Investigation of the Causes of Aquatic Animal Health Problems in the Gladstone Harbour and Nearshore Waters

Dr Matt Landos BVSc(HonsI)MANZCVSc

Director, Future Fisheries Veterinary Service

1 October, 2012

Report commissioned by the Gladstone Fishing Research Fund

Contents

List of Figures

List of Tables

Abbreviations

ANZECC-	Australian and New Zealand Environment Conservation Council
ARMCANZ-	Agriculture and Resource Management Council of Australia and New Zealand
BTEX-	Benzene, Toluene, Ethylbenzene, Xylene
DERM-	Department of Environment and Resource Management, now DEHP
DEHP-	Department of Environment and Heritage Protection, formerly DERM
EIL-	Environmental Investigation Level
FFVS-	Future Fisheries Veterinary Service
FQ-	Fisheries Queensland
GLNG	Gladstone Liquefied Natural Gas
GPC-	Gladstone Ports Corporation
GAWB-	Gladstone Area Water Board
PASS	Potential Acid Sulfate Soil
PCIMP	Port Curtis Integrated Monitoring Program
Port Curtis-	Gladstone Harbour is used alternately in documentation
LOR-	Limit of Reporting
SEL-	Sound exposure level
SPL-	Sound pressure level

1. Details of Report Author

Dr Matt Landos BVSc (Honsl) Sydney University, MANZCVSc,

Director, Future Fisheries Veterinary Service

- Full time registered veterinarian in Australia since 1995.
- Since 2000, my practice has focused entirely on aquatic animals.
- NSW DPI Aquatic Animal Health Veterinary Officer 2000-2005 (Pathology Laboratory and Field Based Fish Health Investigations)
- Member, by examination, of the Australian and New Zealand College of Veterinary Scientists, Aquatic Animal Health Chapter, since 2002.
- Private consultancy investigating aquatic animal disease outbreaks in farmed and wild stocks of fish (tuna, kingfish, barramundi, trout, salmon, murray cod, silver perch, cobia, catfish), crustacean (prawns) and molluscs (oysters, scallops) from 2005. Aquaculture clients in all Australian State's and the Northern Territory.
- Sydney University, Faculty of Veterinary Science, lecturer and associate researcher aquatic animal disease projects (FRDC ornamental fish virus project)
- Co-researcher on student investigations into barramundi and cobia parasitic diseases
- Lead investigator of Southern Bluefin Tuna Sudden Death Syndrome 2008.

- Lead field investigator of Noosa two-headed bass pesticide drift deformity case 2008-2012.
- University of Queensland, associate researcher, barramundi disease research ongoing
- Co-investigator with SARDI, Vic DPI trout disease research
- Co-investigator on Seafood CRC disease investigation (Yellowtail Kingfish Industry)
- Design and run training courses in disease identification and management for prawn, barramundi, mulloway and silver perch industry
- Presenter of aquatic animal health cases at numerous veterinary, wildlife pathology, aquaculture and waste water national and international conferences and meetings
- Co-author on several peer-reviewed aquatic animal disease papers
- Consultant to Brunei Darussalam for development of national aquatic animal biosecurity system
- Co-author of AQUAVETPLAN, national disease emergency response manuals
- Co-author of NSW DPI Emergency Disease Response Simulation, exercise 'kilpatrick'.
- Invited presenter, International Animal Welfare Conference: Fish Welfare 2008
- Numerous presentations on fish health at Australian Veterinary Association conferences 2005-2011, Australasian Aquaculture Conference 2012, Australian Wildlife Health Network Conference 2012.

2. Executive Summary

The Western Basin Dredging and Disposal Project is being undertaken in Gladstone Harbour.

On a weight of evidence scientific assessment (summarised in Table 1), the re-suspension of contaminated sediments by the Western Basin Dredging and Disposal Project has had significant consequences including:

-resuspension and mobilisation of contaminants from sediments causing toxic exposures in aquatic animals;

-increased parasitism of aquatic animals due to stress, immunosuppression and external irritation from poor water quality and toxicosis;

-generation of toxic algal blooms due to disturbance of sediments and release of nitrogen, iron and other nutrients.

-increased boat traffic increasing risk of boat strike to turtles and marine mammals;

- increased noise stress from increased boat traffic, pile driving, drilling and dredging

Currently, resuspension is spreading sediments over tens of kilometres around the dredging and disposal operations. Some of these sediments contain high loads of metals, metalloids and nutrients. These sediments and associated contaminants are likely to be harming reproduction of a range of local species including seagrass. Ongoing harm is being reported by local media outlets and commercial fishermen.

