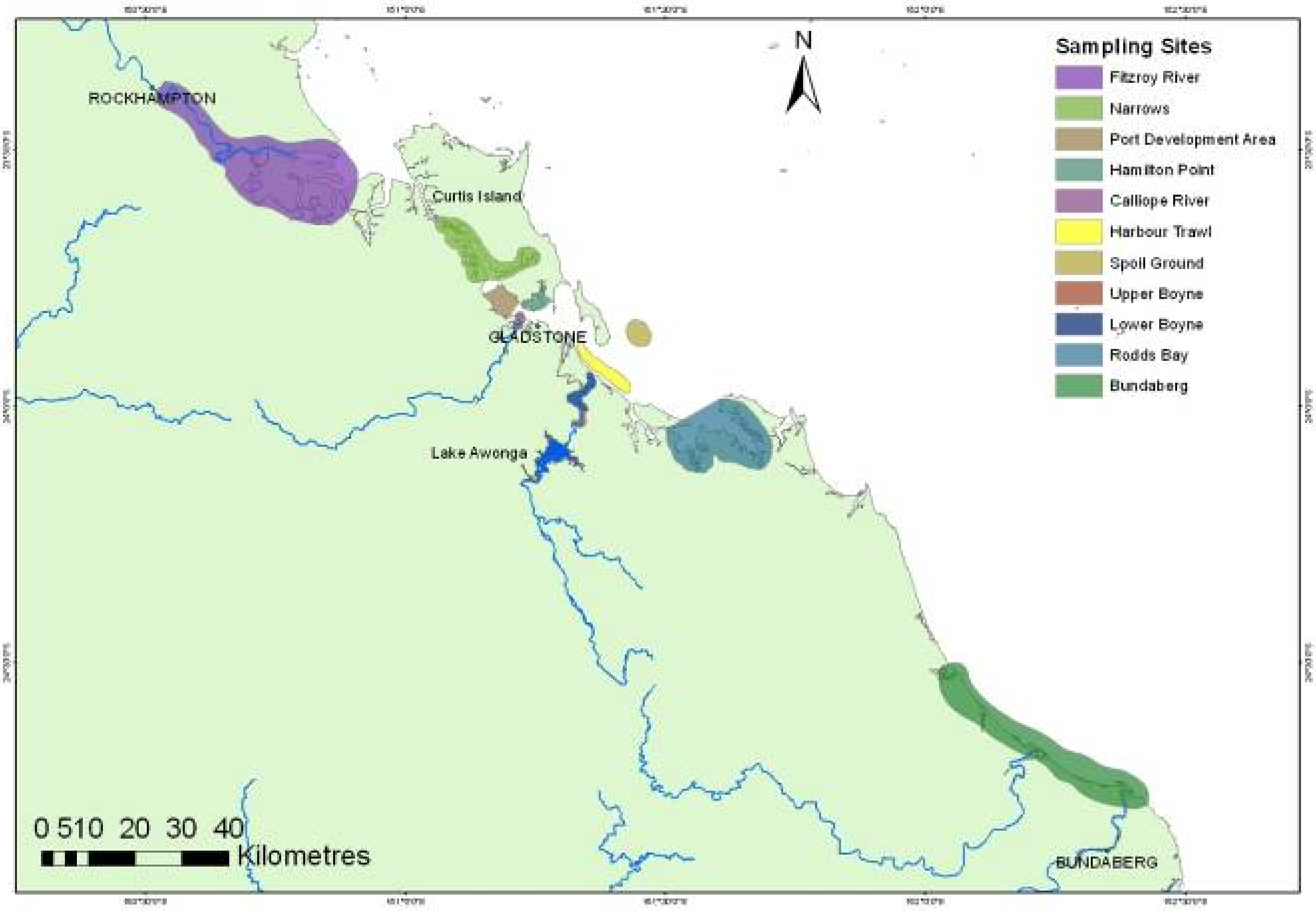

Figure 2. Sampling sites within the principal study area.

Candidate species

A range of species were sampled, and observations recorded in accordance with procedures in Appendix A. Most observations were done as part of normal fishing activities. Subject to limiting the impact on commercial fishing activities, all species caught were examined.

Sampling regime

Timing: Sampling commenced in September 2011 and continued until February 2012. All species caught during sampling events were assessed for signs of ill health.

Although Fisheries Queensland stopped sampling for Phase 1 in February 2012, the general public continued to provide samples for testing by Biosecurity Queensland up until April 2012.

Gear type: Multiple gear types were used during the sampling program depending on the site and fishing activities. The gear types were consistent with commercial fishing apparatus and included:

- gill net / haul net – finfish and sharks
- prawn trawl gear – to target banana prawn, demersal finfish
- crab pots – mud crabs
- electrofisher – fish species in freshwater.

Data collection and processing

Fisheries Queensland observers were placed on commercial fishing vessels during normal commercial fishing trips and asked to record information on gross signs of ill health observed in the catch. On occasion, commercial fishers were contracted to fish in designated locations when no normal commercial fishing activity was taking place at that time.

Fish were observed upon capture and assessed visually for signs of ill health. Data collected included an assessment of skin discolouration, eye condition, lesions and presence of ectoparasites.

Prawns were assessed visually for signs of ill health including shell erosion and the presence of parasites. Crabs were assessed for shell abnormalities. Shell lesions were graded according to the methods described by Andersen (2003).

When fish or crustaceans showed signs of ill health, samples were collected (either as whole animals or tissue samples) and forwarded to Biosecurity Queensland for further histopathology investigations and residue testing.

Some samples were submitted to Biosecurity Queensland by members of the public.

Phase 2: Expanded Gladstone Fish Health Survey (March 2012–September 2012)

Overview

Based on the Independent Scientific Advisory Panel's recommendations, the Queensland Government conducted an expanded integrated program to understand the causes of fish health issues in Gladstone Harbour through further monitoring and research.

The objectives of the expanded Gladstone fish health sampling program were:

- To continue monitoring the fish health in Gladstone Harbour and the surrounding waterways so that Fisheries Queensland had 12 months of data to account for seasonal influences.
- To determine the health status of fish and crustacean species in the Gladstone Harbour and adjacent waterways. For the purposes of this study, health status was defined as the observed prevalence and severity of significant infectious diseases and pathological lesions.
- To determine whether the health status of fish and crustacean species in Gladstone Harbour and adjacent waterways was significantly different to other areas along the central Queensland coast.
- To provide information for the conceptual model being developed by the Queensland Government to help narrow the range of possible causes for the observed health issues, and provide focus for further investigations.

The Extended Gladstone Fish Health Survey (Phase 2) continued and improved on the fish health investigations conducted by Fisheries Queensland during Phase 1 in the Gladstone Harbour, and adjacent waterways and reference sites. Phase 1 sampling events had provided important information for a range of health issues being displayed by fish and crustacean species, in particular the relationship between parasite burden (*Neobenedenia sp*.) and skin discolouration in barramundi. This information was used to guide the structure of the extended sampling program (see *Fish health survey report 1 March 2012*, available from www.qld.gov.au/gladstoneharbour).

A more intensive sampling program was required to better understand the variation in temporal (seasonal) and spatial prevalence of symptoms displayed in fish and crustacean species. Phase 1 sampling provided important information on the health status of a number of fish and crustacean species during late spring and early summer. The extended sampling program was completed by 30 September 2012 and, when combined with results from previous investigations, provided Fisheries Queensland with observations for a 12-month period from both within and outside Gladstone Harbour.

The intensive and structured nature of the sampling program has facilitated more robust statistical analysis of results than was possible in Phase 1. It has also provided a reference point for any future monitoring that considers longer-term temporal variation (e.g. annual) in health status of fish and crustacean species in Gladstone Harbour and adjacent waterways.

Conceptual model

A conceptual model was developed to document and graphically display the potential factors that may influence the health of fish and crabs in the waterways around Gladstone. These factors and pathways were documented and assessed by reviewing scientific literature and from advice from a panel of experts. Next, the likelihood that they were affecting (positively or negatively) the health of fish and crabs was considered. Development of the model considered the impact on fish health of changes to the ecosystem, such as changes to the food web and competition among predators, as well as the introduction of toxicants that could directly affect fish health.

The model allows for different hypotheses (e.g. competition for food versus effects of turbidity) to be visualised and their likelihood to be compared. It can also be used to identify which steps in the pathway can be monitored to determine whether or not the particular pathway is actually affecting fish health. For example, to test the 'competition for food' pathway, the abundance of prey fish should be assessed and compared to other systems.

The model was never intended to be a quantitative tool with the sophistication to predict the health of fish given various levels of driving factors. It can, however, be utilised to explain the thinking and justification for the likely pathways affecting fish health and help improve the understanding of the ecological processes that potentially result in a reduction in the health of fish in Gladstone waterways.

The conceptual model indicated that water quality, including turbidity, was unlikely to be the primary contributing cause for the fish health issues observed in Gladstone Harbour and adjacent waterways.

Study area

The study area during Phase 2 was the same as the study area described for Phase 1, with sampling sites illustrated in Figure 2.

Candidate species

The monitoring program focused on seven species of finfish, one species of shark, one species of prawn, and one species of crab (species listed below). These species represent a range of different life cycles (e.g. catadromous and estuarine) and trophic levels (e.g. predatory, omnivorous detritivores and scavengers). They are species that were encountered during Phase 1 sampling in the principal study area and reference areas, and had displayed a variety of abnormalities.

Barramundi (*Lates calcarifer*): Barramundi is a predatory species and a principle target in the region for recreational line and commercial net fishers. It is catadromous (live in fresh and salt water), but must migrate to salt waters to spawn. Barramundi fingerlings are stocked into freshwater impoundments throughout the Port Curtis and Fitzroy River catchments, including Awoonga Dam.

Mullet (*Mugil sp.*): Mullet is an omnivorous detritivore with a catadromous lifecycle. This species is caught mainly by commercial net fishers, although smaller numbers are also caught by recreational fishers (mainly for bait) using cast nets. Sea mullet have been stocked into Lake Awoonga.

Banana prawn (*Fenneropenaeus merguiensis*): Banana prawns are omnivorous detritivores and a principle target for the commercial trawl fishery in the region, as well as for recreational fishers using cast nets. Banana prawns use the numerous intertidal mangrove-lined creeks as nursery habitats, then move into waters including Gladstone Harbour as they grow.

Mud crab (*Scylla serrata*): Mud crab is the principle target species for recreational and commercial crabbers in the region. The species is an active omnivorous scavenger that occurs in estuarine and coastal habitats with mud substratum.

Bull shark (*Carcharhinus leucas*): Bull shark is a predatory estuarine and coastal species caught frequently in the region by commercial net fishers and recreational line fishers. They are known to inhabit freshwaters, particularly as juveniles.

Trawl fish species – Grinner (*Saurida* sp), Australian threadfin (*Polydactylus multiradiatus*) and Castelnau's herring (*Herklotsichthys castelnaui*): These three taxa are small in size and common in the local demersal fish assemblages, which makes them common in bycatch of trawlers operating inside the study area. They are caught occasionally by recreational anglers.

Pelagic species - Queenfish (*Scomberoides sp.*): Queenfish is a pelagic fish occurring throughout the region and commonly caught in commercial nets.

Sampling regime

Timing: The expanded sampling regime commenced in April 2012 and was repeated in June/July 2012.

A third sampling event was scheduled for September 2012, however observations made between April and July 2012 indicated that fish health was improving compared to observations made in 2011. Therefore, sampling in September 2012 was refocused on investigating whether *Neobenedenia sp.* identified as affecting barramundi in the Boyne River in October 2011 reoccurred in the Boyne River at a similar time in 2012.

Gear type: Multiple gear types were used during the sampling program depending on site and candidate species. The gear types are consistent with commercial fishing apparatus and include:

- gill net / haul net – barramundi, sea mullet, bull sharks and queenfish
- prawn trawl gear – banana prawn, grinner, Australian threadfin, Castelnau's herring
- crab pots – mud crab
- electrofisher – fish species in freshwater.

Data collection and processing

Fisheries Queensland contracted commercial fishers to sample at designated sampling locations using appropriate fishing apparatus to catch candidate species (as described above). Exceptions to this included barramundi collected from Lake Awoonga using an electrofisher, and some mud crab/finfish sampling that involved Fisheries Queensland staff accompanying commercial fishers during normal commercial fishing activities.

Observations in accordance with procedures in Appendix A were made of all fish and crustaceans at the time of capture by Fisheries Queensland staff. A sub-sample of the catch was forwarded to the lab where observations were made by Biosecurity Queensland staff. The samples underwent necropsy examination, and tissue samples were collected for further histopathology investigations and chemical residue testing. The sub-sample selected for further testing included individuals with and without obvious abnormalities. Individuals without obvious abnormalities were included to ensure that health conditions not apparent by external observation alone could be detected during further laboratory investigations.

Statistical analysis was performed to determine significant relationships and trends in the field and necropsy data, histopathology data and chemical residue data (Appendices B, C and D respectively).

Field observations by Fisheries Queensland: Fish were observed upon capture and assessed visually for external signs of ill health. Data collected included an assessment of skin discolouration, eye condition, lesions and presence of ecto-parasites. For the purpose of this data collection, skin lesions were distinguished from skin discoloration by observable signs other than colour change, such as missing scales, ulceration or bleeding (see Appendix A).

Prawns were assessed visually for signs of ill health including shell erosion/lesions and the presence of parasites. Crabs were assessed for shell abnormalities. Shell lesions were graded according to the methods described by Andersen (2001).

Fish collected using trawl methods (prawns, herring, grinner, Australian threadfin) were necropsied while onboard the vessel by a veterinarian, and tissue samples were collected for further histopathology investigations and residue testing.

Laboratory observations by Biosecurity Queensland: Fish were assessed visually for signs of ill health once received in the lab by a veterinarian. Data collected included, but was not limited to, an assessment of skin discolouration, eye condition, lesions and presence of ecto-parasites. Observations of internal organs were made during necropsy, and tissue samples were collected for further histopathology investigations and residue testing.

Crabs were assessed for external shell abnormalities once received in the lab by an aquatic veterinary officer. Shell lesions were graded and location on the carapace recorded according to the methods described by Andersen (2003). Further observations were made of internal organs during necropsy and, in order to allow a statistical comparison of the fish from different sites, discrete categories were assigned to capture these observations based on Adams et al. (1993). Chemical residue test results were interpreted by the Department of Science, Innovation Technology, Innovation and the Arts. For a detailed description of the sampling methods, refer to Appendix A.

Histopathology and chemical residue testing

A subset of individuals were selected for histopathology examination and chemical residue testing (Table 1).

For barramundi, gills, liver and skin (with muscle) were chosen for microscopic investigation, due to their known sensitivity as indicators of environmental stress. Specifically, the following abnormalities were rated from zero to three to enable quantitative comparison between sites and time points: gill hyperplasia; gill parasites; liver melanomacrophage centres; skin lesions; and muscle lesions.

Barramundi gills and liver were used for chemical residue testing, as they were deemed the most likely tissues to show evidence of short to medium term exposure to contamination. Since there were no significant findings in organic testing of barramundi tissues during Phase 1, only metals were tested in Phase 2.

For mud crabs, hepatopancreas, gills and muscle were examined histopathologically. Hepatopancreas tissue was also used for chemical residue testing, which included testing for metals and organic contaminants.

Grinner were chosen due to their benthic and relatively immobile nature, size (ease / feasibility of processing) and previous evidence provided by members of the public of red / sick grinner from Gladstone Harbour. Since no grinner were caught from Bundaberg during April, a total of 25 fish were tested. Gill, liver and muscle were used for histopathology examination. For chemical residue testing, only muscle tissue could be sampled in sufficient volume due to the small size of these fish.

Since insufficient numbers of sharks were caught for a balanced statistical analysis, they were used for histopathology only.

Chemical residue test results were interpreted by the Department of Science, Information Technology, Innovation and the Arts.

Table 1. Number of fish from each site and sampling trip used for histopathology and chemical residue testing. Note: [1] Denotes Gladstone Harbour and adjacent waterways, [2] denotes reference sites. Note due to low sample numbers, sharks were used for histology only.

Site	Species collected per trip 2012						
	Shark	Mud crab		Barramundi		Grinner	
	April/ May	April/ May	June/ July	April/ May	June/ July	April/ May	June/ July
Lake Awoonga				5			
Upper Boyne River[1]				5	5		
Port Dev't Area[1]		5	5				
Hamilton Point[1]	1			5	5		
The Narrows[1]		5	5				
Gladstone Trawl[1]						5	5
Fitzroy River[2]	5	5	5	5	5	5	5
Bundaberg[2]	1	5	5	5	5		5
Calliope River[1]	3						

Summary of samples collected

During the course of fish health investigations conducted by Fisheries Queensland in Gladstone Harbour and surrounding waterways, approximately 9000 fish, crustaceans and molluscs were sampled. Observations were made across a wide range of species and are summarised in Table 2. Samples provided to Biosecurity Queensland for further testing have been summarised in Tables 3 and 4. Biosecurity numbers include some samples provided by sources other than Fisheries Queensland during Phase 1 (Table 3).

Table 2. The number of fish, crustaceans and molluscs observed during Phase 1 and 2 of the Gladstone Fish Health Investigation. Note * indicates multiple species included in category

Fish species (taxonomic group eg. Family or *Genus* or *species*)	Phase 1	Phase 2	Total
Anchovies * (Engraulidae)	355		355
Australian threadfin * (*Polydactylus* spp.)	361	545	906
Barramundi (*Lates calcarifer*)	281	216	497
Batfish * (Ephippidae, Drepaneidae)	3	1	4
Beach salmon (*Leptobrama muelleri*)	2		2
Black jew (*Protonibea diacanthus*)	1		1
Black pomfret (*Parastromateus niger*)	3		3
Blue threadfin (*Eleutheronema tetradactylum*)	51	1	52
Bony bream (*Nematalosa erebi*)	42		42
Bream * (*Acanthopagrus spp.*)	46		46
Butter bream (*Monodactylus argenteus*)	2		2
Catfish (Ariidae)	129		129
Cod/Groupers * (*Epinephelus spp.*)	17	1	18
Flathead * (*Platycephalus spp.*)	9	1	10
Mackerels and Bonitos* (Scombridae)		7	7
Grinner * (Bathysauridae, Synodontidae)	80	179	259
Herring * (Clupeidae, Pristigasteridae, Elopidae)	72	346	418
Javelin fish * (*Pomadasys spp.*)	35		35
King threadfin (*Polydactylus macrochir*)	21		21
Milk fish (*Chanos chanos*)	1		1
Mullet * (Mugilidae)	149	125	274
Ponyfish * (Leiognathidae)	366		366
Queenfish * (*Scomberoides* spp.)	89	71	160
River jew *(*Johnius spp.*)	419		419
Scad * (Carangidae)	18		18
Scats * (Scatophagidae)	10		10
Sharks and rays * (multiple Families)	227	26	253
Silverbiddies (Gerreidae)	24		24
Snappers (Lutjanidae) *	2		2
Snubnose dart (*Trachinotus blochii*)	42		42
Sole * (Soleidae, Cynoglossidae)	1		1
Sweetlips and emperors* (Haemulidae, Lethrinidae)	3	2	5
Trevally * (Carangidae)	49	5	54
Tripletail (Lobotidae)	3		3
Whiting * (*Sillago spp*)	303		303
Total	**3211**	**1526**	**4737**

Crustaceans and molluscs	Phase 1	Phase 2	Total
Moreton Bay bug * (*Thenus spp.*)	1		1
Scallops (Pectinidae)	23		23
Banana prawns *(Fenneropenaeus merguiensis)*	266 (+ an additional 85kg not quantified)	574	840
Other prawns * (Penaeidae)	294		294
Crabs - Not mud crabs * (Portunidae)	5		5
Mud crabs (*Sylla serrata*)	1435	1599	3034
Total	**2024 + (+ = 85kg banana prawns)**	**2173**	**4197+**

Table 3. Numbers of samples tested by Biosecurity Queensland during Phase 1.

Fish Species	Necropsy	Histopathology	Microbiology	Residue	Held frozen
Barramundi (*Lates calcarifer*)	33	69	30	6	27
Black jew (*Protonibea diacanthus*)	0	1	1	0	0
Bony bream (*Nematalosa erebi*)	1	1	0	0	1
Catfish (Ariidae)	9	6	6	6	0
Flathead (*Platycephalus sp.*)	0	0	0	0	2
Grinner (Bathysauridae, Synodontidae)	1	1	0	0	1
Mullet (Mugilidae)	2	2	3	4	2
Queenfish (*Scomberoides* spp.)	2	2	2	0	2
River jew (*Johnius spp.*)	7	7	3	7	0
Scats (Scatophagidae)	2	2	0	0	1
Spangled emperor (*Lethrinus nebulosus*)	1	1	1	0	1
Cod/Groupers (*Epinephelus sp.*)	1	1	1	0	0
Threadfin (Polynemidae)	2	2	0	0	2
Trevally (Carangidae)	2	2	0	0	1
Whiting (*Sillago spp*)	1	6	1	4	1
Sharks (multiple families)	5	21	21	0	2
Total	**69**	**124**	**69**	**27**	**43**
Crustaceans and molluscs	**Necropsy**	**Histopathology**	**Microbiology**	**Residue**	**Held frozen**
Crabs (Portunidae)	9	8	8	0	2
Moreton Bay bug (*Thenus sp.*)	1	0	0	0	3
Prawns (Penaeidae)	2	11	2	7	1
Scallops (Pectinidae)	0	7	0	63	0
Total	**12**	**26**	**10**	**70**	**6**

Table 4. Numbers of samples used for necropsy examination during Phase 2.

Location	Barramundi	Bull shark	Mullet	Queenfish	Other pelagic	Mud crab	Total
Bundaberg	17	1	20	20		21	**79**
Fitzroy River	20	7	21	11	2	21	**82**
Calliope River	20	3	17			18	**58**
Hamilton Point	20	1				20	**41**
Lower Boyne	20		20	1			**41**
Rodds Bay	9		20			20	**49**
Spoil Ground				5	15		**20**
Upper Boyne	16		20				**36**
Narrows						20	**20**
Port Dev't Area						21	**21**
Lake Awoonga	5						**5**
Total	**127**	**12**	**118**	**37**	**17**	**141**	**452**

Significant findings and discussion

Analysis of all species combined

The structured sampling in Phase 2 allowed a statistical analysis to be done on field observations and necropsy data from all species combined (Appendix B). Overall, location had a significant effect on most variables, and for many variables there was a significant interaction ($P < 0.05$) between location and trip, indicating a changing pattern through space and time.

Health assessment index (HAI) was calculated based on the methods of Adams et al. (1993), as a coarse measure of overall health, based on combined scores from a range of internal and external observations made during necropsy. Higher scores indicated a higher prevalence and/or severity of abnormalities. In Trip 1, average HAI was highest in Bundaberg (32.8), followed by the Lower (32.7) and Upper Boyne (30.9) respectively. In Trip 2, the Upper Boyne had by far the highest HAI score (38.8) followed by Lower Boyne (28.2) and Bundaberg (24.6). There was no significant difference between pooled Gladstone sites[2] and pooled reference sites for HAI in either Trip 1 or 2.

In Trip 1, external observations and measurements showed that Gladstone fish had significantly lower condition factor (weight$\times10^5$/Length^3) and higher proportion of tucked abdomen (poor condition) than fish from the reference sites ($P \leq 0.05$). Internal observations support this finding with a significantly lower proportion of mesentery fat in fish from Gladstone. In particular, fish from Hamilton Point had the lowest condition factor and lowest levels of mesentery fat in both trips, while Bundaberg fish had the highest condition factors in both trips.

The estimated annual growth rate (2011) of barramundi in Gladstone (Boyne River 44.0±50.5mm and Calliope River 49.1±40.0m) compared with the Fitzroy River (169.8±67.6mm) (Sawynok, et al 2013) for barramundi greater than 650 mm could indicate limited food supply that results in poor condition as found in Trip 1.