The contrary view that a freshwater influx led to the observed impacts on aquatic animal health is scientifically unsupportable. The reality is that Queensland coastal estuaries have for centuries received large freshwater influxes. The types of diseases observed recently in Gladstone have not been a feature of previous freshwater influxes.

The timing of onset of disease is consistent with the ramp-up of Australia's largest ever dredging project for Harbour development from May 2011 (dredging 25 million cubic metres of sediment, and ocean spoil disposal of 5 million cubic metres of sediment). The spatial distribution of disease in Gladstone Harbour is consistent with the distribution of resuspended sediments from dredging and disposal based on interpretation of satellite images (Petus and Devlin, 2012). There was a synchronous outbreak of disease in a wide range of aquatic species (fish, sharks, rays, crabs, shellfish, turtles, dolphins and dugong) as well as humans, from May 2011 onwards. The health of aquatic animals in Gladstone Harbour was compared to those from a reference site, 250km to the north. The extent and intensity of disease did not, and is not, occurring at the reference sites of Future Fisheries Veterinary Service (FFVS), nor those of Fisheries Queensland.

This report uses examples gathered from peer reviewed scientific literature to identify the likely mechanisms through which various risk factors have impacted the health of aquatic animals in Gladstone Harbour. There is sufficient evidence presented, and available, to ascribe likelihood to the roles various factors have played.

Table 1: Weight of evidence assessment of the plausibility and likelihood of various risk factors in disease induction in Gladstone Harbour

Proposed risk factor for observed diseases	Plausibility of risk factor to cause the observed lesions based on peer reviewed literature	Available evidence to support proposed risk factor as casual	Likelihood of significant causal role
Freshwater	Nil. Based on the lesions observed in fish and crabs detailed in histological reports which cannot be caused by freshwater exposure.	Nil. Timing does not fit with outbreak. Areas remote to Gladstone with similar or larger volume of freshwater influx had no similar aquatic animal disease outbreaks. Previous freshwater influxes into Gladstone have not precipitated any similar events. Freshwater alone does not cause rust spot lesions in crabs, nor dermatitis in fish. Freshwater is lethal to the marine parasites observed on barramundi and bull shark skin, yet the parasite intensities were high.	Very low.
Metals exposure immuno-suppression	Yes. Exposure and uptake can trigger immunosuppression.	Yes. Multiple exceedances of ANZECC guideline of multiple metals over extended periods of time coincident with dredging. Elevated metals levels detected in some tested biota (low sample numbers limit power of analysis). Dissolved metal levels in water were lower prior to dredging. High parasite intensities identified grossly and in histology are consistent with fish, turtle and scallops that are immunosuppressed.	Very high.
Metals exposure direct tissue effects	Yes. Exposure to elevated levels of dissolved metals can cause acute effects on fish skin and impairment of olfactory function.	Yes. High levels (inc aluminium, zinc, arsenic and copper) recorded by DERM/DEHP, sufficient to cause observed symptoms in fish sharks and rays, including increased mucous secretion, skin lesions, and thin body condition due to failure to feed.	Very high.
Metals exposure shell disease in crabs	Yes. Uptake of elevated levels of copper and zinc can induce rust spot lesions in crabs.	Yes. Prevalence survey indicates elevated prevalence of rust spot in Gladstone mud crabs compared to reference site. FFVS pathology demonstrated lesions in Gladstone crabs are morphologically consistent with metal induced endocuticle shell disease. Post dredging elevations in dissolved copper and zinc reported by DEHP.	Very high.
Toxic algae (fish impact)	Yes, plausible cause of fish skin and gill damage, and elevated blooms can trigger irritation/stress/ death.	Limited, details of reports of algal blooms have not been released by GPC/DERM. Yes, elevated Chlorophyll monitoring levels. Blooms not detected in all locations where FFVS identified sick fishes. Toxic algae not known to cause shell lesions in crabs.	Uncertain (likely only partial role)
Toxic algae (human impact)	Yes. *Lyngbya* known for its dermatotoxins.	Yes. *Lyngbya* identified in association with sick fishermen, and in algal monitoring study by FFVS. Lesions in many people consistent with *Lyngbya* exposure after the commencement of major dredging and	Definite.

		never before in Gladstone Harbour fishers.	
Turbidity-increased suspended sediment directly causing stress	Yes. Exposure and uptake of fine sediment can change behaviour and impact on fish and mollusc immune response. Yes, elevated turbidity can smother seagrass with sediment and reduce its health. Yes, elevated turbidity can reduce light available to seagrass.	Yes. Numerous exceedances of permit conditions for turbidity levels, in the absence of significant rainfall. When dredges broke down prior to UNESCO March 2012 visit, turbidity in Harbour improved. When dredging increased to seal bund wall in late July early August 2012, turbidity had a sustained increase. Leaking bund wall acknowledged by GPC to be contributing to elevated Harbour turbidity. JCU satellite study suggests dredge plume extends 34km.	High (however contaminants associated with this resuspended sediment likely to be much more important).
Noise	Yes. Documented to impact on communications between fish, and potentially impact reproductive behaviours. Affects mollusc feeding.	Yes. Massive increase in boat movements across Harbour to Port Curtis estimated to have exceeded 20,000 per month since Curtis Island development commenced.	Very high (however contaminants likely to be much more widespread impact, extending up estuaries eg Colosseum, Boyne and 7 mile.
Habitat/food loss	Yes. Nutritional stress can cause immunosuppression.	Yes. Large area of seagrass documented to have declined and large benthic area lost to reclamation area, and mangroves cleared for wharf development. Loss of seagrass reduces marine ecosystem productivity through diminishment of food web contribution	High (however contaminants likely to be much more important in short term acute diseases). Long term loss of productivity highly likely.
Parasitism	Yes. Can cause lesions observed, but typically requires a concurrent environmental/ husbandry stressor.	Yes. High parasite loads on some, but not all diseased animals. Higher intensities and prevalence of infestations compared to reference site. Indicating elevated parasite intensity may have been secondary to primary immunosuppression in Gladstone.	Very high – but only as a secondary factor to immunosuppression and skin damage.
Other sediment based toxicants	Yes. Ammonia, hydrogen sulphide and hydrocarbons can all cause similar disease conditions to those observed. Yes, ammonia and hydrogen sulphide known to occur in intertidal sediments in particular, and legacy hydrocarbons from spills and local shale oil deposits known to be present.	Insufficient monitoring of these parameters has taken place to determine if exposure has taken place or not. Many of the monitoring sites are too remote to the site of dredging and development on Curtis Island to detect volatile compounds.	High – but effects likely to be more localised.
Primary bacterial disease	Yes. *Streptococcus agalactiae* association with wild Qld Groper mortalities – but unlikely	No. Negative bacterial cultures on numerous sick animals, and pathology does not indicate the presence of primary bacterial infections in diseased animals.	Very unlikely that primary bacterial infection can explain the

	to affect so many different species at the same time. Yes, bacterial disease in molluscs can result in high level mortalities.	Where present, infections are considered to be secondary opportunists on compromised or damaged hosts (scallops, and mud crabs).	observed sickness and mortalities.
Viral disease	Yes. Can cause wild fish disease outbreaks, but unlikely in fish, turtles, dolphins and crabs simultaneously.	A few sharks had pancreatitis of unknown cause (potentially viral). However the bulk of the animals examined had no evidence from large number of histological samples of viral disease.	Very unlikely to be the primary cause of observed sickness and mortality.

3. Introduction

Future Fisheries Veterinary Service (FFVS) was commissioned by the Gladstone Fishing Research Fund to undertake a veterinary investigation into the health problems reported, from May 2011 to the present, in aquatic animals around Port Curtis, Gladstone, Queensland Australia. The project took place from January–August 2012.

4. Aim

To undertake a veterinary disease investigation of aquatic animals in and around Gladstone Harbour, with comparison to a remote reference site; and to assess the likely causes of observed diseased aquatic animals using field and laboratory diagnostic tools.

5. Methods

a) Collection of case history

Information was gathered by interviews with commercial fishers and Gladstone Area Water Board management and hatchery staff. Further history was obtained from Gladstone Ports Corporation website, Queensland Fisheries website, Department of Environment and Resource Management website and information supplied by the Gladstone Area Water Board.

b) Sampling sites

Sites were selected to be representative of the spatial extent of the fish health problems reported around Gladstone Harbour. Gladstone sampling of aquatic animals and water took place in January and late February 2012, for all samples except a sample of saucer scallops which were collected in June 2012, due to previous trawl efforts in January and February failing to catch any saucer scallops.

Gladstone aquatic animals and water were compared to samples of similar species of aquatic animals at reference site, Stanage Bay, Thirsty Sound ~250km north of Gladstone. This site was picked because it had significant freshwater influx at the same time as Gladstone, however there are no Harbour development activities in this location. The reference site was sampled mid-April 2012. Sampling of saucer scallops, coral and prawns did not take place at the reference site due to equipment restrictions and cost constraints.