In Trip 2, the proportion of fish with parasites identified during necropsy was significantly greater in fish from Gladstone ($P < 0.05$), with the highest level observed in barramundi from Hamilton Point.

Prevalence of lesions and fin abnormalities was significantly higher in Gladstone than in the reference sites for both trips ($P < 0.05$). The lesions in particular can be largely explained by the high numbers of barramundi with lesions observed from the Boyne River during both trips during Phase 2.

Externally visible health ‘status’ was assessed in the field for every individual fish. Necropsy data were tested for differences between apparently normal and abnormal fish. Liver abnormalities were significantly higher in apparently abnormal fish ($P < 0.05$). However, despite significant differences in lesions and fin abnormalities between Gladstone and reference sites, the only significant difference in livers between Gladstone and reference sites was in Trip 2 when liver colour abnormalities in fish from Gladstone were significantly less prevalent than at the reference sites.

[2] Gladstone sites refers to Gladstone Harbour and adjacent waterways including the Boyne and Calliope Rivers and the Narrows.

There were significant differences between the two reference sites for most conditions in Trip 1, Trip 2 or both ($P < 0.05$). Some differences would be expected due to the difference in latitude and habitat structure. Reference sites were chosen to the north and south of Gladstone to balance these effects.

Skin condition: Skin redness has been reported and investigated in a wide range of fish species including sharks. Pathology investigation of skin redness in Phase 1 showed inflammation, erosion, scarring, bleeding, cell death and swelling, but no causative agent was identified.

Skin redness was further investigated in Phase 2. The broad range of time taken to process samples allowed changes in observed skin redness over time to be documented. Fish were photographed in the field as they were caught, and again in the laboratory as part of the necropsy examination. Fish were stored in plastic bags on ice, and took between zero and 17 hours to process. Data showed that skin condition and fin condition ratings were affected by time since death ($P < 0.05$), and that there was a significant interaction with fish species. This means that changes in skin condition through time are different according to species. In both barramundi and mullet, the proportion of fish with a skin condition other than normal increased over time ($P = 0.010$ and 0.056, respectively). There was no significant difference between the relationships observed in Gladstone and reference sites. This indicates that the reddening of fish over time is not a phenomenon specific to Gladstone Harbour.

Not enough bull sharks were caught for the above statistical analysis, although the limited evidence did suggest a similar pattern. Photographic evidence allowed for visible comparison of the fish over time, and showed that skin redness developed in some sharks and other fish post mortem (Figure 38).

Barramundi

Commercial catch

Between 2000 and 2010, the reported commercial catch of barramundi in the entire Gladstone region (S30) ranged between 3.97 t (2009) and 16.77 t (2005) with an annual average of 10.8 t. In 2011, the influx of barramundi from Lake Awoonga into the system saw the commercial catch rise to an unprecedented high of 248.31 t (Figure 3). Of this total, 130 t was captured between July and September 2011. Logbook data supplied by commercial fishers for 2012 shows that the recorded catch returned to historical levels.

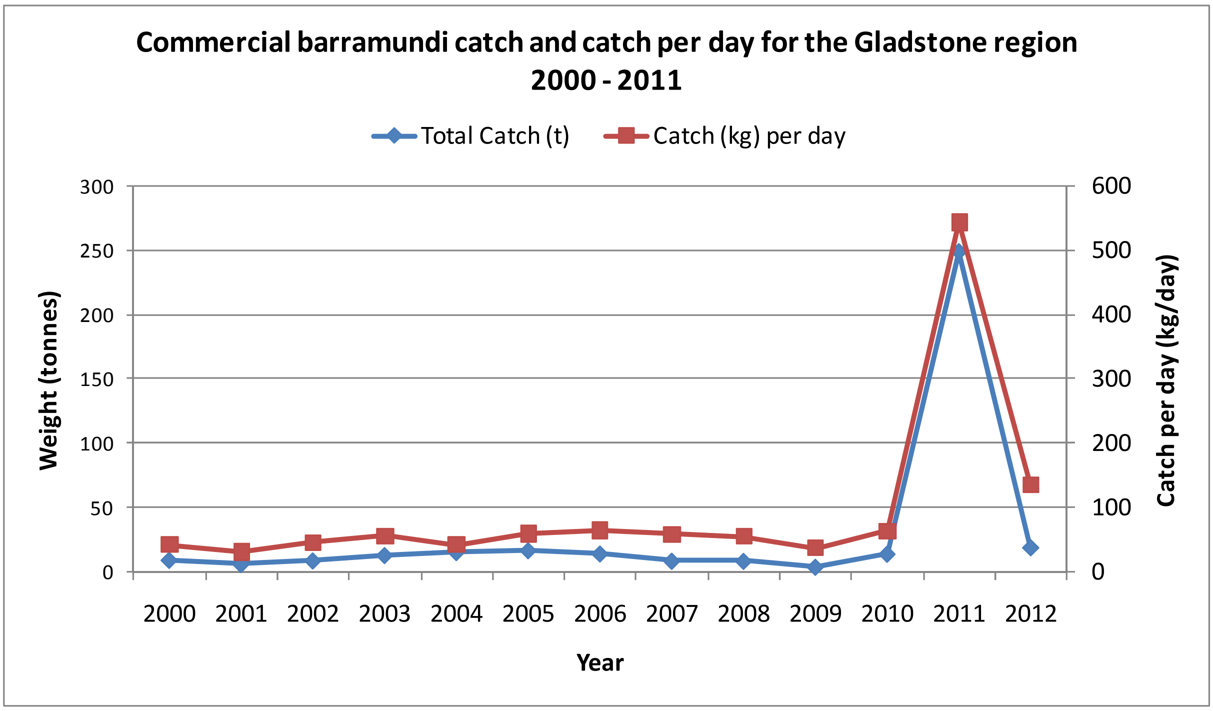

Figure 3. The annual catch and catch per unit effort of barramundi from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2012).

Commercial fishing effort for barramundi in the Gladstone region (days fished with barramundi catch recorded) increased between 2000 and 2004, peaking at 370 days. After 2004, effort dropped steadily to a low of 108 days in 2009 with a rise in 2010 to 223 days. Following the introduction of fish from Lake Awoonga into the Boyne River in 2011, effort increased to a record high of 457 days (Figure 4). Although catches of barramundi for 2012 returned to historical levels, the fishing effort expended (141 days) was the second lowest number of days resulting in the second highest recorded catch rate (135kg/day).

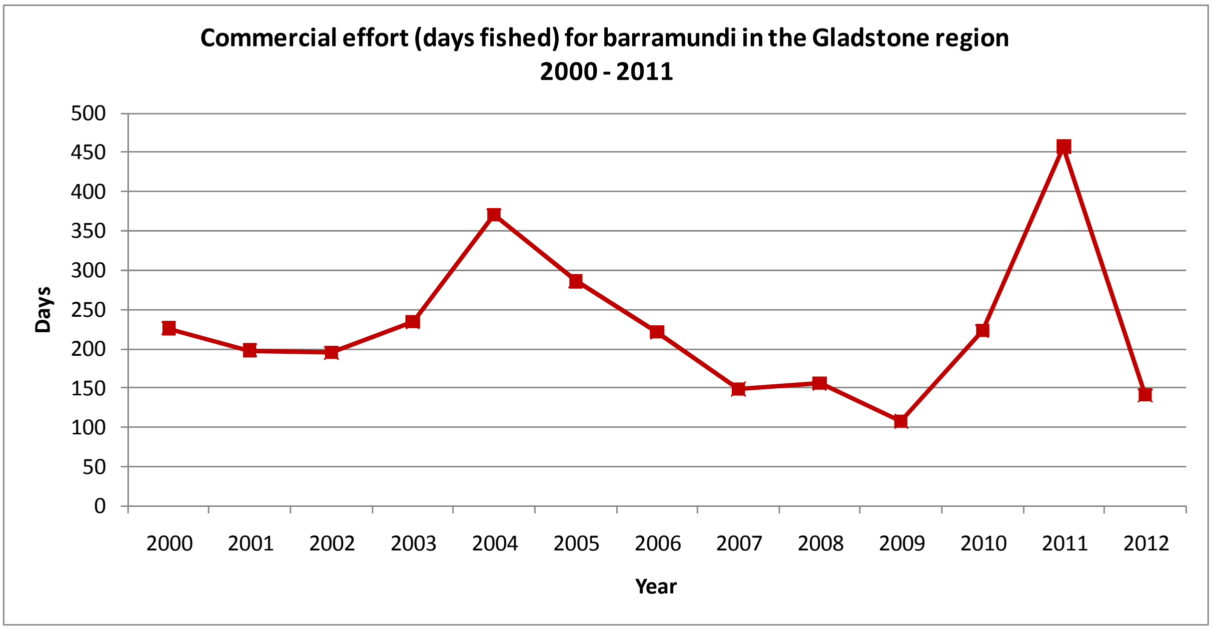

Figure 4. The number of days fishing where commercial fishers have reported barramundi from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2012).

Field observational Phase 1

During Phase 1 of the fish health investigations in Gladstone, 267 barramundi were observed and their conditions recorded. This figure included fish observed at the reference sites, but excluded those sampled during a targeted *Neobenedenia* survey in the Boyne River, which will be discussed separately.

While the deep ulcerative lesions seen on barramundi prior to the commencement of sampling in September 2011 were not detected, a number of barramundi were observed to have lesions comprising areas of detached scales, varying skin discoloration as well as eye abnormalities. The results of observations are summarised in Figures 5 and 6.

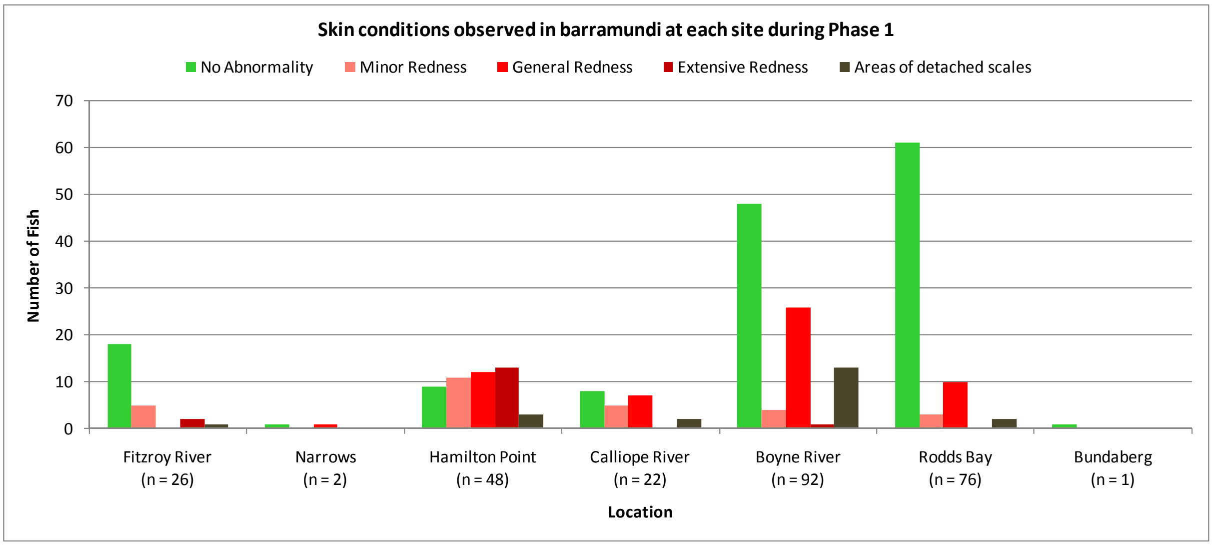

Figure 5. Skin condition observed in barramundi at each site sampled during Phase 1 of fish health investigations, September 2011 – February 2012. Note, (n) refers to the total number of fish examined at each site.

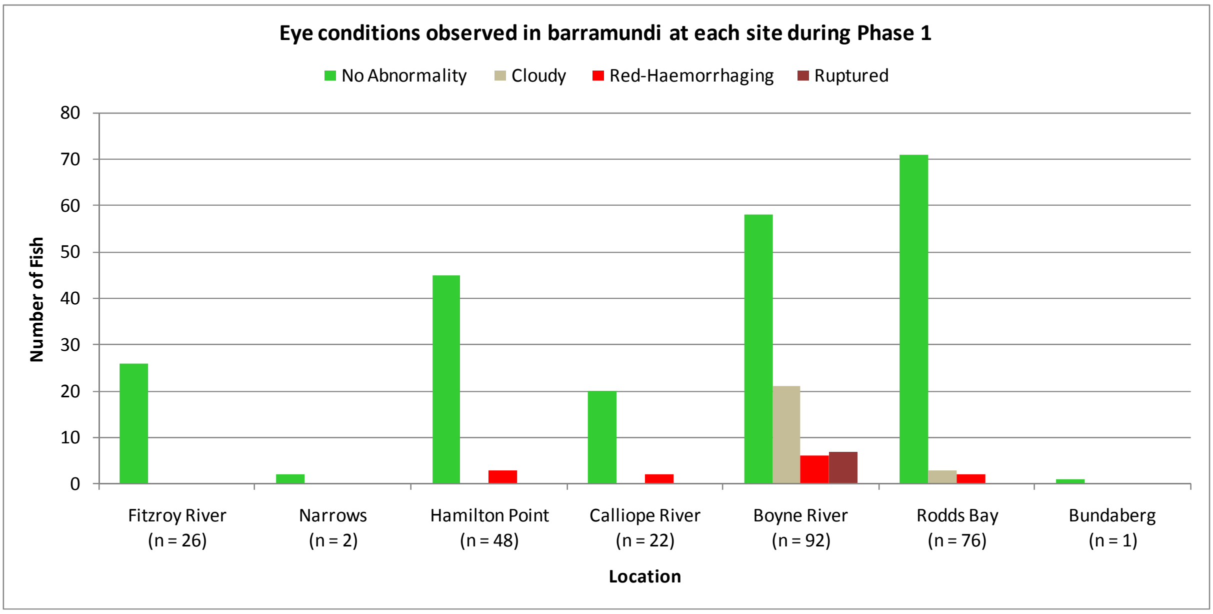

Figure 6. Eye condition observed in barramundi at each site sampled during Phase 1 of fish health investigations, September 2011 – February 2012. Note, (n) refers to the total number of fish examined at each site.

Laboratory findings Phase 1

The majority of the barramundi submitted for necropsy, including fish submitted prior to 16 September 2011, showed eye lesions ranging in severity from inflammation to ruptured eyes. Severe skin lesions and abnormalities including generalised redness were also observed.

Copepods (small crustaceans) were found on the gills of some barramundi in low abundance. Copepods are not considered to be a serious pathogen at the observed level of abundance.

A number of barramundi showed a high number and intensity of melanomacrophage centres (MMCs) in organs (e.g. liver, spleen and kidney). An increase in MMCs is a common histopathological indicator of a range of factors or stressors including poor water quality, starvation, disease or ageing. The general function of MMCs is the focalisation, destruction, detoxification and recycling of internal and external materials. An increase in MMCs is generally likely to be the result of these catabolic physiological processes in the fish, commonly a negative nutritional balance (Agius & Roberts, 1981)

There was no detectable sediment in the gills of the barramundi samples except for two submissions—see P11-76883 (Boyne River) and P11-76885 (Hamilton Point) in reports on www.qld.gov.au/gladstoneharbour

Parasitic flatworm infection – Pathological examination of barramundi by Biosecurity Queensland identified a parasitic flatworm (*Neobenedenia sp.*) as the most likely causative agent of the eye abnormality in the barramundi. Bacteria were excluded as the cause of the lesions. Flatworms can cause eye injuries by way of feeding and attachment over the surface of the cornea. In eyes that are cloudy, eroded, inflamed, swollen or where there is evidence of haemorrhage, vision is likely to be reduced. In severe cases where the eye has been ruptured, this will cause blindness.

The parasite moves over the surface area of the eye and the skin of the entire fish. A number of barramundi showed hyperaemic (reddened) areas on the skin surface, which is consistent with skin damage caused by the parasite feeding on mucous and skin cells on the skin surface. This has been the first report of a significant outbreak of *Neobenedenia sp.* in wild barramundi.

Skin lesions and abnormalities – The causes of the deep, ulcerative lesions in barramundi collected in the early stages of the investigation from Gladstone Harbour were not able to be determined. Specific histopathology testing showed that Epizootic Ulcerative Syndrome (EUS), which is caused by a fungus, was not present in the samples collected from Gladstone Harbour, therefore EUS has been ruled out as the cause of these lesions. Only two cases of EUS were found in testing: a barramundi taken in September 2011, which was collected from Port Alma (at the mouth of the Fitzroy River), and in a whiting from the Bundaberg region.

Chemical residue testing – Barramundi samples were taken from five locations – Port Alma, Lake Awoonga and Fitzroy River (reference sites), and China Bay and Boyne River (Gladstone Harbour sites). Testing was conducted on the gill, liver and muscles of each of the fish or pooled fish samples.

Samples were tested for more than 160 organic chemicals. Only two organic chemicals were detected in the fish samples analysed. These were p'p'-DDE and DEET. p'p'-DDE is a degradation product of the organochlorine pesticide DDT, which was a commonly used pesticide in the agricultural sector, but has not been permitted in Australia for more than 20 years. The samples were from barramundi from China Bay and Turkey Beach in Gladstone. DEET is a very commonly used ingredient in personal insect repellents. Given that it was found in only one sample, it is possible that this fish sample may have been contaminated.

Concentration of metals, aluminium, barium, copper and zinc were slightly higher in the fish tissue from the reference sites than Gladstone Harbour sites. Cadmium, nickel, selenium and silver were very similar among sites. Iron and arsenic were present at higher levels in the Gladstone barramundi population. Further details can be found at www.qld.gov.au/gladstoneharbour

Observational findings Phase 2

April 2012: A total of 111 barramundi were collected from all planned netting sites. No *Neobenedenia sp.* or eye abnormalities were observed on fish collected from any of the sampling locations. In the Boyne River, a high prevalence (50-75%) of physical damage was observed on the barramundi caught. Conditions described as physical damage appeared as graze–type injuries and included large areas of scale loss often on one side of the body, operculum damage and some mandible fractures. Physical damage (Figure 7) was attributed to fish passing over the Awoonga spillway earlier this year (Awoonga overtopped in late January to early February and late March 2012). The observed damage is consistent with injuries seen in fish after being washed over spillways of other impoundments, and consistent with observations made by recreational anglers following the 2011 overtopping of Awoonga Dam.

Seven barramundi caught at the reference sites and six barramundi caught in the Gladstone area (Hamilton Point and Rodds Bay) showed varying levels of redness on the skin. This ranged from pinpoint marks to more general redness over areas of the body and fins. No other signs of ill health were observed, with most fish considered to be in good condition. Observations are summarised in Figure 8.

Seven barramundi were caught incidentally during trawl sampling in Gladstone Harbour (Figure 9). The commercial fisher involved in the sampling identified this as an unusual capture, which may indicate a larger number of barramundi than normal being present in Gladstone Harbour. No significant signs of ill health were observed on these barramundi.

Figure 7. Barramundi caught in the Boyne River showing physical damage consistent with being washed over the Awoonga spillway.

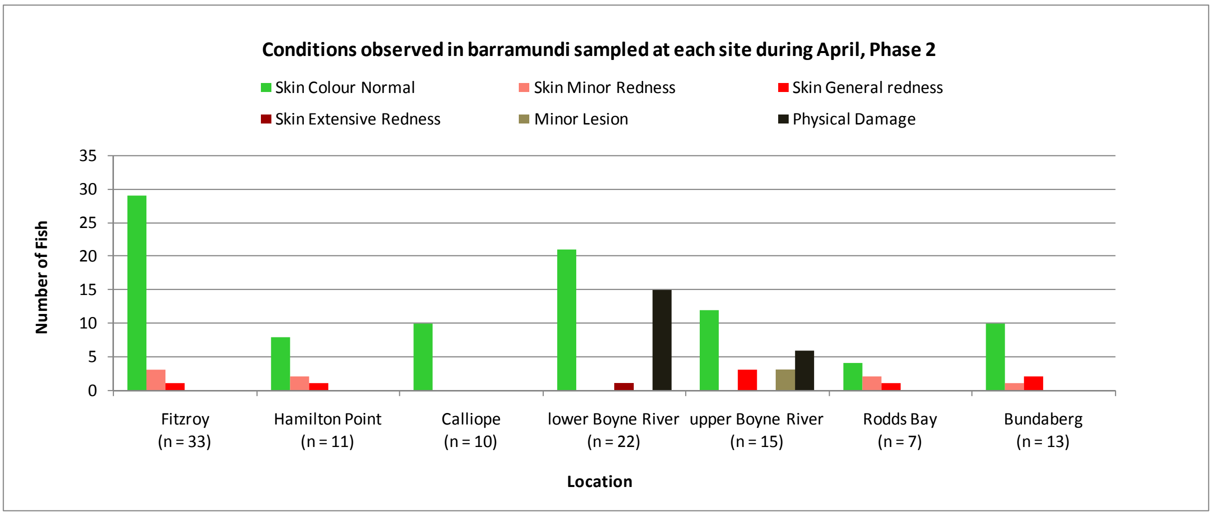

Figure 8. Conditions observed (skin colour and lesions) in barramundi sampled at each site during April 2012, Phase 2. Note, (n) refers to the total number of fish examined at each site. Some fish may have been observed with multiple conditions.

Figure 9. Barramundi caught while trawling in the Gladstone Harbour.

June/July 2012: A total of 93 barramundi were collected from all planned netting sites. No *Neobenedenia* or eye conditions were observed on barramundi at any of the sampling locations.

Most notable in the barramundi samples collected was the presence of two fish from the upper Boyne River showing recovering lesions. The lesions were well infiltrated with connective tissue, indicating recovery from injuries that have occurred at least one month ago, as described by the attending veterinarian. The recovering injuries observed in these fish were consistent with the fresh injuries observed on fish during the April/May sampling event in the Boyne River, which were attributed to fish passing over the Awoonga Dam spillway in late January to early April 2012 (see Figure 10).

Barramundi were also caught in the Calliope and Burnett Rivers with injuries in advanced stages of healing. New skin had completely covered the old injuries and new scales were forming over the affected areas (see Figures 11 and 12). Given the advanced stage of the healing, it is believed these injuries did not happen during 2012.

Most barramundi sampled in Gladstone in June/July were considered to be in good condition. The majority of skin discolouration or lesion conditions observed during June/July were from barramundi caught in the Fitzroy River and were represented by red pin point marks, individual scales detached or those attributed to physical injuries (Figure 13).

Figure 10. A barramundi caught in the Boyne River during the June/July 2012 sampling event with recovering injuries to the jaw and body.

Figure 11. A barramundi caught in the Burnett River, Bundaberg, during the June/July 2012 sampling event with an old injury in an advanced stage of healing. Note the new skin completely covering the old injury with new scales forming over the affected area.

Figure 12. A barramundi caught in the Calliope River during the June/July 2012 sampling event with an old injury in an advanced stage of healing. Note the new skin completely covering the old injury.

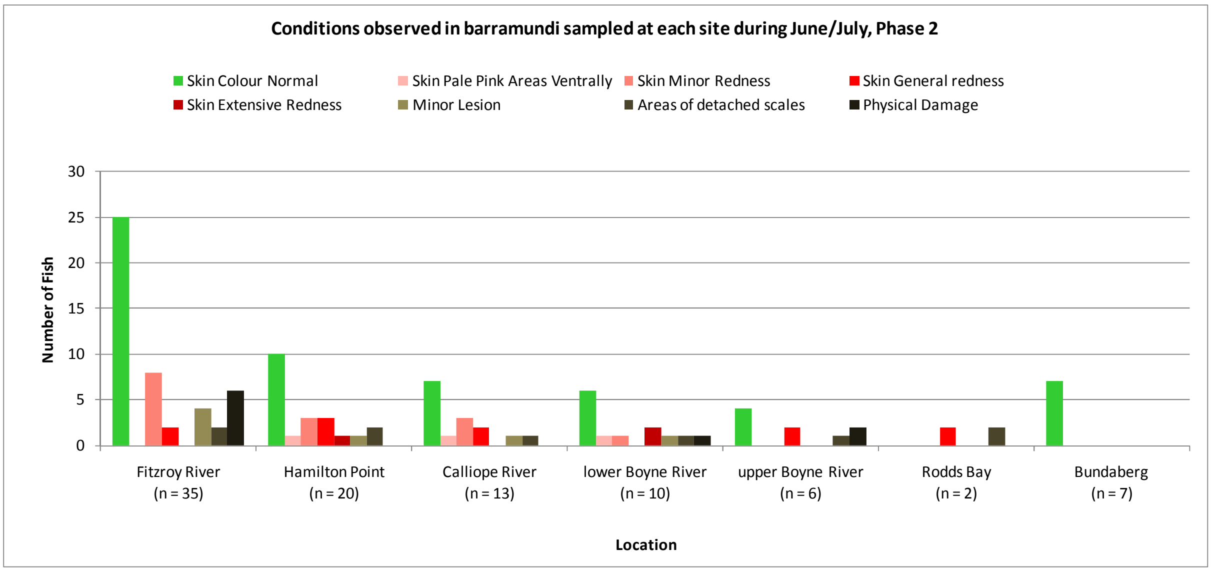

Figure 13. Conditions observed (skin colour and lesions) in barramundi sampled at each site during June/July, Phase 2. Note, (n) refers to the total number of fish examined at each site. Some fish may have been observed with multiple conditions.

Targeted barramundi surveys to examine *Neobenedenia sp.* infection

Targeted barramundi surveys in the Boyne River were undertaken by Fisheries Queensland in October 2011 and September 2012 to investigate parasitism by the monogenean *Neobenedenia sp. Neobenedenia* had been implicated as a causative agent for some of the observed conditions affecting barramundi during Phase 1 of the fish health investigation. *Neobenedenia* is known to exist naturally in the marine environment in the Gladstone area given previous occurrences of *Neobenedenia* on barramundi broodstock in the Gladstone Area Water Board fish hatchery (pers. comm. Kirt Hutchby, Hatchery Manager). *Neobenedenia* does not survive in freshwater.

Neobenedenia are known to cause disease and mortalities in aquaculture. Deveney et al. (2001) documented an outbreak of *Neobenedenia* that occurred in the Hinchinbrook Channel on barramundi cultured in sea cages where parasite burdens exceeded 400 specimens per fish, however these parasites have not previously been associated with documented fish health issues in wild populations.

In October 2011, approximately 28 barramundi were caught during sampling. Of these, 14 fish were retained for closer study. The remaining fish were tagged and released alive. These fish were tagged to determine upon recapture if they moved out of the Boyne River and if their health status changed.

Retained fish were selected based on their external appearance. The objective was to collect fish that exhibited the widest range of health conditions, including fish that appeared healthy, to determine if a relationship existed between parasite burden and signs of ill health.

Of the 14 retained fish, one had distinct skin lesions, 11 fish had cloudy, red, swollen or ruptured eyes, eight fish had general skin discolouration, while a further five showed minor skin discolouration. Samples of the gills and skin were taken from each of the 14 fish. Water samples were also collected at the sampling site.

Neobenedenia counts conducted on each of the retained fish ranged from none to 187 parasites per fish. The eye damage observed in many of these fish was consistent with documented *Neobenedenia* infestation. Although bacteria have been excluded as the cause of eye conditions observed, the severity of some eye conditions may have been further affected by opportunistic secondary bacterial infections.

There was no clear correlation between observed severity of skin conditions and parasite count, other than in the extreme cases where the healthiest fish caught had no *Neobenedenia* while the fish with 187 *Neobenedenia* had the most severe skin conditions.

During sampling in September 2012, only three barramundi were caught. All sampling was undertaken at the same locations and with the same sampling effort (two nights fishing with a commercial fisher) as in October 2011. All fish caught were observed to be in good health with no eye or skin conditions observed. One fish had an old wound on the caudal peduncle (tail) that was in an advanced stage of healing with new skin and scales present. All fish were retained for closer examination, however no *Neobenedenia* were found on these fish.

The *Neobenedenia* outbreak described by Deveney et al. (2001) in the Hinchinbrook Channel was thought to be caused by environmental stress (uncharacteristically low water temperatures of 19°C for three weeks) compromising the ability of barramundi to combat infestation. This stress, combined with the high stocking densities used in aquaculture environments, allowed the number of *Neobenedenia* to reach epidemic proportions quickly.

The density of barramundi in Gladstone was extremely high, as shown by catch rates. The average commercial catch of barramundi in Gladstone in 2011 was 543 kg / day and was recorded as 900 kg / day in August 2011, whereas the average catch rate for the period 2000-2010 was 50 kg / day for Gladstone (Figure 4). Based on these catch rates, the density of barramundi was 10 times higher than normal levels in 2011 and 18 times in August 2011.

There were a number of factors that would have been stressful to the barramundi in Gladstone, rendering them more susceptible to infestation. Although some of the stressors would have been different to the husbandry practices and seasonal conditions identified by Deveney et al. (2001), many are the same for barramundi in the Boyne River in the winter of 2011. The densities of Gladstone barramundi were more than 10 times and possibly up to 18 times higher than that of natural barramundi populations. Many of the fish had physical damage caused by being washed over the Awoonga Dam spillway. Food supply would have been limited due to the large number of large predators present for a lengthy period. The Boyne River recorded temperatures below 19°C for July and August 2011 from the mouth to 8.6 km upstream. The lowest temperature measured was 18.1°C at 4 m depth at the site

8.6km upstream from the mouth of the Boyne River. Finally, these cold water temperatures provided another environmental stress. Under these conditions, *Neobenedenia* populations were able to multiply, resulting in the symptoms observed.

The outbreak of *Neobenedenia* infection in Hinchinbrook cage culture resulted in the loss of an estimated 200 000 fish. All sick, dying and dead fish were removed from the cages and disposed of on land. Wild sick barramundi in Gladstone were not removed as would occur in an aquaculture operation, but remained in Gladstone waters. Although low numbers of barramundi were reported to have died in the second half of 2011, it is believed that most of the infected fish survived.

Given the severity and high frequency of health conditions observed in barramundi in October 2011, the fish in Gladstone Harbour and adjacent waterways would have showed signs of poor health for an extended period of time. This is what was observed for the remaining samples taken in 2011 by Fisheries Queensland. In 2012, Fisheries Queensland observed fish recovering from the loss of significant areas of scales (Figures 11 and 12).

As the water temperature increased, the *Neobenedenia* infection resolved in the population of barramundi in the Gladstone area. Barramundi from Gladstone Harbour and adjacent waterways sampled by Fisheries Queensland after October 2011 and reported by Landos (2012) were not identified as being infected with *Neobenedenia*.

Although more barramundi entered the Boyne after the Awoonga Dam spillway overtopped again in late January to early April 2012, it is thought that the number of barramundi that may have entered the system was less than during the flooding event of 2010-2011. *Neobenedenia* are not expected to cause further issues in wild barramundi in the area under normal environmental conditions.

Laboratory findings Phase 2

Necropsy

Necropsy examination of barramundi in both trips one and two found a significant association between field allocated disease status and liver abnormalities ($P < 0.05$) (Appendix B). Those fish that were allocated as diseased in the field (based on externally visible signs) were almost four times more likely to have liver form abnormalities than externally normal fish. It is not clear from this data whether the liver abnormalities are a consequence of the skin trauma or whether they are linked to another common factor, such as water quality or diet.

Field-based disease assessment was higher in fish from Gladstone than the reference sites. This was driven by the greater proportion of lesions in barramundi from the Boyne River in particular. Interestingly, observed liver abnormalities in fish from the Boyne River were only significantly higher than reference sites during Trip 2, despite significantly higher prevalence of lesions in both trips. In Trip 2 only, skin condition, fin abnormalities and parasites were significantly higher in Gladstone barramundi than reference sites ($P = <0.05$).

Gladstone barramundi had a significantly lower condition factor (weight×10^5/Length^3) than those from the reference sites ($P < 0.05$), and on Trip 1 the Gladstone barramundi also had a significantly higher proportion of sunken, or 'tucked' abdomens and significantly lower levels of mesentery fat. Condition factor is usually a reflection of nutritional status and has been used as an indicator of overall health, however variation can also occur in association with disease, chemical exposure, gonadal status, season and location (Dethloff and Schmitt, 2000).

Barramundi found in freshwater impoundments generally have high fat levels. Within Gladstone Harbour, high levels of mesentery fat appeared to be an indicator of fish that had recently passed over the Awoonga Dam spillway. All five fish from Lake Awoonga had greater than 50% mesentery fat. Of the fish examined from the Boyne River, those with high mesentery fat tended to also have lesions. In total, 19 of the 20 fish with lesions in the Boyne River had greater than 50% mesentery fat. Of the remaining 16 fish from the Boyne River with no observed lesions, only one had greater than 50% mesentery fat. Elsewhere in Gladstone Harbour, 43 of the 49 barramundi examined had no observable mesentery fat (see Figure 14). The proportion of fish with notable mesentery fat decreased across all sites between April and June/July 2012. In both reference sites, barramundi had relatively high levels of mesentery fat, which did not appear to be related to lesion scores.

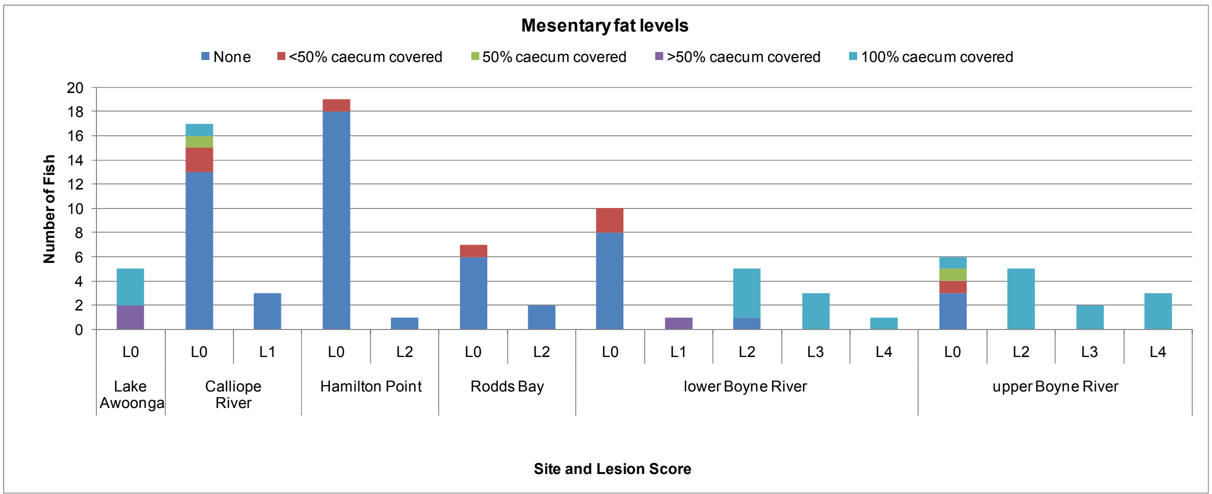

Figure 14. Levels of mesentery fat in barramundi from Gladstone sites, according to their lesion scores as recorded during necropsy.

Histopathology

Due to technical difficulties, there were some samples of barramundi that were not able to be properly evaluated for the analysis of skin, so the skin histology data could not be included in the statistical analysis. All other values were combined to allow comparison of cumulative score as a very crude measure of overall health for each fish. Liver lipid scores were also categorised in this way, but were not tallied with other scores because liver storage vacuolation is not an abnormality.

Due to the variation in time taken to transport barramundi specimens to the lab and to perform necropsies, there were varying levels of post-mortem degeneration in the tissues. Pathologists were provided with time between capture and necropsy for each specimen to enable estimation of this effect, and quantified only the abnormalities deemed not attributable to post-mortem degeneration.

The barramundi from Lake Awoonga had the lowest score for gill hyperplasia, gill parasites, muscle lesions and cumulative score. All five fish from Lake Awoonga were rated moderate for liver lipid, which is consistent with necropsy findings of relatively high condition factor, mesentery fat and hepato-somatic index in these fish. The differences between fresh and salt water fish are expected, given the substantial difference between habitats.

Gill hyperplasia was significantly higher in barramundi from the reference sites compared to Gladstone in April 2012 sampling. There were no significant differences between sites in the

June/July sampling trip. Gill hyperplasia is an abnormal increase in the cell numbers and can be a sensitive indicator of water-borne irritants. The sensitivity of this analysis in barramundi was somewhat reduced because post-mortem degeneration can mask subtle changes in gill tissue. This problem was much reduced in grinner and mud crab, because tissues were fixed immediately following euthanasia of the specimens.

Melanomacrophage centres (MMCs) are distinct clusters of cells containing pigment that are known to increase in size or frequency during conditions of environmental stress or in response to starvation or disease (Agius and Roberts, 2003). Age is also known to have a similar effect on MMCs, however this has been accounted for in our analysis using length as a proxy for age and adjusting the data as though all fish are the same length.

Liver MMCs were rated significantly higher ($P < 0.05$) in barramundi from Gladstone than the reference sites during June/July 2012. They were similarly higher in Gladstone fish than those from reference sites caught during April, but the difference was not statistically significant. The fish from Hamilton Point were rated highest for MMCs in both trip one and two. There was a significant relationship between MMC scores and mesentery fat ($r = -0.57$; $P < 0.01$). Barramundi with lower levels of mesentery fat tended to have higher MMC scores. Barramundi caught from Bundaberg had the lowest ratings for liver MMCs for both sampling trips.

The barramundi from Hamilton Point also had low levels of mesentery fat, low condition factor and low hepato-somatic index. This evidence combined suggests that normal feeding may not have been occurring at the time of capture or in the recent past in the fish caught from Hamilton Point.

For further details and statistical analysis of histopathology findings, see Appendix C.

Chemical residue testing

The detailed report outlining chemical residue analysis of barramundi and interpretation is found in Appendix D. This section is a summary of the main findings. The measured concentrations of metals in barramundi gills from Gladstone Harbour were similar or less than those from the reference sites. The one exception was arsenic, which was twice as large in the Gladstone sites than the reference sites; however, this was not a statistically significant difference ($P > 0.05$). There are no historical data for metals in barramundi livers and gills for similar Queensland estuarine and near-coastal sites available to compare with the results from the current study.

Analysis of barramundi livers showed that the mean measured concentrations of iron and cadmium in fish collected during the first sampling round were significantly ($P \leq 0.05$) higher at some Gladstone sites compared to reference sites, and arsenic and zinc were significantly higher in Trip 2. The significantly higher levels of these metals were driven by results from specimens sampled at Hamilton Point. However, the small number of samples taken at each site and trip ($n = 5$) means that these results should be interpreted with caution. There was no relationship between the concentrations of these metals and the external signs of health.

Arsenic was regularly detected, and iron and zinc were occasionally detected in water samples collected during the water quality monitoring conducted by the Queensland Government. In contrast, cadmium was rarely detected during the Water Quality Monitoring Program. The measurement of elevated concentrations of these metals does not necessarily indicate a toxicological problem, but does indicate exposure to them.

Comparison of concentrations of these metals in barramundi livers with toxicological effects data did not, overall, indicate that the concentrations in the barramundi liver were of concern. In addition, as there were no significant associations between these metals and observed fish health, it is unlikely that they were associated with the fish health issues in Gladstone Harbour. Overall, there is no strong evidence of a link between observed fish health at the time of sampling and tissue residue concentrations.

Mud crabs

The monitoring program collected information on catch composition (size and sex), presence and severity of observed shell damage and abnormalities, as well as commercial catch information supplied by crabbers via commercial logbooks. This information was compared with historical data sets when possible to provide perspective.

Sampling sites for mud crabs during Phase 1 included the Narrows, Port Development Area, Calliope River, South Trees Inlet (joins the lower reaches of the Boyne River), Rodds Bay and the Fitzroy River (reference site).

Sampling sites for mud crabs during Phase 2 included the Narrows, Port Development Area, Hamilton Point, Calliope River, Rodds Bay, as well as the Fitzroy River and Bundaberg (reference sites).

Commercial catch

The commercial catch of mud crabs in the Gladstone region between 2000 and 2012has varied, with the highest catch recorded in 2003 (163.11 t) and the lowest catch in 2012 (67.65 t). In 2011, 112.66 t was landed, the fourth highest catch in the period (Figure 15).

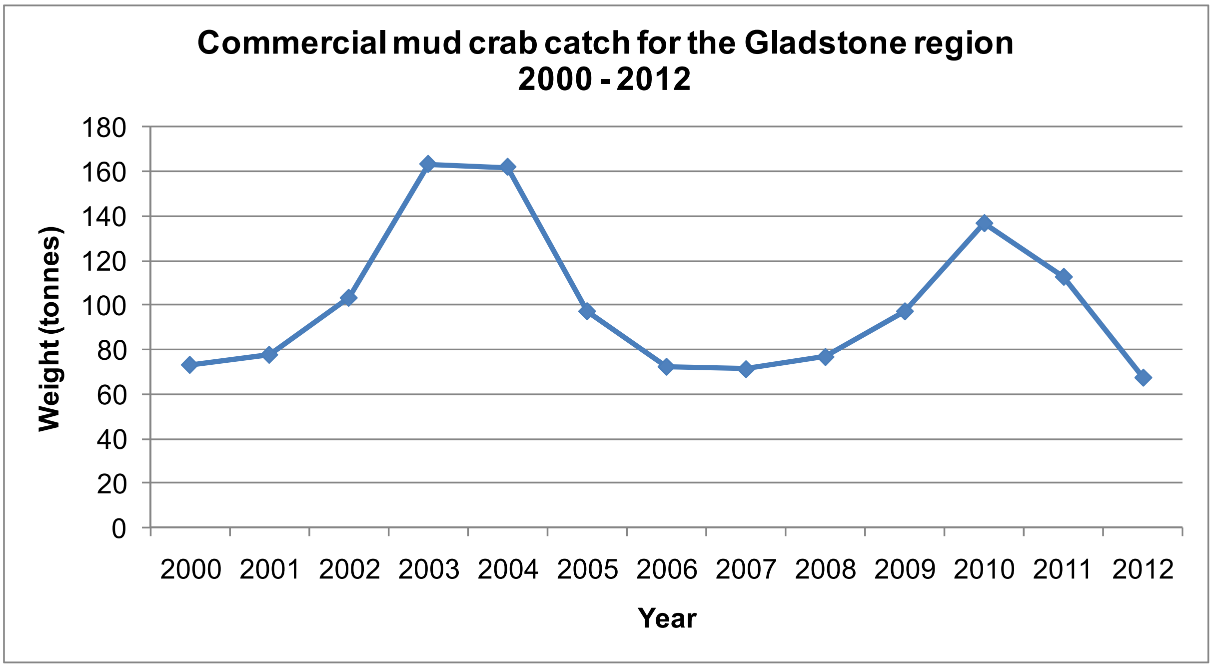

Figure 15. The annual catch of mud crabs from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2012).

Commercial fishing effort for mud crab in the Gladstone region (days fished with mud crab catch recorded) closely follows the trend in catch, with peaks in catch corresponding with peaks in fishing effort (Figure 16). Logbook data supplied by commercial fishers for 2012 shows effort and catch has reduced from 2010 to a level similar to the period 2006 to 2008.

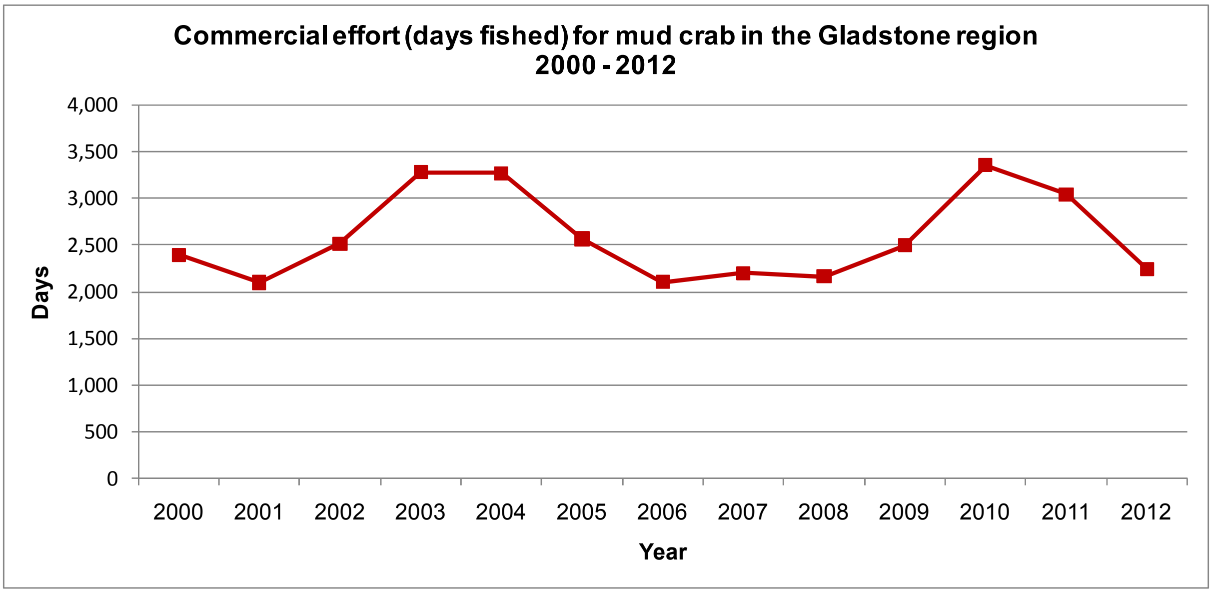

Figure 16. The number of days fishing where commercial fishers have reported mud crab from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2012).

Crab size structure

Concerns were raised that fishers were not catching large numbers of juvenile (undersize) crabs during normal commercial fishing activities. These concerns were investigated, as they relate to recruitment and mud crab catches into the future for the Gladstone region.

To illustrate the size structure of the catch, crabs caught during the program were categorised as legal male, large female (≥ legal size limit for males) or undersize (males and females combined).

Phase 1: During Phase 1, mud crab sampling was conducted between December 2011 and January 2012. A total of 1435 crabs were caught with their size and sex recorded. The percentage of undersized crabs varied between sites within Gladstone, with the percentage of undersized crabs ranging between 34.67% (Rodds Bay) and 50.91% (South Trees Inlet). The percentage of undersized crabs caught in the Fitzroy River was 42.14%, while no sampling of mud crabs was conducted in Bundaberg during this phase of the program (see Figure 17).