Details of sample site locations and dates are found in Appendix 11 of this report. Maps showing the areas of sampling are illustrated in Figures 1-7.

Figure 1: Algal sample sites- Gladstone

Figure 2: Algal sample sites- Reference site: Stanage Bay.

Figure 3: Fish sample sites- Gladstone

Figure 4: Fish sample sites- Reference site, Stanage Bay.

Figure 5: Crab sample sites- Gladstone

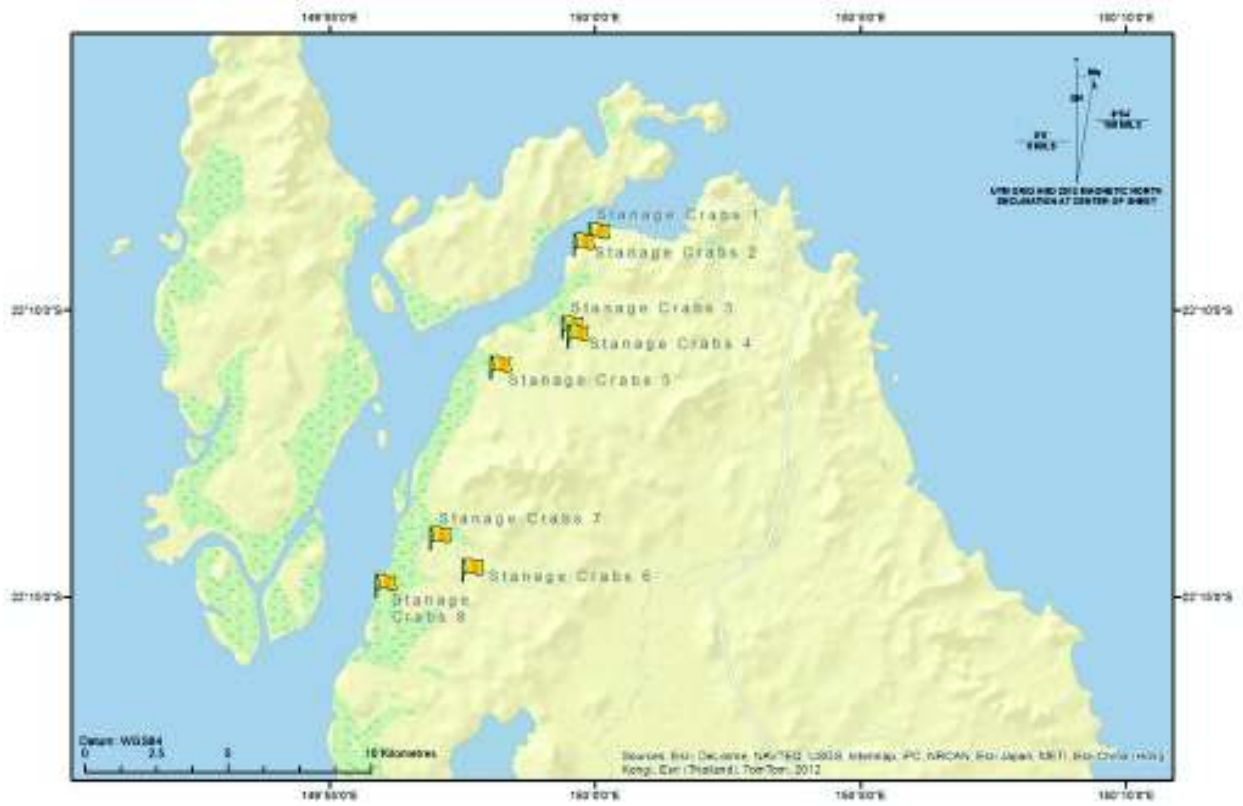


Figure 6: Mud crab sample sites- Reference site, Stanage Bay

Figure 7: Saucer (trawl 1, 5, 6) and rough shelled scallop (trawl 3) sample sites- Gladstone

c) Sampling equipment

Water quality was measured with a YSI oxygen probe, TPS pH/salinity/temperature probe and a secchi disc.

Algal samples were collected with a 25µm plankton net, and preserved with Lugol's iodine.

Fish were captured with commercial gill nets, cast nets or prawn trawl nets. Fish sampled were typically alive immediately prior to sampling, and were preserved in 10% buffered formalin on board the sampling vessels.