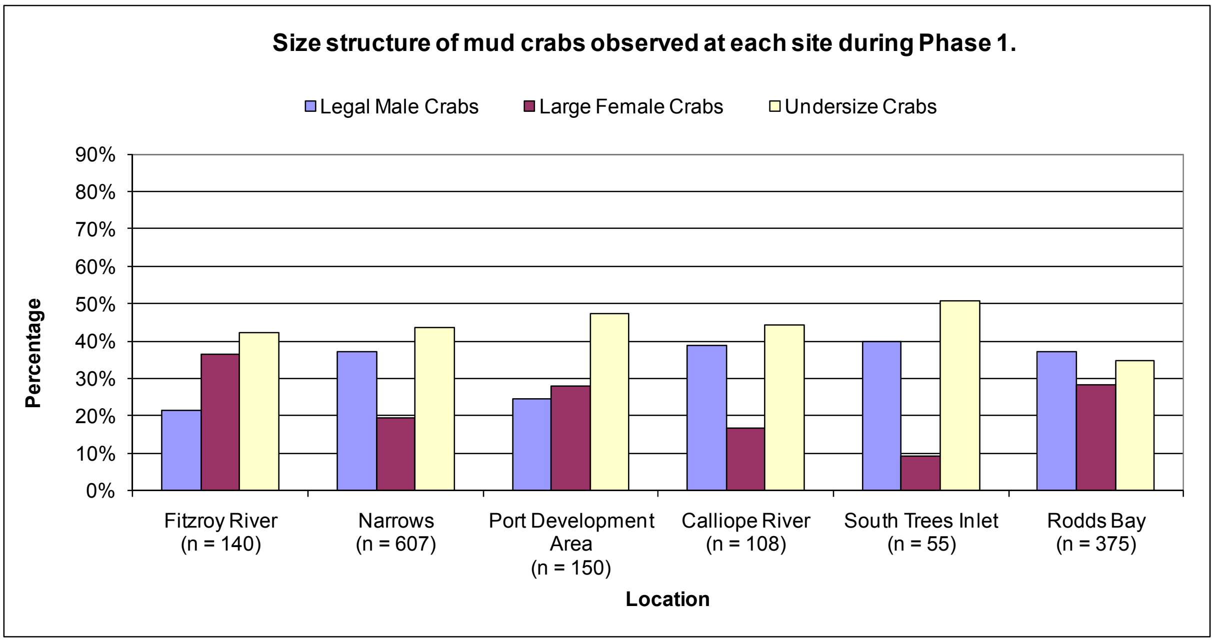

Figure 17. The size structure of mud crabs observed at each site during the Gladstone Fish Health Monitoring Program Phase 1, December 2011 – January 2012. Note, (n) refers to the total number of crabs examined at each site.

Phase 2: During Phase 2, mud crab sampling was conducted between April 2012 and July 2012. A total of 1599 crabs were caught, with their size and sex recorded. Results show that a large proportion of the crabs caught within the Gladstone region were undersized crabs (43.59%) (Figure 18). The percentage of undersized crabs varied between sites within Gladstone, with numbers of undersized crabs ranging between 35% (Rodds Bay) and 55% (Port Development Area). The percentage of undersized crabs caught in the Fitzroy River and Bundaberg were 49% and 78% respectively (Figure 19).

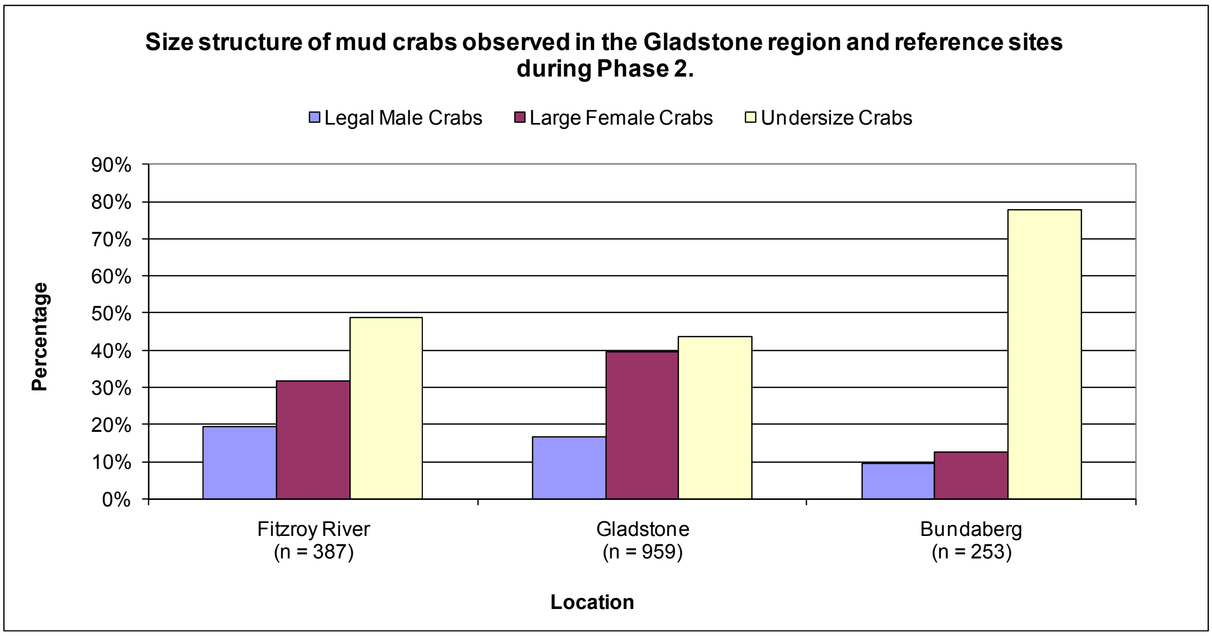

Figure 18. The size structure of mud crabs observed in the Gladstone region (sites combined) and the reference sites during the Gladstone Fish Health Monitoring Program Phase 2, April 2012 – July 2012. Note, (n) refers to the total number of crabs examined at each site.

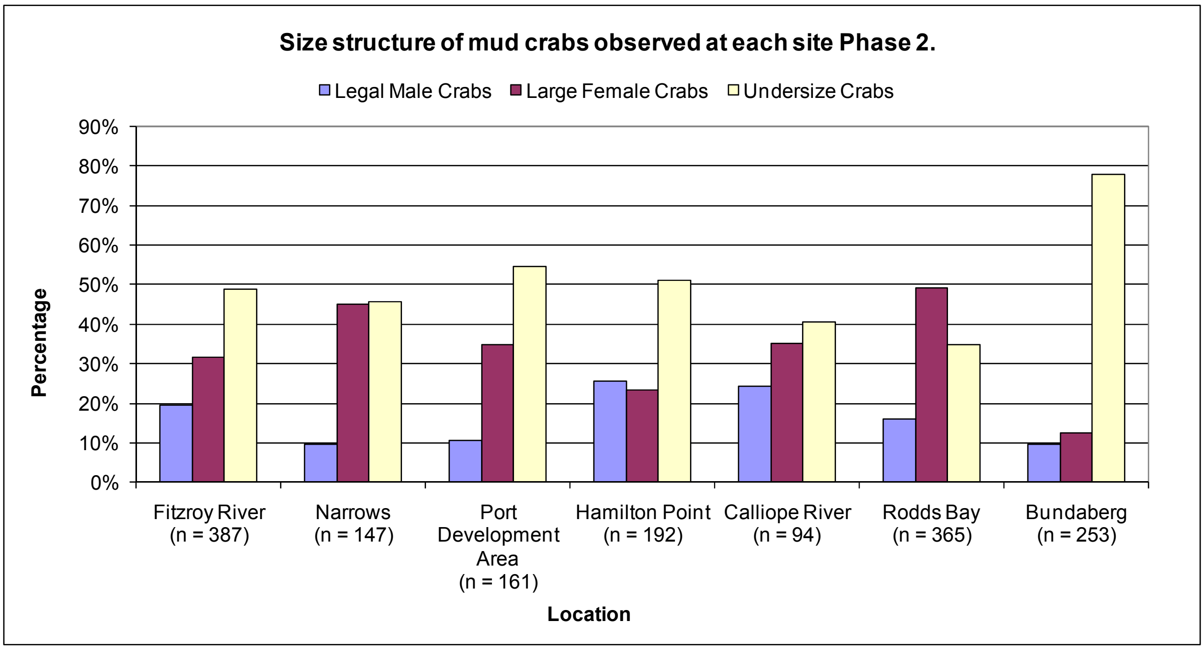

Figure 19. The size structure of mud crabs observed at each site during the Gladstone Fish Health Monitoring Program, Phase 2, April 2012 – July 2012. Note, (n) refers to the total number of crabs examined at each site.

A notable difference in the size structure between Phase 1 and 2 is the higher proportion of all crabs caught that were legal male crabs during Phase 1 (summer), highlighting the seasonal nature of the mud crab fishery.

The percentage of undersize crabs observed during Phases 1 and 2 of the program is very similar to information collected from a long-term, fishery-independent sampling program conducted between 2000 and 2009 along the Queensland coastline where 12 280 crabs were caught and their size and sex recorded. The number of crabs caught within the Gladstone region during the fishery independent survey was 1492 with 40.48% undersize (see Figure 20).

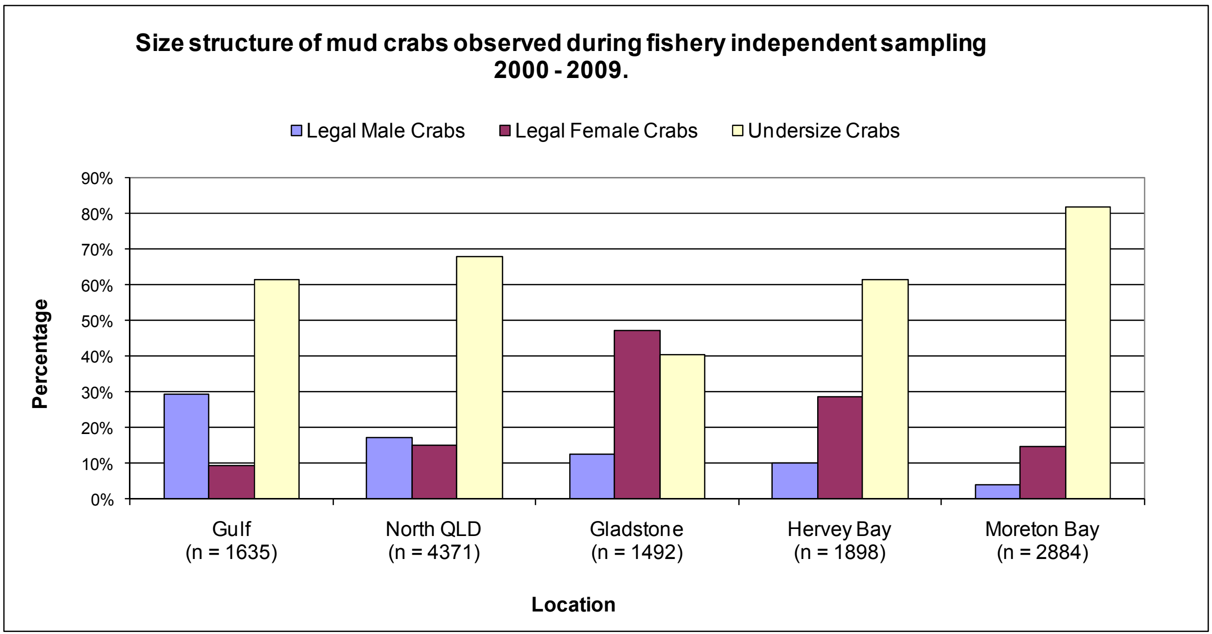

Figure 20. The size structure of mud crabs observed at sites monitored during a fishery independent sampling program conducted along the Queensland coastline 2000 - 2009. Note, (n) refers to the total number of crabs examined at each site.

As the size structure from this investigation is similar to historical data for Gladstone there is no evidence to suggest issues with local recruitment.

Shell disease and abnormalities

In late 2011, commercial fishers were reporting that the occurrence of shell (carapace) abnormalities within the Gladstone mud crab fishery was increasing. These concerns were investigated, as they relate to the health of crab stocks and the viability of commercial crabbing in the region.

Symptoms reported in the abnormal mud crabs were similar to those documented by Andersen (2000) for the description of rust spot shell disease. Lesions on the shell, called ‘rust spots’, due to their orange colour, were described by Andersen (2000) along with the associated histopathology.

Rust spot shell disease was first reported by commercial crab fishers in 1994 in mud crabs from the Port Curtis area. Investigations into the prevalence of rust spot disease in the Port Curtis area across three sampling occasions between 1998 and 2001 determined prevalence had declined from 18.3% during 1998/99 to 14.5% in 1999/2000 and dropped further to 10.2% by the 2000/2001 sampling event (Andersen and Norton 2001). Although the authors acknowledge that prevalences reported for 1998/1999 may have been inflated slightly due to the recording of lesions caused by other factors. The average prevalence across all sampling events was 14.3%. Andersen and Norton (2001) restricted their analysis to observations of lesions on the dorsal carapace. This information was used to provide a baseline for the present survey program.

It should be noted that Fisheries Queensland figures include all shell abnormalities, as it was not possible to definitively identify rust spot shell disease in the field. Fisheries Queensland also documented shell abnormalities regardless of where they occurred on the crab shell (e.g. legs, claws, carapace). Examples of shell abnormalities observed in the field are shown in Figure 21.

Figure 21. Examples of shell abnormalities (circled) observed in mud crabs during the Gladstone Fish Health Monitoring Program.

Observational findings Phase 1

Of the 1435 mud crabs observed during Phase 1, 5.33% were identified as having shell abnormalities from sites within the Gladstone region and 2.14% from the Fitzroy River. No samples were collected from Bundaberg during Phase 1.

The percentage of crabs showing abnormalities varied between sites within Gladstone, with numbers of abnormal crabs ranging from 1.87% (Rodds Bay) to 9.26% (South Trees Inlet) (Figure 22).

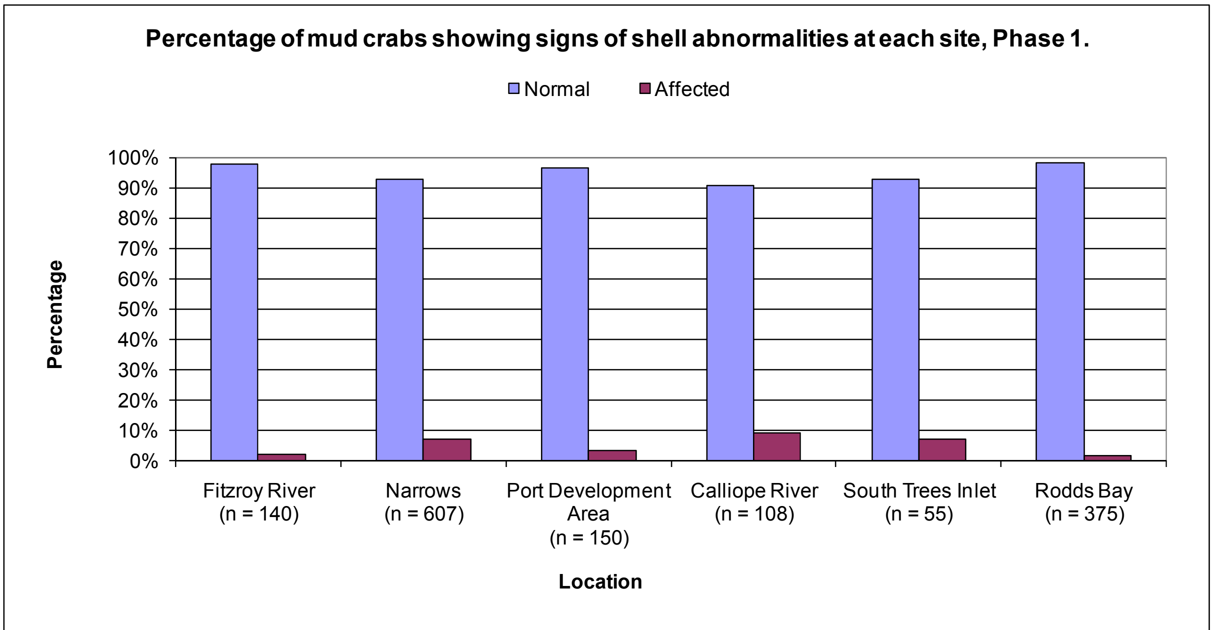

Figure 22. The percentage of mud crabs with shell abnormalities observed at all sites during the Gladstone Fish Health Monitoring Program, Phase 1. Note, (n) refers to the total number of crabs examined at each site.

Laboratory testing Phase 1

The mud crabs examined showed evidence of erosive shell disease. Signs of this disease are consistent with bacterial infection by Vibrio spp., which are naturally occurring in marine waters. *Photobacterium (Vibrio) damaselae* was isolated in one mud crab sample. These bacteria are opportunistic and proliferate on damaged shell to cause erosion due to their chitinolytic enzymes.

Observational findings Phase 2

Mud crab sampling during Phase 2 was conducted in April/May 2012 and was repeated in June/July 2012. The results from these sampling events are recorded below, along with a summary for Phase 2.

April/May 2012: A total of 853 crabs were observed during sampling from seven sites within the study area (385 in the reference sites and 468 within Gladstone). The prevalence of shell abnormalities ranged from 2.1% in the Rodds Bay area to 8.1% within the Port Development Area and averaged 4% in the reference sites and 4.4% in the combined Gladstone sites.

June/July 2012: A total of 746 crabs were observed during sampling in June/July 2012 from seven sites within the study area (491 in Gladstone and 255 in the reference sites). The prevalence of all shell abnormalities ranged from 1.4% in the Fitzroy River area to 4.7% within the Narrows, and averaged 1.96% in the reference sites and 3.26% in Gladstone.

Phase 2 summary: In total, 1599 crabs were observed during Phase 2 sampling from seven sites within the study area (640 in the reference sites and 959 within Gladstone). Of these, 58 crabs (3.6%) were observed to have shell abnormalities.

The prevalence of shell abnormalities as shown in Figure 23 ranged from 2.4% in the Rodds Bay and Bundaberg Reference sites to 6.2% within the Port Development Area, and averaged 3.2% in the reference sites and 3.9% in Gladstone. Grade 5 lesions (most severe shell damage – perforated partially or fully > 20 mm in diameter) were found throughout the study area, including both reference sites (Fitzroy River and Bundaberg).

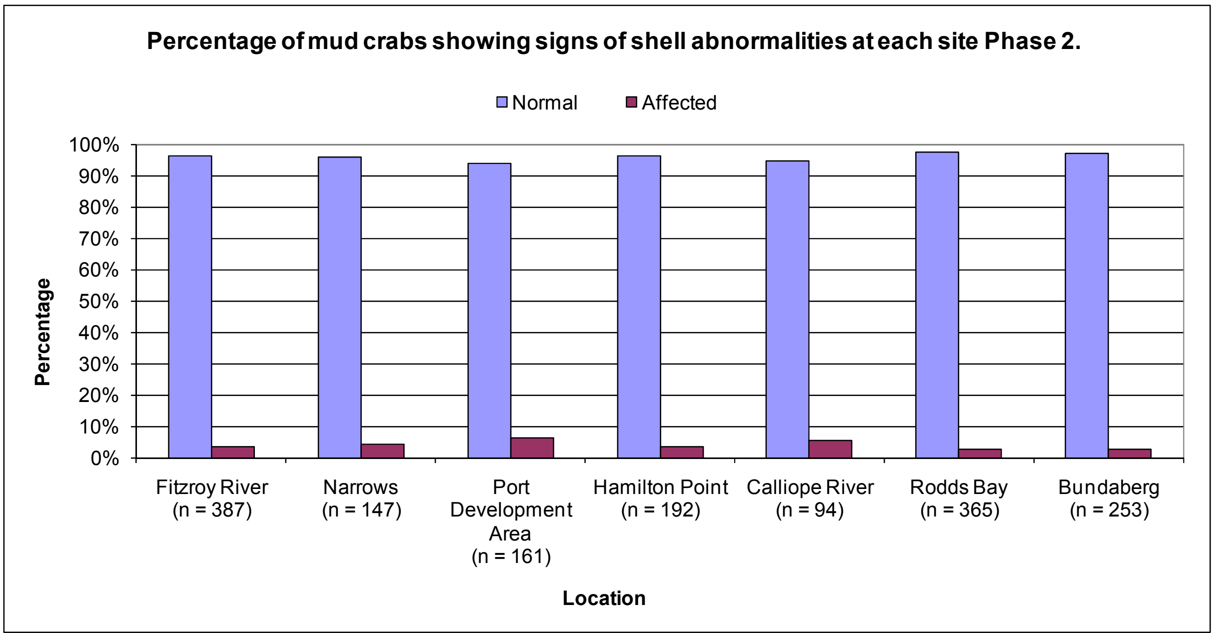

Figure 23. The combined percentage of mud crabs with shell abnormalities observed at all sites during the Gladstone Fish Health Monitoring Program, Phase 2. Note, (n) refers to the total number of crabs examined at each site.

Of the 58 crabs observed to have shell abnormalities in the field, 9 (15.5%) had lesions located on areas of the shell other than the dorsal carapace (legs, claws, abdomen and thorax).

Lesion grade ratings recorded by Fisheries Queensland field staff and those recorded by Biosecurity Queensland from June/July sampling showed a significant ($P < 0.001$) degree of association, with the counts listed in Table 5. There was agreement between field and laboratory assessments in 79.71% of these cases. Interestingly, the field assessment was higher than the laboratory assessment in 14.49% of these cases, whereas the reverse only occurred in 5.8% of the crabs. These results provide confidence for the field assessments made by Fisheries Queensland staff when recording the presence and severity of lesions in the field for both Phase 1 and Phase 2 of the program.

Table 5. Highest lesion grade for each crab as assessed by Fisheries Queensland in the field and Biosecurity Queensland in the laboratory. The grey numbers indicate agreement between the two assessments.

Laboratory Assessment Lesion Grade (0 – 5)	Field Assessment Lesion Grade (0 – 5)					
	0	**1**	**2**	**3**	**4**	**5**
0	47	0	2	4	1	0
1	2	1	2	0	0	0
2	0	1	2	0	0	1
3	0	0	0	1	0	0
4	0	0	1	0	1	1
5	0	0	0	0	0	3

Considering the prevalence of rust spot shell disease documented by Andersen and Norton (2001), the potential bias by Fisheries Queensland staff to overestimate the prevalence of shell lesions during field observations, and that observations of shell lesions were not restricted to the dorsal carapace during this program, these results of 3.9% shell lesions suggest that the prevalence of rust spot shell disease has decreased from levels of 10.2% previously recorded by Andersen and Norton (2001).

It was assumed that the number of crabs with shell lesions that were reported in Phase 1 were not under reported, as the above statistical analysis of Phase 2 for mud crabs did not identify under reporting, and the staff and field methods were the same between Phases 1 and 2. The combined results of Phase 1 and 2 do not indicate changes in the frequencies of occurrence over the sampling period or sampling location in the Harbour. The level of shell lesions observed during Phase 1 and 2 was significantly lower than the 37.8% reported for mud crabs from Gladstone by Landos (2012), and lower than Andersen and Norton (2001). Andersen and Norton (2001) found the highest average percentage in Gladstone of 18.3% in 1998/99, which reduced to 10.2% in 2001.

Laboratory findings Phase 2

Necropsy

There was no significant difference ($P = 0.68$) between the hepato-somatic index of apparently normal and abnormal crabs (0.057 ± 0.002 vs. 0.054 ± 0.002). Hepato-somatic index (HSI) is defined as the ratio of liver weight to body weight. HSI is generally used as an indication of energy stores, and these results would suggest that the presence of shell lesions is not having a significant effect on feeding.

The key variables analysed were the percentage abnormal carapace assessed in the field using all 1599 observations (a Binomial proportions model), the distribution of counts according to highest lesion class (a Poisson linear model), and HSI (Normal model). In these analyses, ‘crab category’ was always significant ($P \leq 0.05$), justifying its inclusion as a stratifying term. The relevant means are listed in Table 6.

Table 6. Mean values and standard errors (s.e.) for the crab categories.

	Hepato-somatic index	s.e.	Percent abnormal	s.e.
Female large	0.059	0.002	3.8	0.8
Female small	0.064	0.004	1.8	0.7
Male legal	0.043	0.003	7.1	1.7
Male undersize	0.056	0.002	2.5	0.7

Regarding locations and sampling events, the only significant ($P \leq 0.05$) effect was for the HSI, where combined results for June/July (0.061 ± 0.002) were higher than those for April/May (0.050 ± 0.002). Results for HSI are to be expected given HSI is generally highest during the non-reproductive period for crabs (winter), and then decreases as crabs move into their reproductive season and energy stores are utilised in gonad production (Table 7).

Table 7. Mean values and average standard errors for hepato-somatic index and per cent abnormal for each location and sampling event.

Location	Hepato-somatic index		Per cent abnormal	
	April/May	June/July	April/May	June/July
Fitzroy River	0.046	0.060	4.5	1.4
Bundaberg	0.050	0.061	3.7	3.1
The Narrows	0.055	0.059	3.2	5.2
Port Development Area	0.057	0.068	8.8	4.4
Hamilton Point	0.050	0.061	3.1	3.7
Calliope River	0.049	0.063	5.8	2.7
Rodds Bay	0.043	0.056	2.3	2.4
	(average s.e.)	0.004	(average s.e.)	2.0

June/July also sees similar or lower percentages of abnormal crabs than April/May for all sites except for the Narrows, while no significant difference was detected between Gladstone and the reference sites.

Possibly due to the low number of abnormal crabs (58), there were no significant effects for location or trip on the distributions of these counts ($P = 0.14$ and 0.13 respectively). The distribution of observed lesions across sites is shown in Table 8.

Table 8. Lesion categories as observed in the field, according to categories described by Andersen, 2001. Where individual crabs had more than one lesion, only the most severe lesion was included.

Location	Lesion Category				
	L1	L2	L3	L4	L5
Fitzroy River	2	6	1	1	4
Bundaberg	0	1	1	2	2
The Narrows	1	0	2	2	1
Port Development Area	2	5	1	1	1
Hamilton Point	0	2	1	2	2
Calliope River	1	1	2	1	0
Rodds Bay	1	3	0	4	1

Histopathology

Lipid storage vacuolation in the digestive organ (hepatopancreas) was evaluated according to the categories none, mild, moderate or severe. The location had no effect on these values, however there was a significant reduction (*P* = 0.006) in lipid between April 2012 and June/July 2012 sampling.

When comparing the combined Gladstone sites with the combined reference sites, there were no significant differences for any findings for the June/July 2012 sampling. However, in the April 2012 sampling, crabs from the reference sites had significantly higher ratings of gill parasites (*P* = 0.015) and muscle lesions (*P* = 0.000) than the score for the crabs from the reference sites (*P* = 0.015). The findings of muscle lesions should be interpreted with caution because there were only five crabs that were rated any score other than zero.

The crabs assessed were essentially normal based on histopathology findings, other than some shell lesions, which are not specific to Gladstone Harbour and occur naturally as discussed previously.

Residue testing

The concentration of aluminium and selenium was found to be significantly higher in crabs with shell lesions than crabs without. As this occurred at all sites, these elevated metal concentrations may be associated with mud crab with shell lesions in general, not just in Gladstone Harbour.

Comparison of measured concentrations of metals and metalloids in the hepatopancreas collected from the Gladstone Port Development Area and the Narrows were similar to other catchments along the east coast of Australia when compared to historical data. The fact that the tissue concentrations from Gladstone were typical for the area indicates that the metals investigated in tissues are not responsible for health issues particular to Gladstone Harbour.

None of the 114 organic chemicals analysed were detected in the crabs from either of the Gladstone harbour sites.

For a detailed analysis of chemical residue results, please see Appendix D.

Mullet

The monitoring program collected information on mullet size and sex, presence and severity of observed signs of abnormalities, as well as commercial catch information supplied by fishers via commercial logbooks.

Several species were encountered during sampling including sea mullet (*Mugil cephalus*), diamond scale mullet (*Liza vaigiensis*), goldspot mullet (*Liza argentea*) and bluespot mullet (*Valamugil sp.*). These species are commonly caught by commercial fishers in the region and are often known under different common names, so examples of each are shown in Figure 24.

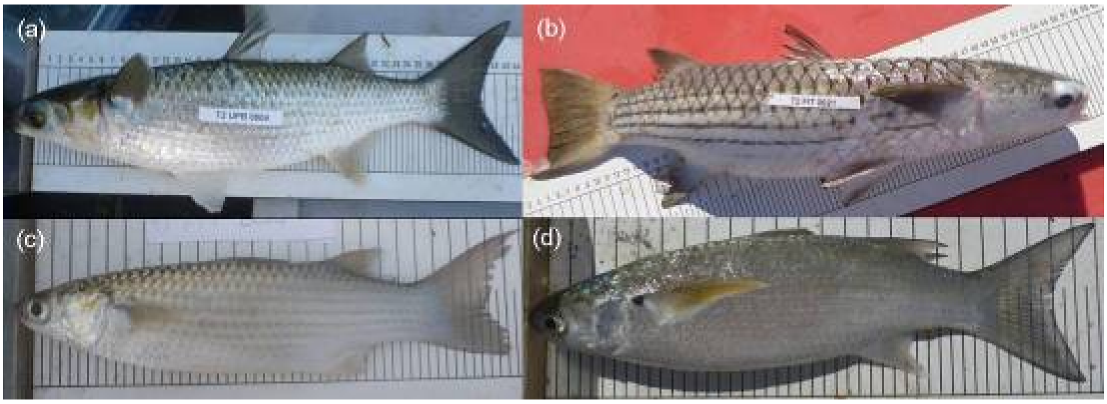

Figure 24. Mullet species encountered during sampling (a) sea mullet; (b) diamond scale mullet; (c) goldspot mullet; (d) bluespot mullet.

Sampling sites for mullet during Phase 1 included, Calliope River, Boyne River, Rodds Bay in the Gladstone area and Bundaberg and the Fitzroy River (reference sites).

Sampling sites for mullet during Phase 2 included the Calliope River, Boyne River (upper and lower reaches), Rodds Bay, as well as the Fitzroy River and Bundaberg (reference sites).

Commercial catch

The commercial catch of mullet in the Gladstone region (CFish grid S30, as reported in commercial logbooks) between 2000 and 2012 peaked in 2004 at 30.36 t. Since 2004, the catch has shown a downward trend, with 2012 the lowest year in the period (7.33 t) (Figure 25).

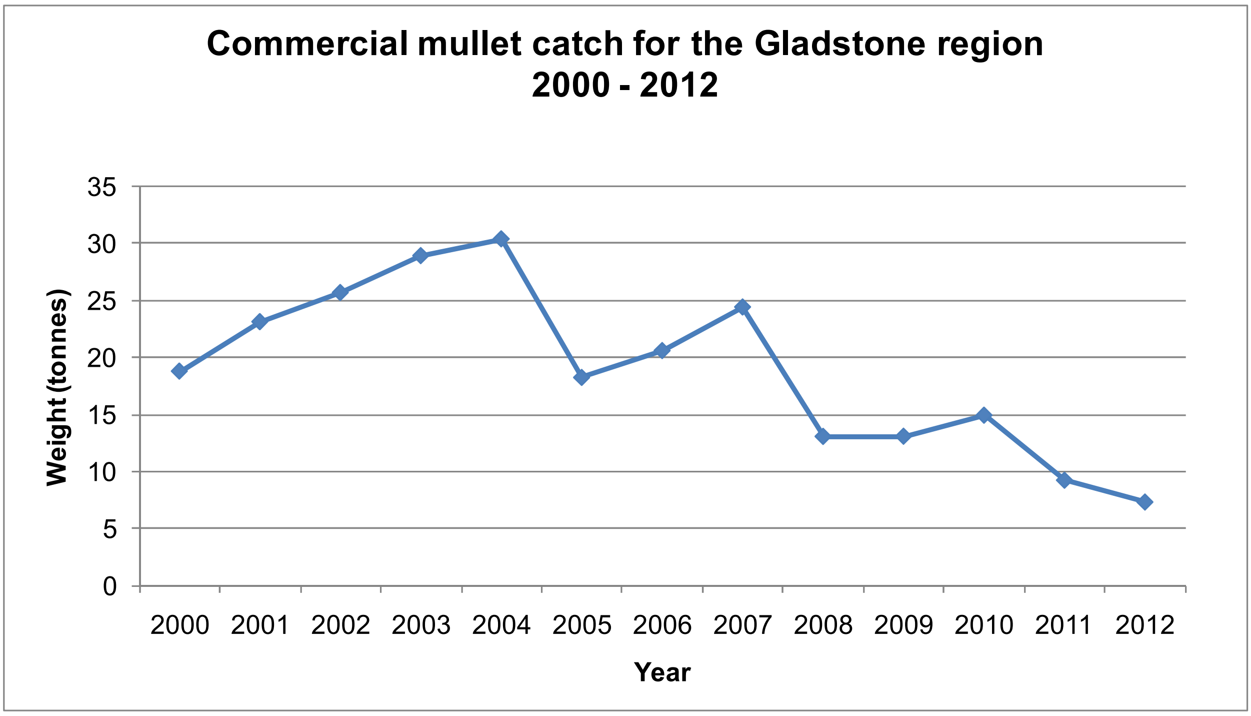

Figure 25. The annual catch of mullet from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2012).

Commercial fishing effort for mullet in the Gladstone region (days fished with mullet catch recorded) has varied since 2000, with a general downward trend. Effort in 2012 was the lowest recorded since 2000 (96 days) and is approximately a quarter of the effort recorded in 2000 (451 days) (Figure 26). The number of fishers catching mullet during this period has also dropped from a peak in 2001 (30 fishers) to lows in 2012 (7 fishers). Although the catch and number of fishers has decreased, the catch per day fished (76.3kg/day) is above average for the period (62.3 kg/day) and is the fourth highest catch rate for the period.

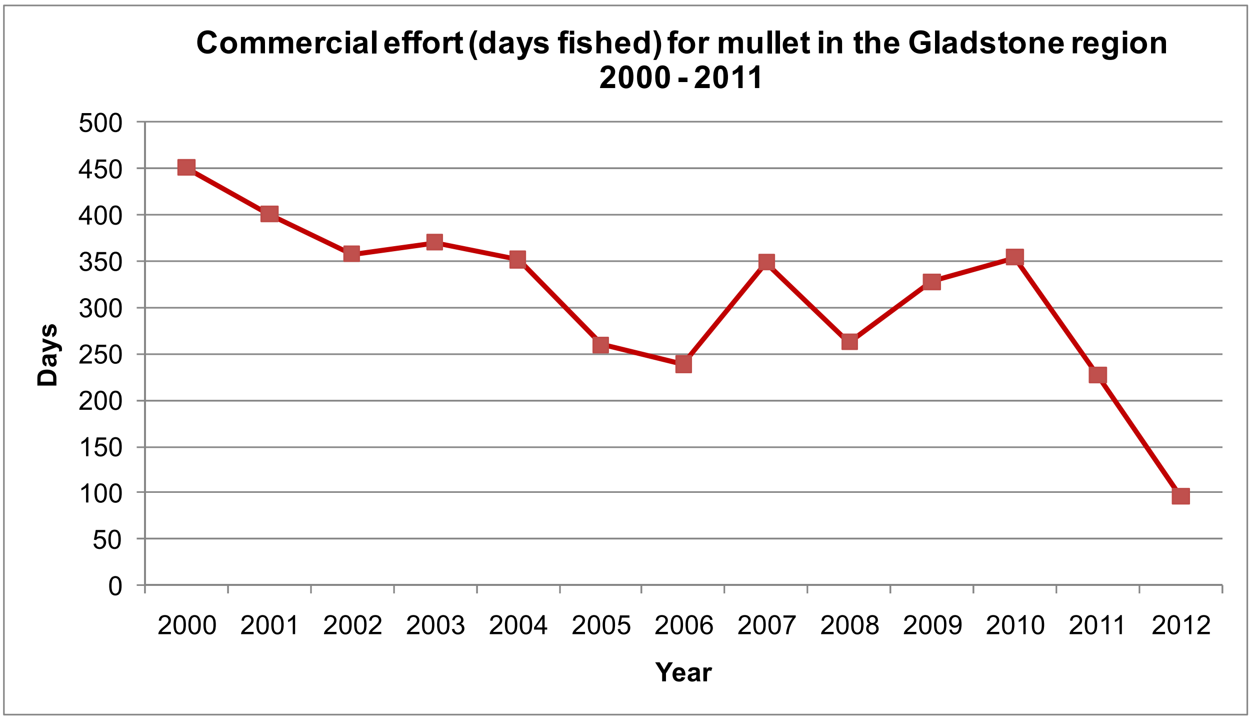

Figure 26. The number of days fishing where commercial fishers have reported mullet catch from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2012).

Observational findings Phase 1

Between September 2011 and January 2012, approximately 149 mullet caught by commercial fishers were examined for external signs of ill health both within Gladstone and at the reference sites. The majority of these fish were caught in Rodds Bay and the Bundaberg area.

All mullet caught at reference sites (37 fish) were observed to be in good condition, while 15 of 112 fish caught within the Gladstone area were observed to have some abnormal skin conditions. These observations were made from a single catch each at Rodds Bay (13 of 40 mullet) and Calliope River (2 of 2 mullet) (Figure 27).

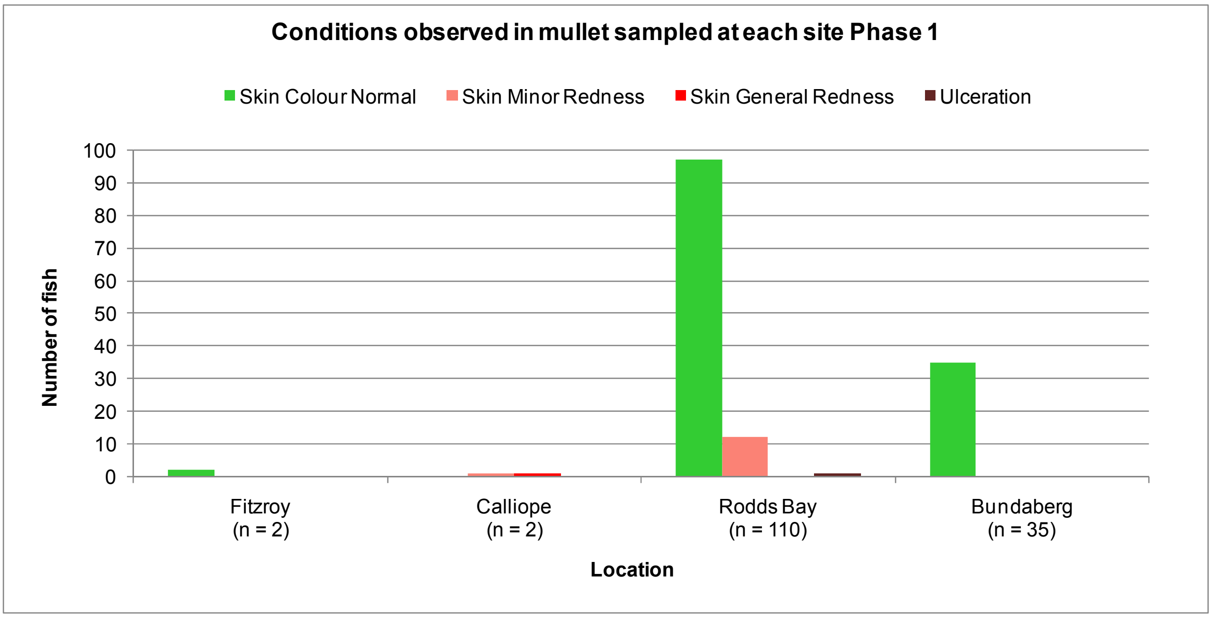

Figure 27. Conditions observed at each site sampled during Phase 1, September 2011 – January 2012. Note, (n) refers to the total number of fish examined at each site. Some fish may have been observed with multiple conditions.

Conditions observed consisted mostly of minor redness on the ventral surface/pin point red marks, with a single fish from the Calliope River having significant skin discolouration. One fish captured in Rodds Bay displayed an ulcerative lesion on the caudal peduncle (Figure 28).

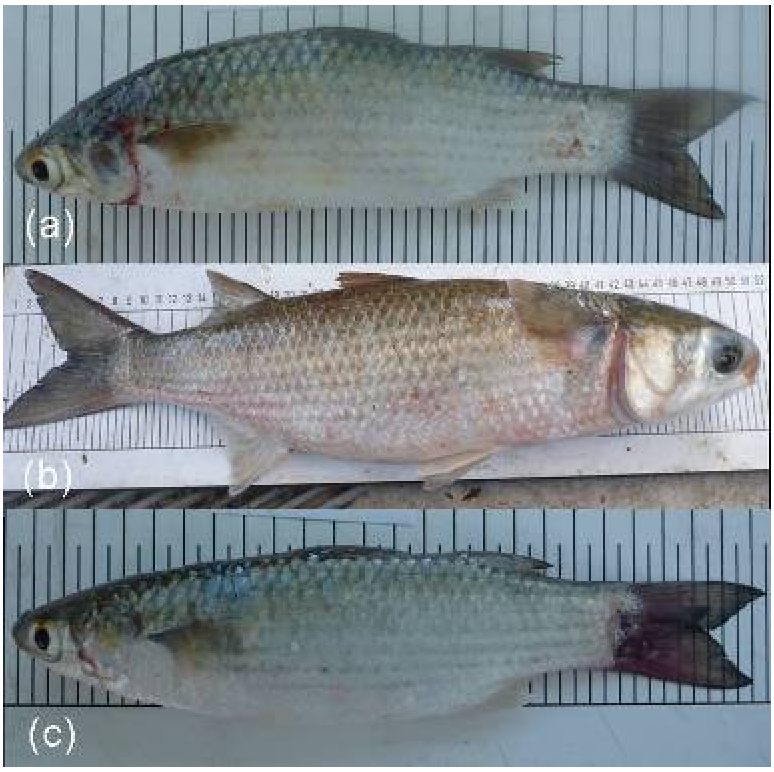

Figure 28. (a) Mullet caught in Rodds Bay displaying minor redness/pinpoint marks on the ventral surface and caudal peduncle. The redness around the operculum was a result of the capture by net; (b) Mullet caught in the Calliope River showing general redness; (c) Mullet caught in Rodds Bay with ulcerative lesion on the caudal peduncle.

Histopathology and residue testing Phase 1

Only two mullet were examined by necropsy and histology during Phase 1 of the investigation. They were submitted by a member of the public from the Gladstone area, but no location was provided. Both fish exhibited skin redness with inflammation of the dermis and loss of epidermis. No bacteria or fungi were present in the skin lesions. Some inorganic sediment was found in association with sloughed epidermal cells and mixed with sloughed cells between gill filaments. A range of minor lesions related to parasites were present internally, however these are normal.

Mullet from the Kolan River (Bundaberg) and Turkey Beach (Gladstone Harbour) were tested for chemical residues using gill, liver and muscle tissue from two fish from each site. The metal concentrations for the reference site (Kolan River) exceed those from Gladstone for most metals (i.e. aluminium, iron, copper, zinc, arsenic, selenium, silver and barium). Other metals, including lead and mercury, showed no obvious difference between the reference and Gladstone Harbour sites. No organic chemicals were detected.

Observational findings Phase 2

Mullet sampling during Phase 2 was conducted in April 2012 and was repeated in June/July 2012. The results from these sampling events are recorded below, along with a summary for Phase 2.

April: Mullet samples were collected from all proposed sites with a total of 63 caught and examined for signs of ill health. The species of mullet sampled at each site varied, with sea mullet dominating the catch in Bundaberg and the Calliope River. The catch in the Fitzroy River and the upper and lower reaches of the Boyne River were almost exclusively goldspot mullet, while bluespot mullet dominated the catch at Rodds Bay. A single diamond scale mullet was also caught at Rodds Bay. No significant signs of abnormalities were observed in any mullet caught during the April sampling events. Minor redness was observed on samples collected from both the Fitzroy River (100% of samples) and Bundaberg (25%) reference sites, but was not observed in samples collected in Gladstone.

The conditions observed in the Fitzroy River and Bundaberg consisted of minor redness and small pin point marks on the body and fins, as illustrated in Figure 29.

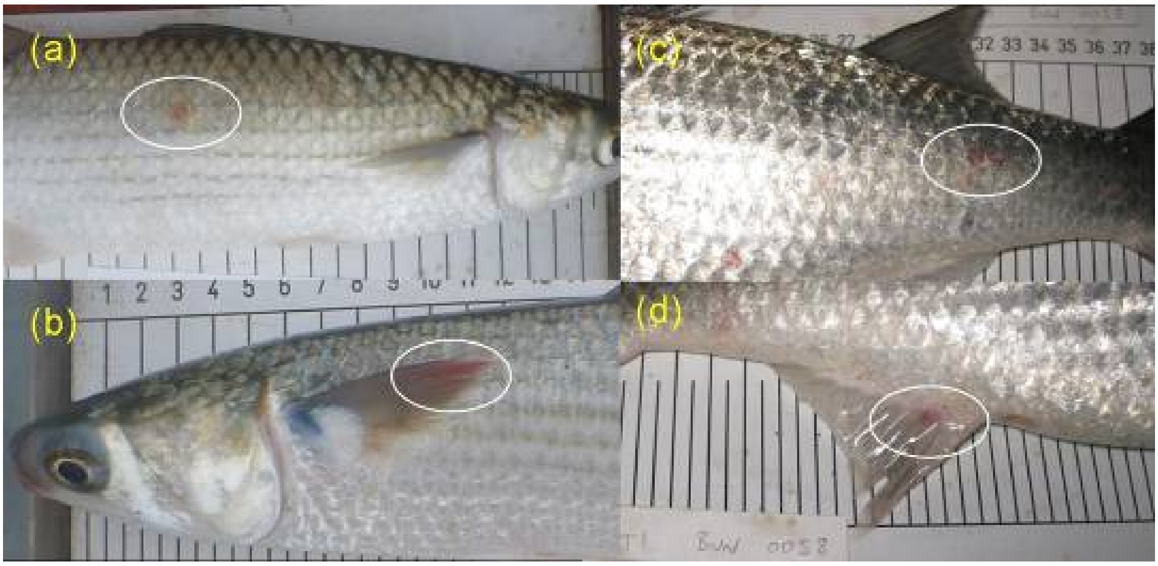

Figure 29. Minor redness and pinpoint marks observed on fins and the body surface of mullet sampled from the Fitzroy River (a) and (b), and Bundaberg (c) and (d).

A single mullet was captured in the lower reaches of the Boyne River with a fresh bite mark. It was most likely that the bite mark occurred while the fish was in the net. Given the type and recent nature of the wound, this fish was recorded as being in good health for analysis (Figure 30).

Figure 30. A mullet captured in the lower reaches of the Boyne River with a fresh bite mark

June/July: Mullet samples were collected from all proposed sites with a total of 62 caught and examined for signs of ill health. The species of mullet sampled at each site varied, with the catch in Bundaberg, Fitzroy and the Boyne River being almost exclusively sea mullet. A single diamond scale mullet was sampled from the Fitzroy River. The mullet catch in the Calliope River and Rodds Bay was exclusively goldspot mullet.

Mullet were observed with minor redness/red pin point marks on the body surface from all sites except Rodds Bay and the upper reaches of the Boyne River. This condition was most prevalent in the Fitzroy River (six of 11 mullet sampled). The ventral pale pink areas observed in three out of 11 mullet caught in the Calliope River were due to the capture process (removal from the net).

A single mullet from the upper reaches of the Boyne River displayed both general redness and lesions comprising of small areas of detached scales (see Figure 31).

Figure 31. A mullet sampled from the upper reaches of the Boyne River displaying general redness and lesions comprised of small areas with detached scales.

A single mullet caught in the Fitzroy River was observed to have ulcerative lesions on both sides of its gill covering (operculum) and a cloudy eye (Figure 32).