Microbiological swabs from fish were plated onto Horse Blood Agar prior to shipping to the laboratory.

Crabs were caught using commercial crab pots. The surface of the carapace was cleaned/scrubbed to ensure all rust spot lesions could be visualised.

Prawns were captured with commercial prawn trawl equipment.

Scallops were captured with commercial prawn and scallop trawl equipment.

A single freshly dead turtle was convenience sampled when observed on sampling trip.

Coral which became entangled in sampling equipment was convenience sampled.

d) Sample selection

Animals exhibiting any visual signs of disease were identified on board commercial fishing vessels, and preferentially necropsied, with tissues preserved immediately for histopathology. A selection of animals exhibiting gross signs of disease also had microbiological swabs collected from them for culture. Where no grossly abnormal animals were observed, as was largely the case at the reference site, sampling took place on a convenience basis of around five fish per selected species (where available).

e) Number of animals observed and/or sampled at each sample sites

Maps demonstrating the location of samples are contained in the body of the report, under each type of aquatic animal sampled. A mud crab (*Scylla serrata*) shell lesion prevalence study was undertaken using the methods and scoring system developed by (Andersen L. , 2003) in response to previous sampling indicating the presence of shell disease in Gladstone mud crabs.

Table 2: Summary of FFVS aquatic animal examinations and sampling

Aquatic Animal Type	Gladstone animals (n)		Reference site animals (n)	
	Gross	Histology	Gross	Histology
Gill net caught fish	293	29	132	16
Cast net caught fish	63	2	0	0
Gill net caught elasmobranch	61	8	6	6
Coral	2	2	0	0
Oysters	>300	0	>300	0
Mud crab	715	15	153	5
Trawl – prawn	~2500	7	0	0
Trawl – fish	1170	8	0	0
Trawl – saucer scallop	10*	10	0	0
Trawl – rough shelled scallop	4	4	0	0

Hawkesbill Turtle	1	1	0	0

* 5 scallop were collected close to the spoil ground, with a second 5, collected on a wider trawl shot East of the spoil ground.

Complete details of all animals sampled, sites, and cross referencing to pathology reports is presented in Appendix 11, Table 11.

6. Results and Weight of Evidence Assessment

a) History timeline

15 Jul to 9 Nov 2010: "Sealift 3", Geotech drilling on Jack Up drill barge, performed between Fishermans Landing and Hamilton Point Curtis Island in the Western Basin (Mariners Notice 594T of 2010).

2 Sept to 16 Sept 2010: Cutter suction dredge Wombat to conduct 24 hours per day dredging to create construction docks in the middle and upper harbour areas in Gladstone (Mariners Notice 582T of 2010). It is not known whether this work was undertaken.

15-29 Nov 2010 and 3-16 Dec 2010: Drill barge "Shine" undertaking borehole drilling with support vessels near Targinnie creek, Western Basin (Mariners Notices 973T, 979T, and 1009T of 2010).

12 Oct 2010 to Feb 2011: Mariners Notice 844T (2010) notes the cutter suction dredge Wombat commenced 24 hour per day dredging activities on 12 October 2010 near Curtis Island and also at RG Tanna wharf.

Dec 2010: 30 000 m^3 of material was removed from the RG Tanna coal terminal aggregate dock and disposed of at the RG Tanna mainland reclamation area. Additional 200 000 m^3 removed – unknown where deposited. This activity was not part of the monitoring program.

7 Dec 2010 and ongoing into 2011: pile driving dock construction at North China Bay, Western Basin. Multiple barges servicing transport of pylons and materials from RG Tanna loading dock (Mariners Notice 1039T of 2010).

Dec 2010–Jan 2011: Large volume of freshwater influx into Gladstone Harbour, with overtopping of Awoonga spillway and release of large volume of hatchery reared, stocked barramundi.

Jan to May 2011: large volume (~200 tonnes) of visually normal barramundi (T. Falzon pers. comm. 2012) were captured. These escapees from Awoonga Dam were captured in and around the Boyne River when it was running with freshwater overflow from the dam.

Jan – Feb 2011: The Wombat dredging was to conclude on 18 January 2012; however workers advise that rock was encountered. Subsequently the backhoe dredge Big Boss was brought up in January 2011 to remove the rock, with this initial dredging at Curtis Island concluding in early February 2011. 343 426 m^3 of material was removed from adjacent to Curtis Island. Disposed of at the Fisherman's Landing reclamation area in absence of monitoring program.