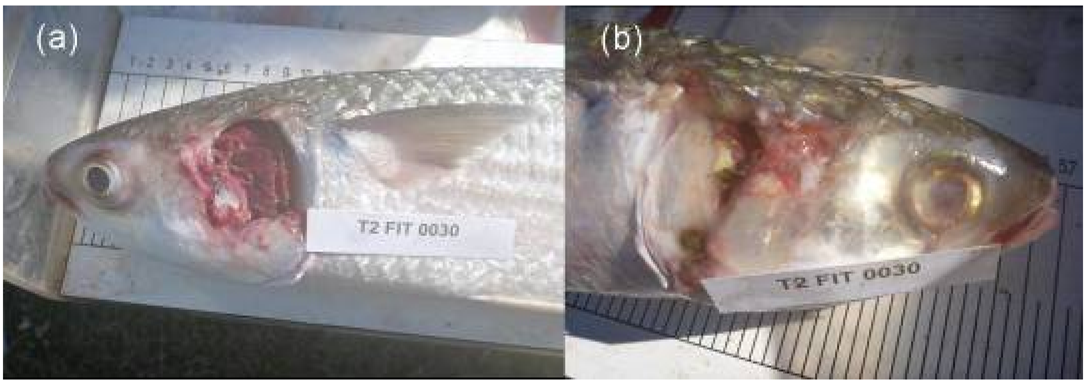

Figure 32. A mullet sampled from the Fitzroy River with ulcerative lesions on (a) the left and (b) right side of the gill covering (operculum), exposing the gills. Note also the cloudy right eye

Phase 2 summary: In total, 125 mullet were observed during Phase 2 sampling from six sites within the study area (47 in the reference sites and 78 within Gladstone). Seven mullet (8.97%) observed from the Gladstone area showed external signs of abnormalities and they were all caught during Trip 2. This value does not include three fish from the Calliope River observed to have pale pink areas ventrally resulting from the capture process. In the reference sites, 22 mullet (46.81%) displayed external signs of abnormalities and these were seen during both trips. The conditions observed are summarised in Figure 33.

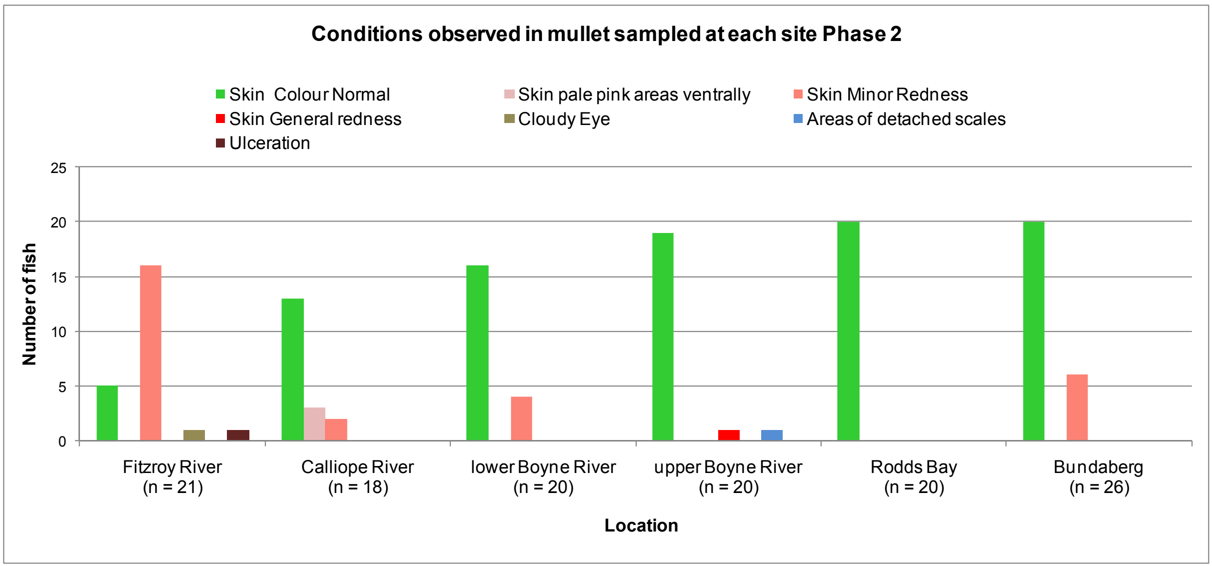

Figure 33. The combined results for conditions observed in mullet at all sites sampled during Phase 2, 2012. Note, (n) refers to the total number of fish examined at each site. Some fish may have been observed with multiple conditions.

In mullet, skin lesions were far less prevalent than barramundi, with only two lesions recorded.

Laboratory findings Phase 2

Collectively, Gladstone sites appeared to contain healthier mullet than the reference sites in April 2012, but in June/July 2012 the trend was reversed.

In April, the only significant differences ($P < 0.05$) between Gladstone and reference sites were that Gladstone mullet had significantly lower incidence of skin redness than the reference sites. The cause of the difference is not known. In June/July, fin and kidney abnormalities were significantly more prevalent in Gladstone sites than reference sites ($P < 0.05$). The hepato-somatic index (HSI) and health assessment index (HAI) were also significantly higher in Gladstone mullet.

HSI has been widely used as an indicator of overall health. Both increases and decreases in size have been linked with exposure to environmental contamination, although changes in liver size can also occur in response to seasonal cycles, nutrition, gonadal status and activity level of the fish immediately prior to death (Dethloff and Schmitt, 2000).

The HAI allows overall quantitative analysis of necropsy data by assigning numerical values to a range of internal and external observations and combining them to record a single number for each individual (based on Adams, 1992). Higher average values in Gladstone sites during June/July indicate a higher incidence of abnormalities. This appears to be driven largely by high rates of liver and kidney abnormalities in the Boyne River. Specifically, a number of kidneys were pale in colour, and livers were commonly discoloured and/or fatty.

Location was a significant determinant of both liver form and liver colour, however there was no consistent trend over time, and overall the Gladstone area was not significantly different to reference sites.

Sharks and rays

The monitoring program collected information on shark species, size, sex, presence and severity of observed signs of ill health, as well as commercial catch information supplied by fishers via commercial logbooks.

During Phase 1, no candidate species of shark had been identified, therefore all sharks and rays encountered during routine, at sea observing or targeted sampling events were observed for signs of ill health. During Phase 2, the sampling identified bull sharks as the candidate species given they were the dominant species in the catch during Phase 1 (Figure 34).

Figure 34. Bull shark

Sampling sites for shark during Phase 1 included the Calliope River, Rodds Bay, Spoil Ground and nearby offshore waters, the Narrows, Hamilton Point, the upper and lower reaches of the Boyne River, as well as the Fitzroy River and Bundaberg (reference sites).

Sampling sites for shark during Phase 2 included the Calliope River, Boyne River (upper and lower reaches), Rodds Bay, as well as the Fitzroy River and Bundaberg (reference sites).

Commercial catch

The commercial catch of shark species in the Gladstone region (CFish grid S30, as reported in commercial logbooks for both line and net combined) between 2000 and 2011 peaked in 2008 at 157.40 t. The recorded catch then declined sharply to 44.55 t in 2009 and has continued to fall to 26.32 t in 2011 (Figure 35). In 2012, minimal catch was reported (0.44 t).

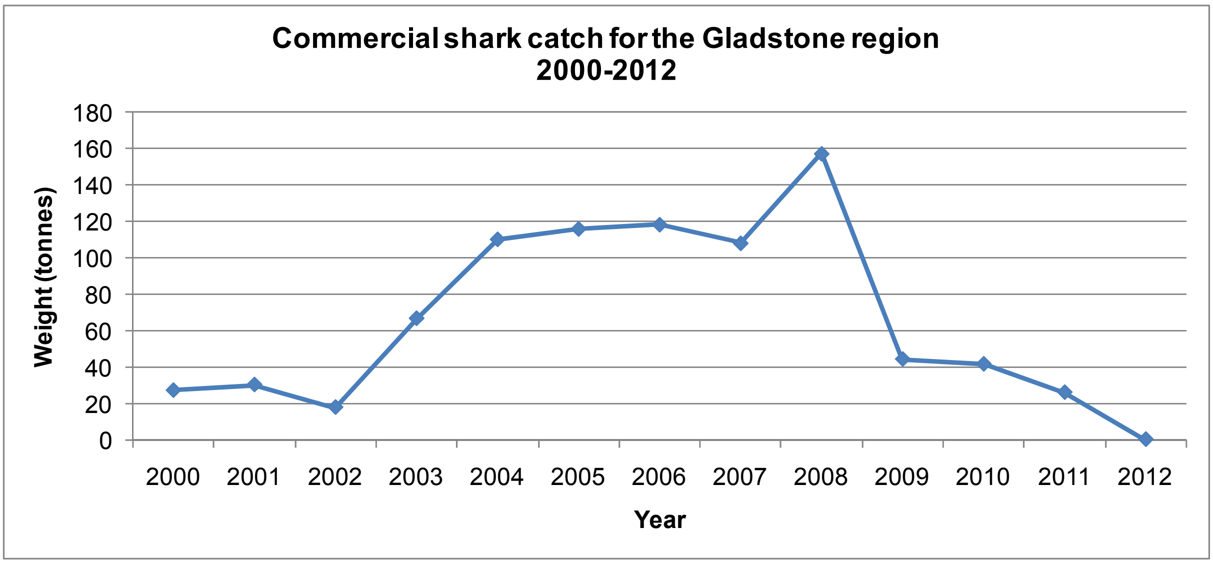

Figure 35. The annual catch of shark from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2011). Data includes both line and net catch for the region combined.

Commercial fishing effort for sharks in the Gladstone region (days fished with shark catch recorded) has varied since 2000 and follows a similar trend to catch with a peak of 706 days in 2008. In recent years, there has been a decline in the number of fishers recording shark catch from a peak in 2004 (34 fishers) to 2011 (14 fishers) (Figure 36). Effort recorded via logbooks for 2012 is very low, with only five fishers recording shark catch for a total of 11 days fishing.

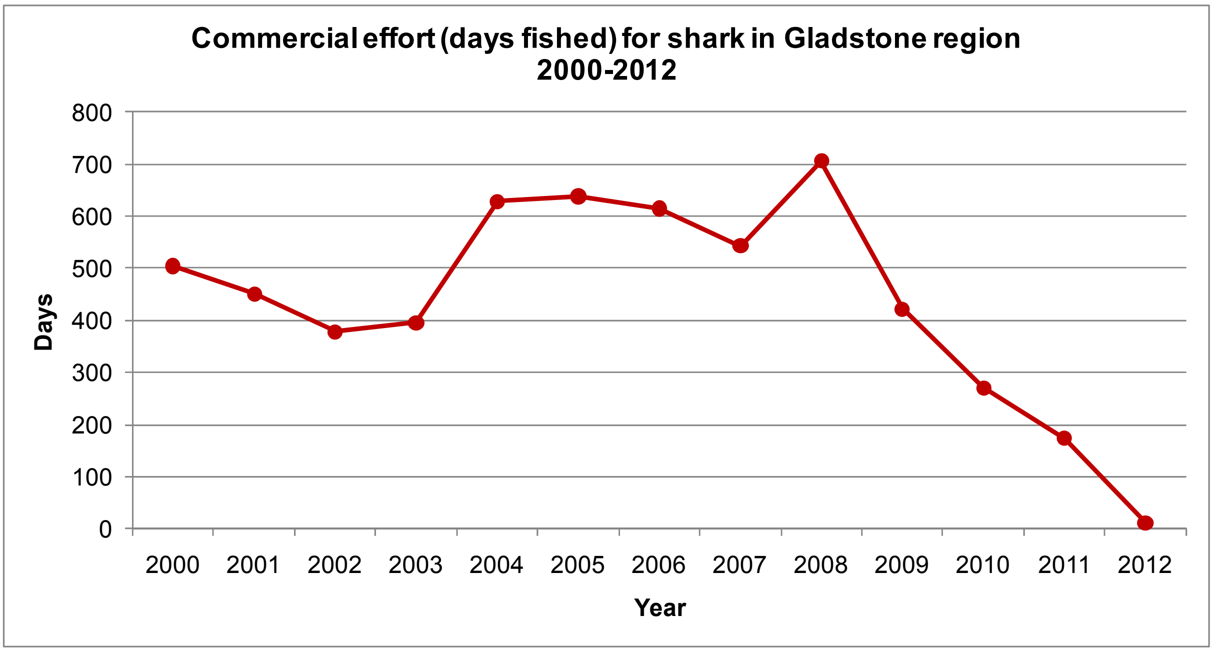

Figure 36. The number of days fishing where commercial fishers have reported shark catch from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2011). Data includes both line and net effort for the region combined.

Observational findings Phase 1

Phase 1 sampling of sharks included observations made between September 2011 and February 2012 across a range of species (227 sharks and rays). Table 9 lists the number of each species encountered during targeted sampling events or during routine, at-sea observing trips.

Table 9. The sharks and rays observed during Phase 1.

Species	Number
Blacktip whaler complex (*Carcharhinus limbatus* & *Carcharhinus tilstoni*)	45
Bull shark (*Carcharhinus leucas*)	65
Creek whaler (*Carcharhinus fitzroyensis*)	5
Eagle ray (*Myliobatidae sp.*)	3
Giant shovelnose (*Rhinobatos typus*)	1
Graceful shark (*Carcharhinus amblyrhynchoides*)	3
Grey carpet shark (*Chiloscyllium punctatum*)	2
Lemon shark (*Negaprion acutidens*)	18
Milk shark (*Rhizoprionodon acutus*)	22
Narrow sawfish (*Anoxypristis cuspidatus*)	2
Pigeye shark (*Carcharhinus amboinenis*)	5
Scalloped hammerhead (*Sphyrna lewini*)	7
Sharks unidentifed in field (*Carcharhinus spp.*)	31
Spinner shark (*Carcharhinus brevipinna*)	2
Spot-tail shark (*Carcharhinus sorrah*)	1
Stingrays (*Dasyatis spp.*)	2
Weasle shark (*Hemigaleus australiensis*)	2
Whitecheek shark (*Carcharhinus coatesi*)	1
Whitespotted guitarfish (*Rhynchobatus australiae*)	9
Zebra shark (*Stegostoma fasciatum*)	1
Total	**227**

During September 2011, nine sharks caught in the Fitzroy River by a commercial fisher were observed to be in good health. The sharks comprised a number of species, including bull sharks (2), creek whalers (3), pigeye sharks (3) and a blacktip whaler.

A total of 87 sharks and rays were sampled in October and November 2011 from a range of sites including the Fitzroy River (5), Narrows (1), Calliope River (16), Gladstone Harbour (21), Rodds Bay (43) and Bundaberg (1). Sharks showed a range of abnormal conditions throughout the study area, including the reference sites. A number of sharks showed varying degrees of skin redness generally on the flanks and underside of the body. Although the cause of the redness is unknown, sharks can display varying levels of red, discoloured skin depending on the length of time in the net and post-harvest handling. One stingray was caught during a trawl survey that had an injured, shortened tail with the barb missing.

A number of sharks also had areas of lighter skin discolouration, particularly between the first and second dorsal fins. Closer examination revealed the placoid scales (tough modified scales found on sharks and rays) were absent, exposing the underlying layers of skin (epidermis and dermis). Some of these areas were bleeding and appeared to have associated scratch marks. Many of these sharks were observed to have numerous flatworm parasites (monogeneans) on the body surrounding the affected areas. While this condition was most commonly observed on bull sharks, it was not restricted to this species.

A report from an independent expert stated ”it is well established that fish infected by monogeneans on the skin may ‘flash’ (i.e. rub affected parts of the body against substrate), presumably in an attempt by the host to remove the source of irritation” (Whittington and Chisholm, 2008). It is possible that the clinical signs (e.g. skin redness and scratch marks observed in sharks) could be caused indirectly by the parasites. These parasites occur naturally, although normal parasite loads are not well documented, so it is not possible to determine whether observed infections are abnormal.

In December 2011, 29 sharks or rays were captured from several sites including the Calliope River (1), the lower reaches of the Boyne River (3) and in Rodds Bay (25). Of these, 14 were assessed as being healthy with no skin discolouration, 13 sharks showed discolouration on a small area of the body, one was described as showing a large area of skin discolouration and one displayed skin discolouration over the majority of the body. No sharks or rays showed ulcerative lesions.

In January 2012, of the 50 sharks and rays captured, 12 were caught in the Fitzroy River (all bull sharks) while the remaining came from sites within Gladstone including the Calliope River (7), Hamilton Point (1), the Spoil Ground (11) and Rodds Bay (19). All the sharks from the Fitzroy River had ecto-parasites and skin discolouration. Within the Gladstone sites, some of the black tip whaler, bull, lemon, milk and weasel sharks caught had skin discolouration. Most of these sharks also had ecto-parasites.

In February 2012, at sea observations were conducted on two net fishing operations for two consecutive nights. The fishing was conducted at Rodds Bay and at Colosseum (halfway between Rodds Bay and the mouth of the Boyne River). Over the four nights, the fishers caught 52 sharks and rays. Of the 21 bull sharks caught, 19 had ecto-parasites and skin discolouration. Four of the other sharks and rays exhibited redness on the ventral surface.

Laboratory testing Phase 1

Tissue from a total of 21 sharks was examined microscopically during Phase 1, with 21 bacteriology tests. Five necropsies were conducted on whole sharks.

Shark species examined during Phase 1 included lemon shark, bull shark, pig eye shark, whitecheek shark, blacktip whaler shark, graceful whaler shark and narrow sawfish. The sharks examined had scale pocket hyperaemia (pockets of reddening), dermal haemorrhage (bleeding of the skin) and epidermal necrosis (cell death). When sections were taken and the skin lesions were observed under a microscope, observations included congestion in the connective tissue immediately below the epidermal basement membrane, occasional haemorrhage into the epidermis and/or skin surface, and a mild inflammatory cell infiltration into the outer layer of the dermis. No bacterial or fungal pathogens were found that could explain these skin conditions on the sharks.

Monogenean parasites found on the skin of several bull sharks were identified as *Dermophthirius maccallumi* from the family Microbothriidae. Similar parasites were found on graceful whaler sharks and pig eye sharks. This genus is known to cause disease in captive sharks, including excess mucous production and ulcerated skin lesions. This disease can lead to secondary infection by bacteria or viruses, but has also been implicated in skin lesions, denticle loss and other clinical signs in wild sharks without co-infection by bacteria or viruses.

Encysted larval parasites were found internally, but were not expected to be causing significant effects on the health of the sharks.

There was minimal or no detectable sediment in the gills of the shark samples.

Observational findings Phase 2

April: Bull sharks were collected from all proposed sites except for Rodds Bay. Sampling of bull sharks proved difficult during the April period with a total of 23 caught. Anecdotal evidence from commercial fishers suggests that bull sharks start leaving the estuaries at this time of year. Three sharks of other species were also caught (two blacktip whaler, one Creek whaler) and their condition recorded.

Conditions observed with sharks caught during the April sampling period consisted of skin redness and areas of detached scales predominantly between the first and seconc dorsal fins exposing the skin, as well as some scratch marks around the affected areas. No other signs of ill health were observed at the time of capture. A summary of results is shown in Figure 37.

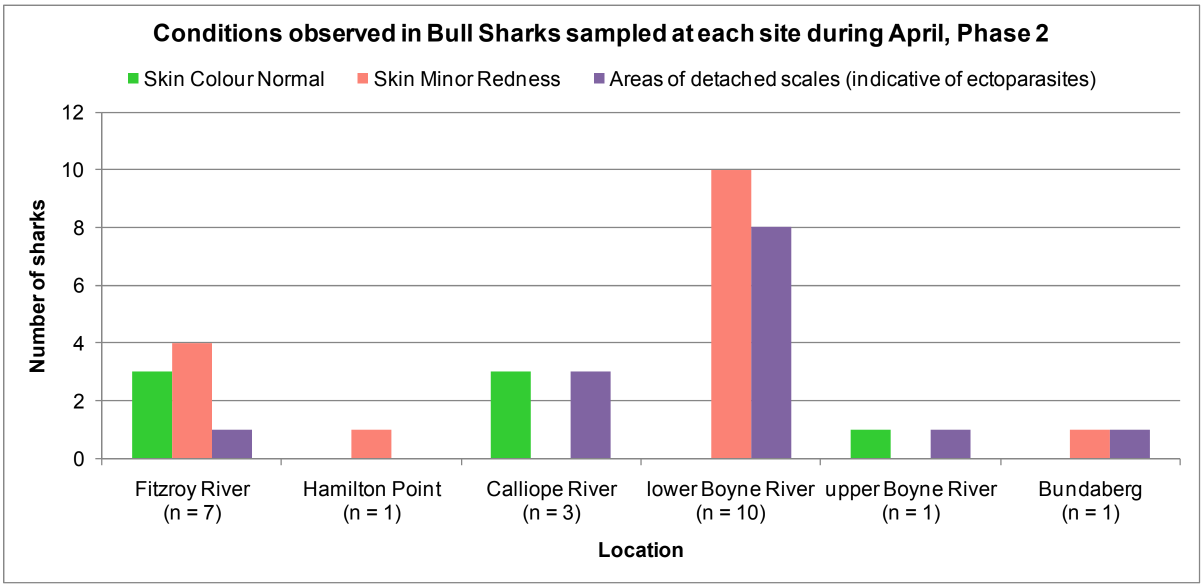

Figure 37. Conditions observed in bull sharks at the time of capture from all sites sampled during April 2012. Note, (n) refers to the total number of sharks examined at each site. Some sharks may have been observed with multiple conditions.

Skin redness was observed on sharks caught from most areas, including those caught at the reference sites. The redness observed at the time of capture was classified as minor redness and was most obvious on the lighter ventral surface of the sharks. The extent of redness was difficult to quantify consistently, given redness increased significantly post mortem as illustrated in Figure 38. The two sharks pictured were captured in the Calliope River and both were classified as having no redness in the field immediately after capture. After being individually bagged and kept on ice for approximately four hours, both sharks displayed significant redness.

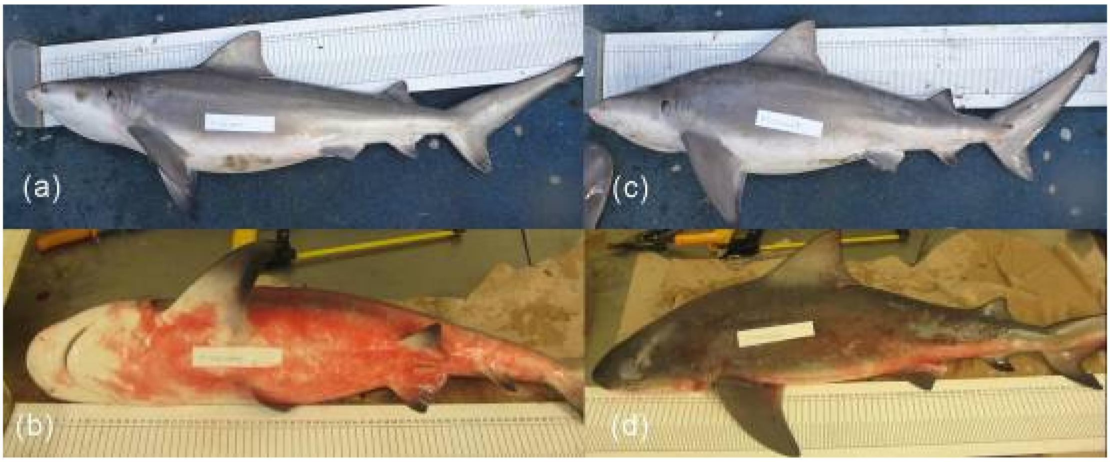

Figure 38. Sharks caught in the Calliope River illustrating the increase in skin redness post mortem. (a) and (c) Shark #0005 and #0007 photographed in the Calliope River at the time of capture; (b) and (d) The same sharks photographed four hours later in the lab.

Areas of skin between the first and second dorsal fin with placoid scales absent were also observed on sharks from most areas, including reference sites (Figure 39). This symptom has been previously associated with the presence of the monogenean parasite *Dermophthirius maccallumi* during Phase 1 sampling. Therefore, it was considered to be evidence that monogenean ecto-parasites were present even when the parasites themselves were not seen. This assumption was also based on personal communications with Dr Ian Whittington, who suggested that only adult monogeneans from the family Microbothriidae would be visible on the skin in the field, as juvenile worms are generally located underneath the scales and would be too small and hidden from sight for visual identification.

Figure 39. Bull sharks caught at Bundaberg and the Calliope River showing evidence of ecto-parasites (lighter areas) between the dorsal fins.

June/July: No bull sharks were collected during the June/July sampling event. Commercial fishers suggested that bull sharks leave the study area during the winter months.

When considering the findings from the sampling program (Phase 1 and Phase 2) the most common abnormalities observed in sharks and rays was skin redness, and lesions located between the dorsal fins. Pathology investigating skin redness during Phase 1 found no associated pathogenic organisms, so the cause of this condition is unknown. Given sharks, in particular bull sharks, have been observed outside Gladstone both during this survey and during routine observer trips, it is known that this skin condition is not unique to Gladstone. Furthermore, the degree of redness increases post mortem. It is thought that capture methods and handling practises of sharks influence the degree of redness exhibited, but further work is needed to quantify this effect.

The parasitic monogenean, *Dermophthirius maccallumi,* identified during the program is known to cause symptoms similar to those observed on many sharks sampled during the program, and may also indirectly be the cause of many of the scratch marks around the affected areas if sharks are 'flashing' or rubbing themselves on hard substrates in an attempt to rid themselves of the irritation. Given there have been no previous studies on *Dermophthirius maccallumi* on wild sharks that quantify a normal parasite load, it is difficult to determine whether the number of parasites observed on sharks during this program are abnormal. What is known is that the presence of these parasites and the associated skin lesions are not unique to the Gladstone area.

Laboratory findings Phase 2

Necropsy

A total of 12 sharks were received for laboratory testing, all during April. Only four of these were from Gladstone Harbour (three from Calliope River and one from Hamilton Point), seven were caught in the Fitzroy River and one from Bundaberg. The sharks from Gladstone Harbour, particularly the three from the Calliope River, had higher skin condition ratings than other sharks, although as discussed above this redness was not apparent on capture. It is possible that subtle differences in handling, such as net mesh size, soak time and time spent in transit, could have influenced this post-mortem skin redness, but particularly with such low numbers it is not possible to draw any conclusions from these observations.

No significant abnormalities were observed internally in any of the sharks during necropsy.

Histopathology

Liver, skin and gills from 10 sharks were assessed histologically, including the only four that were caught from Gladstone Harbour, five from the Fitzroy River and one from Bundaberg. Common pathology indicators for each tissue were rated from zero to three to allow quantitative comparison to be made. There were no significant abnormalities observed in shark tissues, and there were no significant differences between fish from Gladstone and the reference sites for any tissues. Microscopic examination of skin tissue identified mild inflammation and bleeding in one shark from Bundaberg, mild inflammation in a shark from Hamilton Point, and mild bleeding and degeneration of skin tissue in a shark from the Calliope River. No causal agent was identified for the observed skin redness.

Samples collected for chemical residue testing were archived for future testing if required.

Banana prawns

The monitoring program collected information on prawn size, presence and severity of observed signs of ill health, as well as commercial catch information supplied by fishers via commercial logbooks. Samples were collected and provided to Biosecurity Queensland for further testing.

Sampling sites for banana prawns during Phase 1 and Phase 2 included Gladstone Harbour and waters offshore of Bundaberg and the Fitzroy River (reference sites).

Figure 40. Banana prawns, a targeted species during the sampling program.

Commercial catch

The commercial catch of banana prawns in the Gladstone region (CFish grid S30, as reported in commercial logbooks for both otter and beam trawl combined) between 2000 and 2011 peaked in 2003 at 125.53 t. The recorded catch declined to 6.36 t in 2007 and then steadily increased to 109.18 t in 2011, the second highest recorded catch since 2000 (Figure 41). In 2012, the reported banana prawn catch in Gladstone had dropped to 12.88 t.

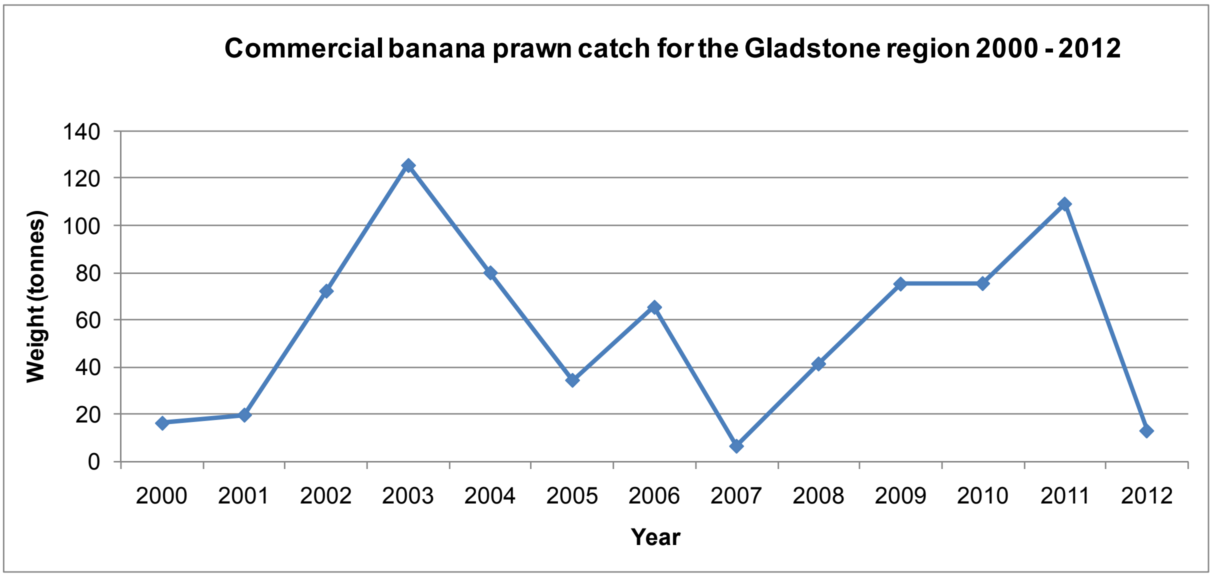

Figure 41. The annual catch of banana prawns from the Gladstone region (CFish grid S30) as reported in commercial fisher logbooks from calendar years 2000 – 2011. Data includes both otter trawl and beam trawl catch for the region.

Commercial fishing effort for banana prawns in the Gladstone region (days fished with banana prawn catch recorded) has varied since 2000 and follows a similar trend to catch however between 2008 and 2011 similar effort was recorded while the commercial catch rose steadily (Figure 42). The number of fishers catching banana prawns during this period peaked in 2003 (30 fishers). Effort recorded via logbooks for 2012 was very low with only seven fishers recording banana prawn catch.

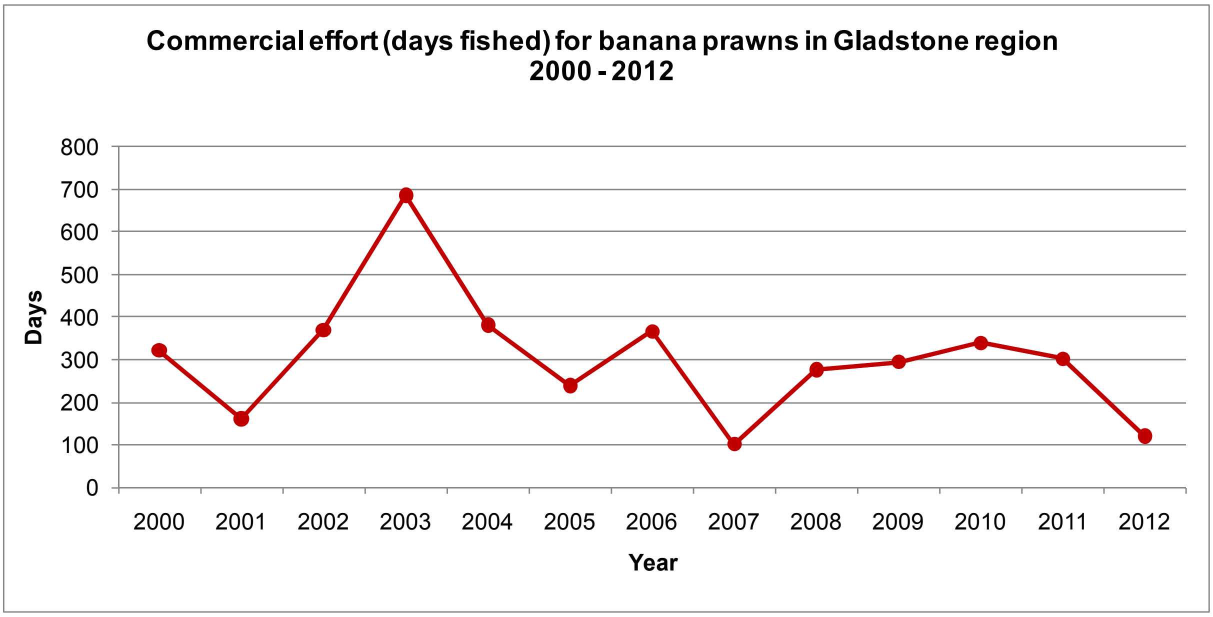

Figure 42. The number of days fishing where commercial fishers have reported banana prawn catch from the Gladstone region (CFish grid S30), as reported in commercial fisher logbooks (calendar years 2000 – 2011). Data includes both otter trawl and beam trawl catch effort for the region.

Observational findings Phase 1

Between October 2011 and February 2012, catches of banana prawns were observed and information on the external signs of health was recorded from a range of sites by Fisheries Queensland observers, as well as during targeted banana prawn surveys.

During October 2011, observers noted the small number of banana prawns caught (six prawns) while aboard a boat fishing for scallops in the Harbour were all in good health.

Trawl sampling in Gladstone Harbour in November 2011 caught 85 banana prawns with a single prawn infected by a parasitic isopod located under the carapace and one prawn showing signs of minor shell erosion. The remaining 83 prawns were observed to be externally normal.

A further four prawns were collected from the Gladstone Fish Markets. These prawns were caught on 11 November 2011 in an area opposite Auckland Point Wharf and provided to Fisheries Queensland by a local seafood wholesaler. A preliminary examination of one of them revealed a large parasitic isopod under the carapace. These prawns were provided to Biosecurity Queensland for further testing.

In December 2011, two banana prawns were caught in the trawl sampling in Gladstone Harbour. These prawns were in good condition. In the Fitzroy River, 173 banana prawns were caught and all observed to be in good condition.

During February 2012, Fisheries Queensland observers working aboard commercial fishing boats noted that of the 25 kg of banana prawns caught in the Gladstone Harbour, two prawns were infected with the gill isopod, while catches in the Fitzroy River (56 kg) and Bundaberg (4 kg) were all observed to be in good health.

While the above results refer specifically to banana prawns, a number of other prawn species were caught during sampling with samples provided to Biosecurity Queensland for further testing. Other species included eastern king, endeavour, rainbow and tiger prawns. Based on the same methods applied in the assessment of banana prawns, no signs of ill health were observed in these other species.

Laboratory testing Phase 1

Prawns showed evidence of shell erosion due to *Vibrio spp*. A number of prawns including banana, eastern king and tiger showed evidence of endoparasite infection by immature stages of tapeworm and gregarines. These parasites are normal in wild prawn populations and would not have had a significant impact on the health of prawns (Owen, 1983; Owen & Rothlisberg, 1985). As noted in the field observations, the isopod observed under the carapace was identified as a bopyrid isopod and is also common in wild prawn populations.

Tails of four healthy looking prawns and three prawns with visible abnormalities caused by bopyrid isopods from Gladstone Harbour were tested for chemical residues. Organics testing found no detectable levels of any chemical. Metal testing showed some differences between the samples, however without replication or any samples from a reference site, meaningful interpretation of this data is not possible.

Please refer to www.qld.gov.au/gladstoneharbour for a complete description of the pathology results, and the chemical residue test results.

Observational findings Phase 2

April 2012: During trawl sampling in April, the catch of banana pawns at each location was 150 kg or greater. Due to the size of the catch, observations were made from a sub-sample of at least 100 prawns at each location.

A single prawn was collected in the Gladstone Harbour and one from the offshore Fitzroy River site with shell erosion. A single prawn at Bundaberg and a single prawn from the offshore Fitzroy River sites were observed to be affected by an isopod parasite under the gill carapace. No other signs of ill health were observed in banana prawns caught during the trawl sampling.

June/July 2012: Observations were made from the complete banana prawn catch at both the Gladstone Harbour (approximately 1.5 kg) and offshore Fitzroy River sites (approximately 2 kg). Due to the size of the catch at Bundaberg (approximately 200 kg) observations were made from a sub-sample of 100 prawns.

Two prawns collected from the Gladstone Harbour and one prawn collected from the offshore Fitzroy River sites had minor shell erosion. Two prawns collected from the offshore Fitzroy River site and a single prawn collected from Bundaberg had isopod parasites located under their gill carapace (see Figures 43 and 44). No other signs of ill health were observed in banana prawns caught during the trawl sampling.

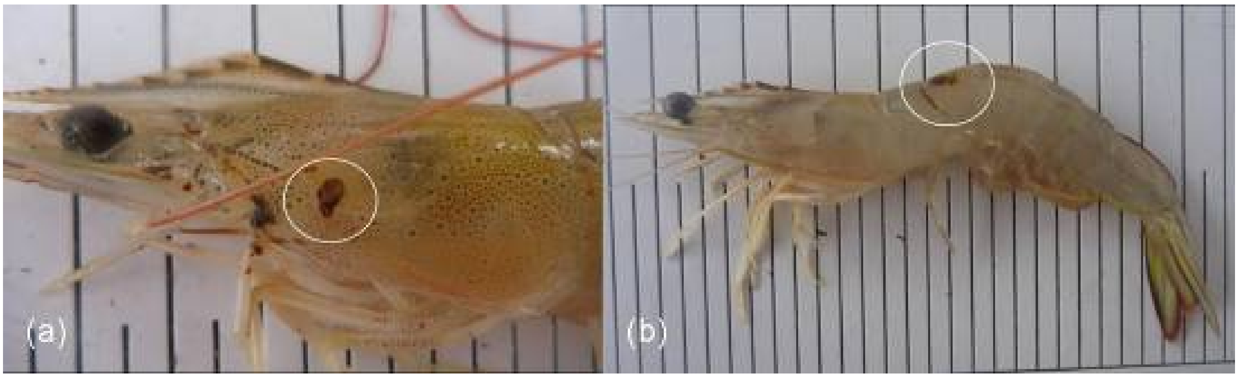

Figure 43. Banana prawns collected during the sampling program from (a) the Gladstone Harbour and (b) offshore Fitzroy River sites showing signs of shell erosion (circled).

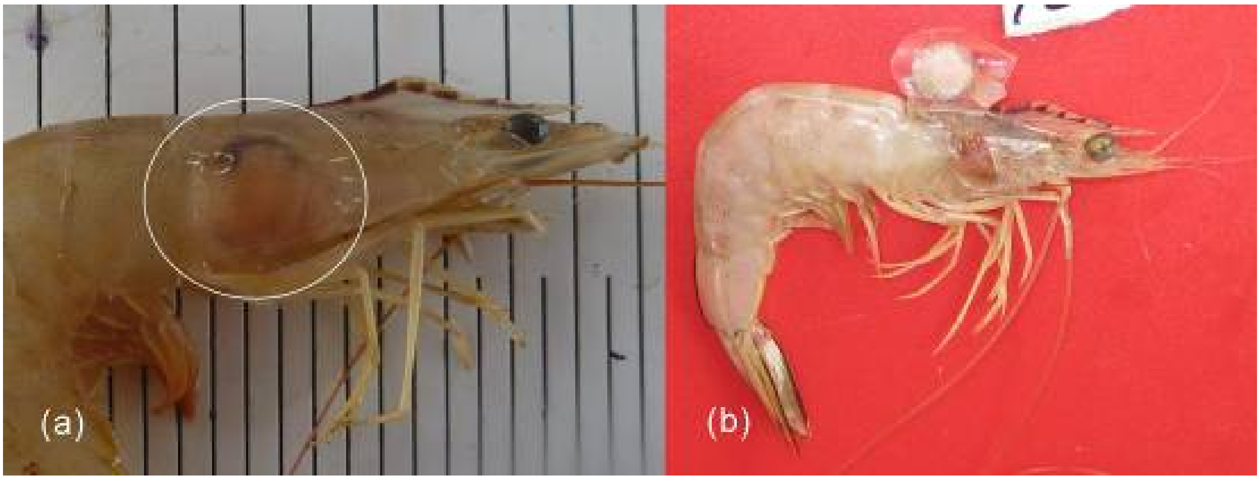

Figure 44. Banana prawns collected during the sampling program with the isopod present under the carapace (a) Prawn caught from the offshore Fitzroy River site with an isopod present under the carapace (circled); (b) Prawn caught at Bundaberg with part of its carapace removed to reveal the isopod.

Bopyrid isopods and larval stages of tapeworms are common parasites of prawns and are a normal occurrence in wild populations along the Queensland coast. The prevalence of bopyrid isopods observed during this sampling program was low across all areas (Gladstone and reference sites), suggesting a normal background prevalence in the population throughout. This information, along with the low prevalence of shell erosion observed throughout the study, provide no evidence of health issues in the wild banana prawn population in the Gladstone region.

Laboratory testing Phase 2

Samples collected for histopathology and residue testing during Phase 2 have been archived for future testing if required.

Trawl caught finfish species

Observational findings Phase 1

In November 2011, trawl sampling was conducted in Gladstone Harbour targeting prawns. Approximately 350 of each of the following finfish species were caught as incidental catch: river jew, anchovy, Australian threadfin and ponyfish. Three river jew displayed some minor redness on the ventral surface, and all other fish caught were in good condition. Three out of 31 herring caught showed injuries on the dorsal side between the dorsal fin and head.

In December 2011, trawled finfish (bycatch) were observed in catches from the Fitzroy River (149) and Gladstone Harbour (62). All fish caught by trawling were assessed as being healthy with no skin discolouration.

In February 2012, Fisheries Queensland observers monitored the health condition of fish caught in Gladstone by trawl net while aboard commercial fishing boats. In total, approximately 490 bycatch fish were observed. None of these fish showed any signs of skin discolouration.

Laboratory testing Phase 1

Seven river jewfish and one ghost grinner were submitted to Biosecurity Queensland in December 2011 from Port Curtis where they were collected floating on the surface in response to a report of a fish kill. Chemical residue testing and pathology investigations were conducted on these fish, and found no likely cause of death. Based on these findings, the fish were most likely caught by fishers and subsequently discarded as bycatch. See www.qld.gov.au/gladstoneharbour for more details.

Observational findings Phase 2

April 2012 sampling: The following numbers of the candidate fish were observed at the three sites during the April sampling event.

- Bundaberg: no grinner; 105 herring; 105 Australian threadfin
- Gladstone Harbour: 20 grinner; 20 herring; 100 Australian threadfin.
- Fitzroy River: 16 grinner; 15 herring; 100 Australian threadfin.

No significant signs of ill health were observed in trawl fish species caught during the trawl sampling from offshore Bundaberg, the Gladstone Harbour or from offshore of the Fitzroy River.

June/July sampling: The following numbers of the candidate fish were observed at the three sites during the June/July sampling event:

- Bundaberg: 18 grinner; 10 herring; 25 Australian threadfin.
- Gladstone Harbour: 26 grinner; 100 herring; 100 Australian threadfin
- Fitzroy River: 100 grinner; 100 herring; 100 Australian threadfin.

Two Australian threadfin caught at Bundaberg and a single Australian threadfin caught in Gladstone Harbour had isopod parasites present either on the gills or body surface. Other than the presence of the isopod parasites, no signs of ill health were observed in the fish species collected during the trawl survey at the three sites.

Laboratory findings Phase 2

The grinner, Australian threadfin and herring specimens were preserved fresh on-board a trawl vessel by a veterinarian with aquatic animal expertise. Other than gross observations mentioned above and length records, no necropsy data was collected for these species. A sub-sample of grinner was chosen for histopathology and chemical residue testing, and all other samples were archived for further testing if required.

Histopathology

Skin, liver and gill samples from each of the 25 fish were assessed histologically and rated in the same way as has been described previously for barramundi. There were no significant or notable differences between Gladstone Harbour and the reference sites for any tissues – the fish appeared normal. These fish tissues were preserved immediately following euthanasia to eliminate any deterioration of tissues and allow detection of very subtle abnormalities. There was no evidence of any unusual gill or skin irritation occurring in the fish from Gladstone Harbour.

Chemical residue testing

Aluminium, iron, copper and zinc were significantly higher ($P < 0.05$) in grinner muscle collected at Bundaberg (reference site) compared to other sites, but there were no other differences in metal concentrations between sites. Differences in metal concentrations between sites are normal and expected. This data indicates that the grinner caught in Gladstone Harbour had not been exposed to any chronic elevated levels of metals.

Pelagics

Sampling sites for pelagic fish species during Phase 1 included the Calliope River, Gladstone Harbour, Boyne River (upper and lower reaches), Rodds Bay, the Spoil Grounds as well as the Fitzroy River and Bundaberg (reference sites).

Sampling sites for pelagic fish species during Phase 2 included the Calliope River, Boyne River (lower reaches), Spoil Grounds, as well as the Fitzroy River and Bundaberg (reference sites). The candidate pelagic species for Phase 2 were queenfish due to conditions observed in this species during Phase 1 (Figure 45).

Figure 45. Queenfish caught at Bundaberg during the sampling program.

Observational findings Phase 1

During October 2011, golden trevally (1), snubnosed dart (2) and queenfish (2) were caught in Rodds Bay while targeting barramundi. These fish were all considered to be in good health with no signs of redness or lesions observed. A giant trevally was also caught in the upper reaches of the Boyne River that had minor redness.

During November 2011, while conducting a targeted prawn survey in the Harbour, one queenfish was captured and displayed damage to the snout and lower jaw, with some associated minor redness. Twenty small trevally (mixed species) were also caught during the trawl survey, but all were described as being in good health with no discolouration or lesions. A single queenfish was also caught during netting activities with no signs of ill health.

During December 2011, an oxeye herring caught in the Boyne River and a queenfish caught in Rodds Bay showed no signs of ill health. Sampling in the Calliope River caught a single oxeye herring observed to be in good health, however two of the six queenfish caught were observed to have skin discolouration with minor redness or red pin point marks on the body or fins.

During net sampling in January 2012, three golden trevally were caught in Bundaberg, two queenfish in the Calliope River and two queenfish in the Boyne River. None of these fish showed external signs of ill health. At the Spoil Ground, 42 queenfish were caught, of which four displayed signs of redness. These fish were also infected with calanoid copepods, with 41 of the 42 queenfish having copepods present on the body surface.

During February 2012, observations were conducted aboard commercial net fishing operations at Rodds Bay and at Colosseum (halfway between Rodds Bay and the mouth of the Boyne River). Two of the 18 golden trevally caught showed minor redness ventrally, while all five giant trevally caught were in good condition. All 40 snubnosed dart and 32 queenfish caught were in good health. No parasites were obvious on the skin of the queenfish examined. These results were recorded as Rodds Bay and summarised with Phase 1 data in Figure 46.