Jan 2011: *"An unprecedented amount of construction activity is being carried out in the middle and upper harbour areas (Barney Point to Grahams Creek) in the Port of Gladstone. Activities include (but are not limited to) movements of barges, ferries, workboats and tugs in addition to normal shipping."* Mariners Notice 19 of 2011

25 Jan to 25 Feb 2011: Seismic Surveying throughout Gladstone shipping channels. (Mariners Notice 059 T of 2011)

Jan to Aug 2011: Construction of 8 km bund wall reclamation area created by dropping thousands of tonnes of large rocks and fill into the Fishermans Landing tidal mudflats and enclosing some areas of

seagrass meadows. 50 metres per day is constructed. Satellite images demonstrate resuspension plume adjacent bund wall during construction in May 2011.

Feb 2011: Gladstone Area Water Board (GAWB) sampled of barramundi in Awoonga dam and found them to be healthy. There was a zero prevalence of red skin rashes.

15 Feb 2011: Fish kill reported in Gladstone Marina, no sampling undertaken (Gladstone Observer, 2011f)

3 Feb 2011 to 22 Feb 2011: Gladstone Harbour Master has confirmed the dredge Brisbane conducted dredging operations in Gladstone shipping channels from 3 February 2011 until 11 February 2011 and then again from 14 February 2011 until 22 February 2011. Dredging was to remove large amounts of flood sediment and was disposed at the East Banks ocean disposal site. No Mariners Notices were found in relation to this dredging activity.

10-25 Mar 2011: Sick and dying turtles reported by commercial fishers around mouth of Boyne River (Falzon, T. pers. comm. 2012)

Apr 2011: Sick fish(shark, cobia, queenfish, jewfish (not barramundi)) reported at spoil dump ground with skin redness with high mucous excretion, and nets coated in 'slime'. Barramundi and other fish in freshwater reaches of Boyne River remain healthy.

10 April 2011: Dead turtles reported at mouth of Boyne River. (Downey, K. 2011)

1 May 2011: 60 day ban on net fishing at mouth of Boyne River announced.

May 2011: Three dead dolphins found around Gladstone Harbour in month of May (Gladstone Observer, 2011c).

1-29 May 2011: Seismic Surveying throughout Gladstone shipping channels (Mariners Notice 373 T of 2011)

May to 30 June 2011: Barge ramp created from 40 metres off Curtis Island by dropping large quantities of rock into the harbour each 2 days (Mariners Notice 455T of 2011).

20 May 2011 and ongoing as at Sept 2012: Two backhoe dredges commence large scale dredging 24 hours per day, 7 days per week, in areas of mapped potential acid sulphate soils (PASS) adjacent Curtis Island. “Big Boss” backhoe dredge was one of the dredges operating which was reported to be removing 3500m^3 per day. Dredge spoil was barged to the East Banks Ocean Disposal site, near the entrance of Gladstone Harbour.

20 May to 26 Aug 2011: Gladstone Liquefied Natural Gas (GLNG) dredging 24 hours per day, adjacent its facility in South China Bay, Western Basin. Equipment used included Jack Up barges, excavators and tugs and barges to dispose of material at East Banks Ocean Disposal site.

May 2011: Large increase in turbidity in harbour with exceedance of 99th percentile at monitoring sites.

Mid-May 2011: Gladstone Area Water Board (GAWB) note marked deterioration in water quality in their hatchery intake in Auckland Creek, adjacent Gladstone Harbour.

May 2011: More sick fish begin being observed near dredge spoil ocean dump ground, with excessive mucous secretions and skin redness. 'Slime' reported on fishing nets.

Jun 2011: Sick ulcerated barramundi captured near hot water outlet in Calliope.

Jun 2011: Two dead dugong reported in Gladstone Harbour (Gladstone Observer, 2011c)

Jun 2011: Ulcerated crabs and increased rust spot prevalence reported by commercial fishers.

6 June 2011: Coral Trout with skin lesions unloaded in Gladstone Harbour (Munn H. Pers. comm. 2012)

8 Jun 2011: Dredging operations were underway throughout the upper reaches of Gladstone harbour between Laird Point and Hamilton Point. Mariners Notice 517T of 2011 confirms numerous dredges were operating 24 hours per day within the area, along with tugs and barges taking spoil to the East Banks Ocean Disposal site.

15-19 Jul 2011: The dredge Brisbane dredging shipping channel for maintenance.

Jul 2011: Dead dugong reported in Harbour, and dead turtle on Facing Island (Sparkes, D. 2011a)

17 July 2011: Dead turtle on Tannum Sands beach, adjacent Gladstone Harbour (Sparkes, D. 2011b)

Mid-Jul 2011: First sick fish with new distinct lesions (pop-eye) reported in Boyne River.