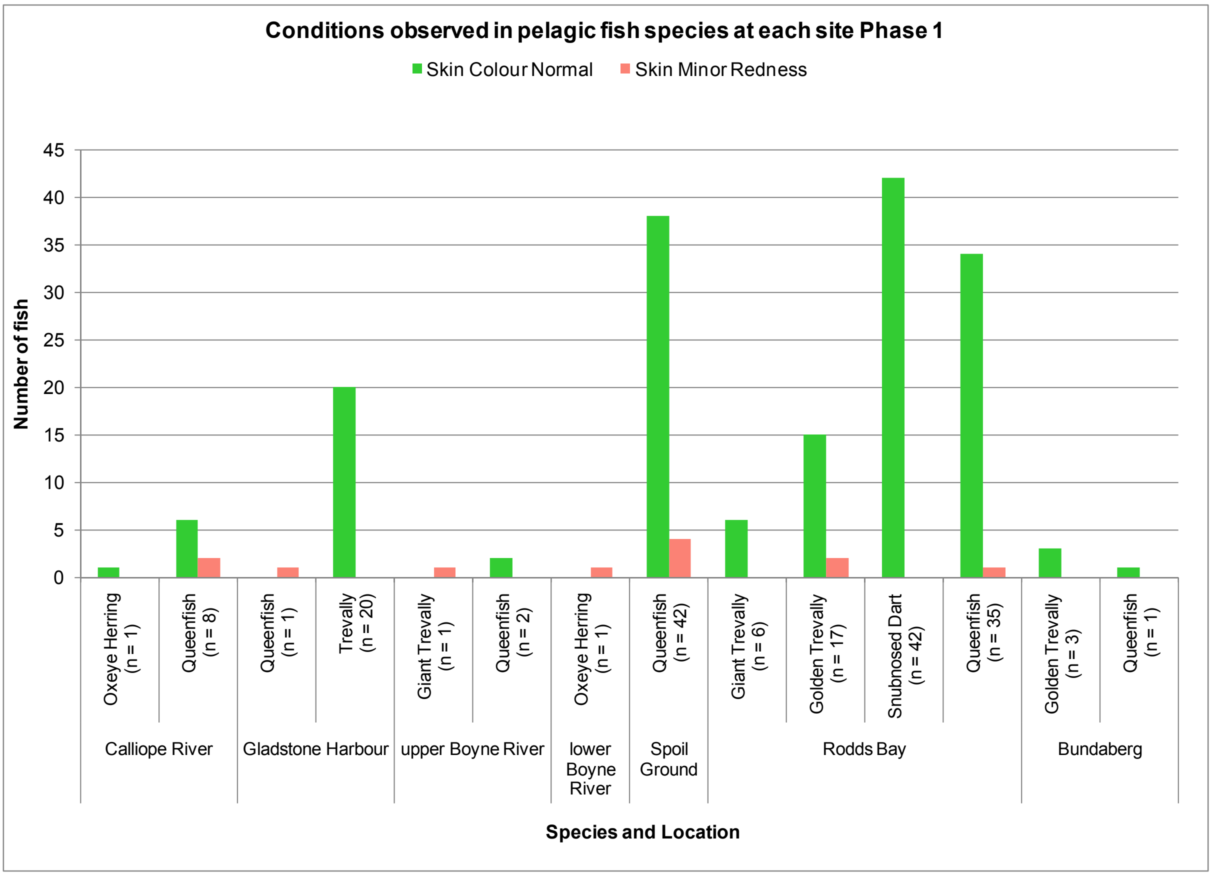

Figure 46. Conditions observed in queenfish and other pelagic species at each site during Phase 1. Note, (n) refers to the total number of fish examined at each site.

Histopathology and residue testing Phase 1

Two trevally and two queenfish were submitted to the Biosecurity Queensland laboratory during Phase 1. The trevally were submitted due to their skin redness. One of these trevally was provided in a private submission. Mild inflammation of the dermis and loss of epidermis was observed, but no bacterial or fungal pathogen was identified in the skin lesions. Two encysted parasites were found in the dermal layer of skin from one trevally.

The queenfish were submitted in response to concerns about cyanide discharge from local industry. The fish were effectively normal based on necropsy and histological examinations.

Observational findings Phase 2

April 2012: It was difficult to collect large numbers of the candidate species (queenfish) from all sites during the April sampling period. Consequently, other pelagic species captured during sampling were also observed and selected for necropsy examination. Examples of other pelagic species included grey mackerel, oxeye herring and giant trevally.

All fish were observed to have normal skin appearance (no redness). A blue threadfin was also caught at the Spoil Ground. Although it might not be considered a pelagic species, it was observed to have a laceration and was retained for necropsy examination.

Four of the five queenfish caught at the Spoil Ground had two to three copepod parasites each. Copepods were also present on the queenfish caught offshore from the Fitzroy River. One queenfish caught at Bundaberg had a minor lesion consisting of a small area of scales missing.

June/July 2012: Queenfish were observed at both the offshore Fitzroy River and Bundaberg sites, but were not captured at the Spoil Grounds during the June/July sampling event. Queenfish were captured during netting activities in both the Calliope and Boyne Rivers. Observations of these fish were recorded and included in the results.

A gold spot grouper, slate sweetlip and brown sweetlip were caught while sampling the spoil grounds. These species are all demersal species and were assessed for external signs of ill health according to the same protocols as all other species. The grouper and sweetlips appeared to be in good health with no signs of skin discolouration or lesions observed.

Queenfish with copepod parasites were observed from all areas except the Calliope River. The single queenfish caught in the Calliope River did have an injury thought to have happened during the netting process and was classified as a physical injury. While the prevalence of copepods observed on fish from two of the four sites was as high as 100%, most fish only had a few visible copepods present. Other than the presence of the copepod parasites, all fish appeared to be in good condition.

Phase 2 summary: Of the 83 pelagic fish observed during Phase 2, only two fish were observed with abnormalities including one queenfish with a small area of scales detached and one queenfish with a physical injury thought to have occurred during capture. Numerous queenfish were observed to be infected with calanoid copepods both from the reference sites and Gladstone. It is thought the copepod is a normal parasite for this host, however the normal parasite prevalence is not known. Results of observations made for pelagic species during Phase 2 are illustrated in Figure 47.

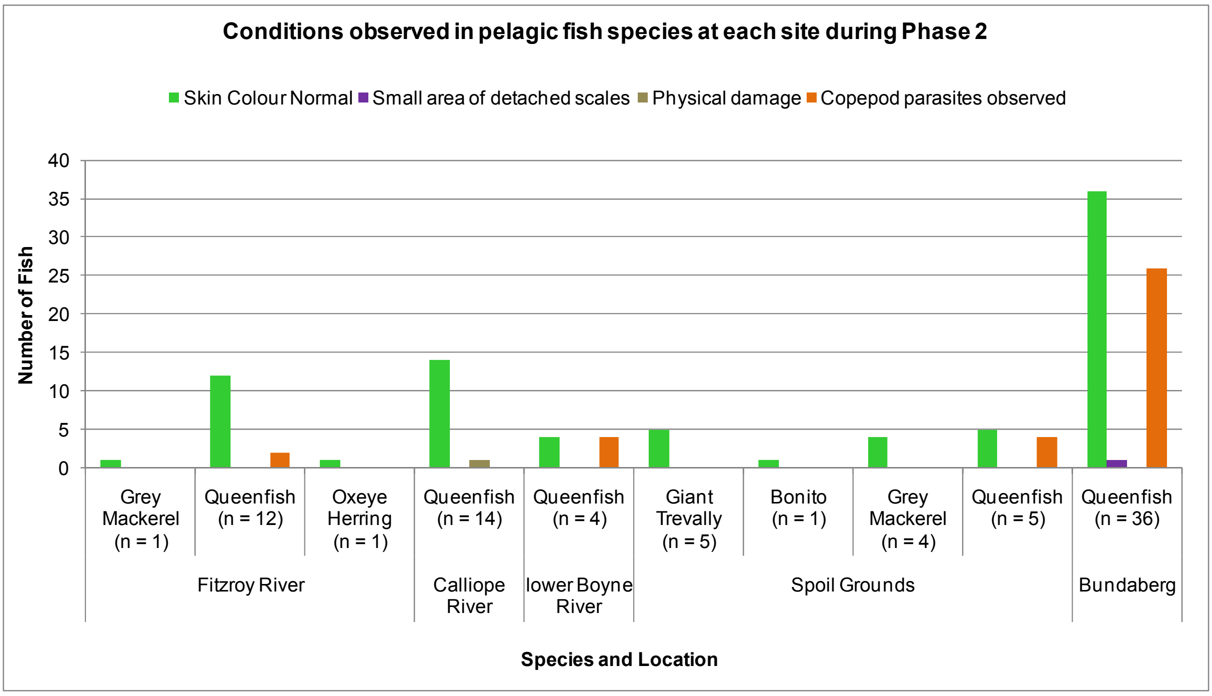

Figure 47. Conditions observed in queenfish and other pelagic species at each site during Phase 2. Note, (n) refers to the total number of fish examined at each site. Some fish may have been observed with multiple conditions.

Histopathology and residue testing Phase 2

Tissue samples collected for histopathology and residue testing have been held in archive for further investigation if required.

Other species

During Phase 1 of the sampling program, a large number of other species were observed, as summarised in Table 2. This included finfish, crustaceans and molluscs. These observations were made during routine observer trips aboard commercial fishing vessels, as well as during sampling trips targeting other species using net, trawl and pot fishing apparatus. The results of observations and associated pathology on other species is summarised below.

Scallops

Observational findings

Targeted sampling of scallops was conducted offshore from Gladstone with a commercial fisher on 29-30 October 2011. Sampling sites included areas adjacent to the Spoil Grounds. Some scallops identified by the fisher as appearing abnormal were preserved for histopatholgy. Signs of abnormalities included discoloured digestive glands and brittle shells. A sample of scallops was also retained frozen for residue testing.

In November 2011, scallops caught in waters offshore of Bundaberg by a commercial fisher were collected and forwarded to Biosecurity Queensland. These scallops were apparently healthy and were transported fresh for histopathology and residue testing.

Further scallop samples were collected in February 2012 from both Bundaberg and the Spoil Grounds offshore from Gladstone. These scallops appeared healthy, were frozen and forwarded to Biosecurity Queensland for residue testing.

Histopathology and chemical residue testing

Scallops collected from offshore of Gladstone and offshore from Bundaberg (reference samples) in October and November 2011 were examined histologically and were normal in all organs and tissues, with some parasitic infestation internally and in the gills of scallops from both sites. Composite samples of scallops from Bundaberg and Gladstone Harbour showed very little difference in tissue metal concentrations. Lead, mercury and silver were all below detection limits and, of the remaining 10 metals, only iron differed to any extent between samples, being higher in the reference site. No organic chemicals were detected.

Scallops collected in February 2012 were tested for relative concentrations of total and inorganic arsenic in tissues using four samples: two with pooled adductor muscles from multiple individual scallops and two with pooled visceral tissues. No organic contaminants were detected in any scallop samples. Metal concentrations were similar in the samples from Gladstone and Bundaberg except for iron, aluminium, nickel and copper, all of which were higher in the Bundaberg (reference) samples.

In testing for metal residue levels, arsenic levels were reported for total arsenic. However, in some instances, the appropriate standards are set for inorganic arsenic, and total arsenic should not be directly compared with standards for inorganic arsenic. On the basis of the first test results, this matter was further investigated to determine if there was a risk to human health. Results showed that the level for inorganic arsenic (As) in the two muscle samples was higher than the maximum level (ML) set in the Australia New Zealand Food Standards Code. The current legal limit for inorganic arsenic in molluscs is 1 mg/kg (wet weight), which is set out in the Table to Clause 2 of Standard 1.4.1 of the Code. The test results reported by the Queensland Health Forensic and Scientific Services Laboratory that are above the ML are as follows: Bundaberg scallop muscle: 1.5 mg/kg as inorganic As (wet weight), Gladstone scallop muscle: 1.9 mg/kg as inorganic As (wet weight).

Inorganic As is the combination of Arsenite (AsIII) as As (wet weight) and Arsenate (AsV) as As (wet weight). For this particular scallop species, the viscera are not considered to be part of the edible portion of the animal and therefore the Code does not apply to these samples. Representatives from Queensland Health, Department of Agriculture, Fisheries and Forestry and Safe Food Queensland discussed the results. To inform their discussion, the group requested additional information including sampling information; history of results from National Residue Surveys; comparisons with As levels in other foods and associated risk assessments; importing country requirements for seafood sourced from Australia; and typical processing procedures involving washing shucked scallops with filtered and sterilised seawater, which may reduce the final level of As in market-ready scallops.

The conclusion reached by these representatives on the significance of the test results was that they do not present an acute health risk. It is the chronic, cumulative effects of the excess intake of As that could potentially be a concern. However, the frequency and level at which scallops are consumed indicates it is unlikely to be a public health issue.

Finfish

Observational findings

During September 2011, 52 fish were caught and observed in the Fitzroy River including beach salmon (2), blue threadfin (20), catfish (11), king threadfin (13), pikey bream (4) and tripletail (2). No skin discolouration, lesions or other signs of ill health were observed.

During October 2011, blue threadfin (3) caught in the Narrows and Rodds Bay and a king threadfin caught in the Narrows were observed to be in good health, while a catfish and king threadfin caught in the Boyne River showed general redness on the skin and fins.

During November 2011, 401 finfish were observed from the Fitzroy River (9), Hamilton Point (1), Rodds Bay (200) and Bundaberg (191). Of these fish, a single whiting caught in Bundaberg was observed to have a lesion that was described as a recovering wound, and 60 of the 200 whiting caught in Rodds Bay displayed some minor skin condition. These mild skin conditions consisted of areas of slight discolouration, often with a single red dot under a scale.

During December 2011, observations were made of 87 finfish caught during both netting (52) and crabbing trips (35). During net fishing trips, other species of finfish were caught in the Calliope River (7), Hamilton Point (1), Rodds Bay (33) and the lower reaches of the Boyne River (11). Of these, eight fish showed signs of minor redness, while a black jew caught at Hamilton Point had more general skin discolouration. During crabbing trips in the Calliope River, the Narrows and South Trees Inlet, three fish were observed to have minor redness. These fish were a pikey bream from South Trees Inlet and two cod from the Narrows.

In January 2012, observations were made of 42 fish from the Gladstone area. Of these, one blubber lip (sweetlip) caught at Hamilton Point showed small areas of discolouration. Of 27 catfish captured from the Calliope River and the upper reaches of the Boyne River, 19 exhibited small areas of skin discolouration with an additional two showing discolouration more broadly across the body. One of these fish also had an abnormal eye. Four catfish caught in the upper reaches of the Boyne River had lesions present on the skin. A further 87 fish were observed from the Fitzroy River and Bundaberg (reference sites). The species were dominated by catfish (27) and bony bream (42) with javelin fish (17), and a spotted scat was also captured. All fish observed in the reference sites were considered to be in good health with no abnormalities observed.

During February 2012, observations were conducted aboard commercial net fishing operations at Rodds Bay and at Colosseum (halfway between Rodds Bay and the mouth of the Boyne River). Blue salmon (11), catfish (7) and a single sweetlip were amongst the catch. A single catfish was observed with redness to the pelvic fins, with the remaining fish showing no signs of ill health.

Histopathology and chemical residue testing

Testing has also been carried out on a wide range of bony fish species including whiting, goldspot grouper, spangled emperor, flathead and scat. Many of these samples were private submissions.

Skin abnormalities from other finfish species were generally mild and not due to *Neobenedenia sp.* Findings included localised skin inflammation, skin erosion, fibrosis (scarring), reddening, dermal haemorrhage (bleeding of the skin), epidermal necrosis (cell death) and oedema (swelling).

No bacterial, parasitic or fungal pathogens were found that could explain the skin conditions on other finfish species.

A range of bacteria were isolated from the fish, for example *Moraxella* spp, *Micrococcus* spp, *Proteus vulgaris*, and *Pseudomonas* spp. The lack of distinct invasion or proliferation on the skin and muscle lesions suggests that these bacteria were opportunistic and secondary colonisers following the initial skin damage. These bacteria are not considered to be fish pathogens (i.e. they are not primary infectious agents that cause disease in fish). They are normal microorganisms of the marine environment.

Conclusions

In 2011, a wide range of fish species in Gladstone were reported with health issues supporting the hypothesis that the Gladstone Harbour ecosystem as a whole was under stress. Barramundi provided the clearest and most consistent evidence for the cause of ill health. In the other species, there appeared to be a range of observed symptoms. Anecdotal reports were received of increased levels of rust spot disease in mud crabs, first characterised by Andersen and Norton (2001).

An investigation began in September 2011 to determine the cause of the reported issues and specifically to address public concern regarding the potential impact of industrial activity including dredging in Gladstone Harbour. The investigation, conducted by DAFF, was part of a whole-of-government response.

Barramundi

In September 2011, an external parasitic fluke *Neobenedenia sp,* was identified in barramundi from the Gladstone Harbour. *Neobenedenia* was identified as the cause of the observed eye problems and contributed to a number of the observed skin conditions in barramundi.

This parasite has previously been found in Queensland waters in the Hinchinbrook Channel between Hinchinbrook Island and mainland Queensland where barramundi are in high densities (Deveney, et al, 2001), and had resulted in the loss of approximately 200 000 cultured fish.

An additional factor was the significant number of barramundi (estimated 30 000) washed out of the Awoonga Dam in the flooding during 2010/11. Many of these fish would have received physical damage as a result of being washed over the spillway. This damage was reported by recreational fishes as physical damage and was documented in necropsies of barramundi undertaken by a veterinarian with specialist skills in fish and aquatic animals in 2012 after a second overtopping of Awoonga Dam. The fish showed damage to the jaw and head and usually presented with unilateral injuries to scales that appeared like a grazing along the body.

In the winter of 2011, the population of barramundi in the Boyne River was under extreme stress. The densities of these large predatory barramundi in the Boyne River were at higher levels than the system could naturally sustain, resulting in fish in poor condition and lower growth rates (Sawynok, et al, 2013). Their immune systems were further stressed by low winter water temperatures, resulting in an environment where the natural population of the parasite *Neobenedenia* could rapidly proliferate, and in turn resulting in observed eye problems and increases in fish exhibiting skin conditions (e.g. redness, loss of individual scales).

As the water temperature increased, and the densities of barramundi were reduced by fishing (130 t of barra were caught by commercial fishers in the third quarter of 2011), the remaining barramundi became healthier. In 2012 and in 2013, other than barramundi showing relatively minor physical damage, no significant health issues have been reported for barramundi caught in the Gladstone region.

These results are consistent with flooding and the significant number of barramundi being washed into the Boyne River. If the cause of the fish ill health continued beyond 2011, the health of the fish would not be improving and *Neobenedenia* infections would have been seen in September 2012.

Mullet and other finfish

Unlike barramundi, there did not appear to be a single symptom that characterised what was observed in other fish including mullet in Gladstone. Symptoms observed included loss of scales, small red pin point wounds, red colouration of fins and body areas, and increased levels of parasites.

One catch of queenfish in Phase 1 had a high frequency of fish with a high intensity infestation of *Lepeophtheirus spinifer* (sea lice). From the total catch of queenfish, 41 of the 46 fish had sea lice. This is the same catch of queenfish that was examined by Matt Landos and presented in his report.

In Phase 1, in addition to this catch, a total of 56 queenfish were observed in Gladstone. Of these fish, two fish were reported as showing no external signs of ill health, but parasites were not recorded as either being present or absent. Of the remaining fish, 13 were recorded as having no external visual parasites. The only time that parasites were recorded on queenfish in Phase 1 in Gladstone was this particular catch.

In Phase 2, of the 23 queenfish examined from the Gladstone region, only eight had ectoparasites. For the reference sites, 26 out of the 36 queenfish from Bundaberg examined had ectoparasites, while two of the 12 queenfish from the Fitzroy River had parasites.

It appears from the sampling conducted by Fisheries Queensland over an extended period of time that the percentage of queenfish with parasites is not unusual (64% in Gladstone compared with 58% in the reference sites). It appears that the school of queenfish caught on the Spoil Ground was unusual in both the percentage of fish infected with parasites and the level of parasitism. If high frequency and high intensity of parasitism was a continuing issue in Gladstone, Phase 2 sampling would have continued to show high levels of ectoparasites on queenfish. This did not occur.

It appears that the ecosystem in 2011 was different from periods before and after the reported fish health events.

The introduction of a large number of barramundi and other fish into the system would have caused a significant imbalance in the Gladstone Harbour in 2011. These large predators would have reduced the number of small fish and other prey items impacting on the food supply of other fish species. In addition, the lower salinity and turbidity caused by the flooding would have increased the stress on the ecosystem throughout Gladstone Harbour.

The fact that the Phase 2 survey of fish health in Gladstone in 2012 did not find a continuing problem in any of the candidate fish species supports the hypothesis that the observed issues were caused by a unique set of circumstances that occurred in 2011. This is consistent with a stressed ecosystem resulting from the freshwater influx, turbidity caused by the flooding and a large biomass of predators.

Sharks

Skin redness and areas of detached scales associated with parasitic infection were observed on a number of shark species caught during the sampling program from Gladstone and the reference sites. It is unknown whether the numbers of parasites observed on sharks during this program are abnormal. However, it is evident that these parasites and associated pathology are not unique to Gladstone. Severe skin redness was observed as a post-mortem effect, and this is also not unique to Gladstone.

Mud crabs

Fisheries Queensland did not observe any increase in the level of shell lesions in mud crabs in Gladstone Harbour compared with the reports in 1998 to 2001 (Andersen and Norton 2001). Fisheries Queensland observed 2253 mud crabs in Gladstone in 2011/12 (Phase 1 and 2 combined).

Summary

It is obvious from all the reports that something happened to aquatic life in Gladstone Harbour in 2011. The findings provided in this study indicate that the fish health in Gladstone Harbour had returned to a more normal situation in 2012. Other than the physical damage or recovery from physical damage, this study found the fish in a health status similar to that observed in the Bundaberg and Fitzroy reference sites.

All industrial activities in Gladstone that were operating in Gladstone in 2011 have continued to operate, including dredging. The only factor different in 2011 compared to previous years was the significant rainfall, the consequent flooding and the introduction of a significant biomass of fish from Lake Awoonga. Flooding has subsequently occurred in 2012 and 2013. However, other than continuing reports of barramundi showing physical damage as a result of being washed over the Awoonga spillway in these flooding events, widespread reports of sick fish have not been received by DAFF.

The introduction of the large number of barramundi into the Gladstone ecosystem would have caused the system to become imbalanced. There are a number of studies that show the impact of introduced and invasive species on ecosystem, which in this situation barramundi could be classified. Eby et al. (2006) found that stocking introduced fish resulted in predation, competition for food, and declines in animal abundance at different trophic levels, which in turn drastically altered these ecosystems.

The change in biomass of stocked fish is a gradual process commencing after the first introduction of hatchery reared fingerlings into new ecosystems. The ecosystem and species composition change slowly over time. This was not the situation in the Boyne River in 2011 where the biomass of barramundi was increased by 300 t in less than three months, having started from a very low base. The changes identified by Eby et al. (2006) were compressed into a short time frame, which did not allow the ecosystem to gradually change to a new equilibrium.

The stocking of Lake Awoonga with 4.5 million fingerlings since 1996 meant that prior to the 2010/11 flood, the Boyne River (including Lake Awoonga) was populated with more fish than the Boyne River would have naturally contained if Awoonga Dam was not present. Consequently, when these stocked fish moved downstream of Awoonga Dam, the biomass of barramundi was far in excess of the carrying capacity of the Boyne River. The population of barramundi in Gladstone Harbour also increased to higher levels than normal, as indicated by seven barramundi being caught while trawling for prawns. This population increase caused stress to the whole ecosystem.

The results in this study support that it was flooding combined with the introduction of a large number of barramundi that stressed the ecosystem in Gladstone. This study cannot rule out the possibility that the activity of dredging and associated turbidity provided additional stress to the ecosystem, but it was not the primary stressor.

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