Jul 2011: Pile driving and marine construction activities continue at Barney Point wharf, 24 hours per day (Mariners Notice 547 T of 2011).

Aug 2011: Dead stingray washed up (Blatchford, E. 2011)

Aug 2011: 20 625 m^3 removed from adjacent to Fisherman's Landing and disposed of to the east bank offshore disposal area.

Aug 2011: Seismic survey work continues in Gladstone waterways. (Mariners Notices 673T, 722T, 740T of 2011)

Aug 2011: Barramundi with eye lesions from Calliope River reported (Sparkes, D. 2011c)

Aug 2011: Reclamation bund wall closure completed (however it leaked dredge spoil, lime, and fill increasing harbour turbidity).

Aug-Oct 2011: Backhoe dredge 595 000 m^3 of material was removed from adjacent to Curtis Island and disposed of to the east bank offshore disposal area. Cutter suction dredge 395 000 m^3 of material was removed from Gladstone Liquefied Natural Gas berth pocket and disposed to the Western Basin reclamation area.

Sept 2011: Dredging at large scale in multiple locations continues 24 hours per day. Involves numerous dredging operations are being carried out simultaneously in Gladstone harbour between Barney Point and Grahams Creek. Various types of dredging plant is being used including cutter suction dredges, backhoe dredges, trailing suction hopper dredges, booster stations, hopper barges, dump barges, tugs and workboats. (Mariners Notice 846T of 2011)

30 Sept 2011: Dredging stopped due to exceedances of turbidity (Sparkes, D. 2011d)

Aug 2011 and ongoing: 700 000 m^3 of material is being removed from the material off-loading facility for Queensland Gas Corporation by bucket dredge Tavos and backhoe dredge Big Boss and disposed to the East Bank offshore disposal area. Substantial area of Western Basin dredge spoil disposed into Fishermen's Landing Bund from cutter suction dredges.

Aug 2011: Barramundi reported in saltwater reaches of Boyne River with heavy parasitic skin infections, and red eyes. Catfish mortalities reported.

Aug 2011: Fish kill Facing Island: involved turtles, crayfish and small crabs (Busteed, B. via Ted Whittingham pers. comm. 2012 and Gladstone Observer, 2011a). Turtle and dugong deaths reported since.

Late Aug–Sep 2011: >40 Fishermen/others develop unusual skin lesions, most undiagnosed at the time.

Sep 2011: The Narrows: involved salmon, triple tails, barramundi, and turtles. (commercial fishers reported incident to EPA)

19 Sep 2011: Hundreds of sick barramundi reported in the Boyne River (Gladstone Observer, 2011d)

Sep–Oct 2011: Toxic algal bloom in harbour reported through leaked document to Courier Mail – reports still not publically available as at September 2012.

Oct 2011: Fish kill Turkey Beach: involved turtles and several species of fish (Nathan Fox, pro fisher, 0488 437 109 reported it to EPA and gave them a bottle of scum they were floating in.)

Nov 2011: Dead turtles reported on Curtis Island. (Gladstone Observer 2011b)

Fish kill Facing Island: involving multi species of fish (source Gladstone Observer)

Dec 2011: Fish kill Boyne River: barramundi and catfish (Chris Sipp, reported incident to EPA)

Dec 2011: Crabs with shell disease from Gladstone Harbour reported (Gladstone Observer, 2011e)

Jan–Feb 2012: FFVS sampling demonstrates high percentages of diseased fish/crabs/scallops across entire area contacted by dredge spoil resuspension.

Apr 2012: FFVS samples reference site, Stanage, Thirsty Sound ~250km north of Gladstone demonstrates low disease prevalence in fish, sharks and crabs.

April 2012: Fish kill Boyne River: catfish (Sparkes, 2012a)

May 2012: Fish kill at NRG Power Station on Calliope River: hundreds of fish of various species (Irving, 2012)

May 2012: Fish kill 7 Mile estuary: turtles, queenfish, barramundi (Gary and Brad Otto, pers.comm. 2012).

Jul 2012: Images of bream with red skin lesions from narrows. Rust spot crabs still present in commercial catch. FFVS measures secchi disc measure of water clarity in Western Basin on moderate tide, in absence of rainfall. Clarity was only 10cm.

Aug 2012: Images of herring and blubberlip bream from 7 mile estuary catch with red erosive skin lesions. 6 turtle strandings in Gladstone Harbour.

Sep 2012: More dead dugongs and turtles reported, barramundi reported floating dead in harbour, barramundi with red skin lesions and eye lesions caught in harbour trawl and Calliope river (Queensland Telegraph, Mikkelsen, 2012).

Temporal and spatial epidemiological observations

- Multiple fish species synchronously affected in Gladstone Harbour, most of which did not come over the Awoonga Dam wall as they are strictly estuarine or marine fish (e.g. Queenfish, blubber lip bream, bronze whaler sharks, threadfin and blue salmon).

- Concurrent with observed fish sickness: turtles, dolphins, dugongs suffer substantial unexplained mortality increases in Gladstone area, well above historical baselines, and well above levels observed along adjacent areas of the coast.
- Concurrently substantial increase in human skin/eye cases linked to fish/Harbour water exposures.
- The documented reports of grossly diseased and dead fishes, turtles and dugongs are ongoing to 5 September 2012. This is now >20 months after the freshwater influx. It is incoherent to suggest that a freshwater influx could still be the cause of ongoing acute ulcerative skin diseases that are occurring on animals living in full strength seawater.
- Time of onset of diseased fish was >5 months after freshwater influx to Gladstone Harbour, and coincident with appearance of marine parasites on some, but not all affected fish. The marine parasite *Neobenedenia* does not survive in freshwater. Similarly the *Dermophthirius* sp. found on some bull sharks does not survive in freshwater. Indicating that the sharks and barramundi only contracted these infestations after contact with seawater from the Harbour. By this time the water quality in the harbour had deteriorated due to the dredging and disposal resuspension plume.
- Time of onset of disease observations in the Boyne River (2nd week of July) was also coincident with cessation of overflow of freshwater from Awoonga Dam, and increasing salinity from incursion of high turbidity Harbour water into the Boyne river and other estuaries.
- Fish/dugong/turtle/dolphin/humans to south at Bundaberg and to north in the Fitzroy (where an even larger influx of freshwater was experienced) remain largely normal.
- No use of silt curtains to control dredge or disposal plumes.
- No additional controls for dredging in high potential Acid Sulfate Soil(ASS) areas.
- No cessation of dredging based on current velocities and large tidal movements.
- 11-month delay to try and seal the leaking bund wall which was contributing to Harbour turbidity.
- Fishermen first report fish (shark, cobia, queensfish and jewfish (but not barramundi)) with increased slime, and slime on their nets when fishing around the ocean spoil dumping ground in April 2011 (T. Falzon and G. Otto pers. comm.) when ocean disposal of dredge spoil was taking place from the Brisbane dredge, which was undertaking channel maintenance and other works on tug mooring areas.
- The coral reef in front of Facing Island was established with several species of corals as noted in a survey prior to the major ocean disposal in April 2011 (Ocean Maritime Consultants Pty Ltd, 2011). This area of coral reef has developed over the past ~10 000 years. During this length of time it is likely that there have been numerous heavy rainfall events of the scale experienced in December 2010 to January 2011. It is apparent that these previous rainfall events were insufficient to kill off the coral. Surveys of the area in April 2011, after the flooding, but before the large scale ocean dumping of dredge spoil, demonstrated that the coral was still alive at that time (Ocean Maritime Consultants Pty Ltd, 2011). However in February 2012 FFVS observed and sampled coral which had proliferative mucous cells on its surface, and bacterial infections, on the oceanic side of Facing Island-Gladstone. This location is exposed to resuspended sediments from ocean disposal of dredge spoil.
- The prevalence of rust spot lesions on mud crabs showed an increasing trend with proximity to the Western Basin Dredging when monitored in January and February 2012. Prevalence of shell disease in all Gladstone Harbour sites was elevated compared to the FFVS reference site.
- The distribution of reported and observed sick aquatic animal biota is similar to the extent of the satellite dredge plume reported by (Petus and Devlin, 2012).
- The distribution of sick aquatic animal biota does not match the distribution of the freshwater influx on the Queensland coast.
- GAWB water testing results (Gladstone Area Water Board, 2011a) indicate a marked deterioration in water quality in their mid-May 2011 sampling. Turbidity, suspended solids, nitrogen, phosphorus all peaked at levels which were above historical data. This peak took place

in the absence of a rainfall event. The peak occurred immediately after the onset of major dredging in the Harbour.

b) Water quality

Turbidity data (examples in Appendix 10) from the GPC/DERM/DEHP monitoring program in Gladstone demonstrates there has been frequent marked elevation since dredging increased in May 2011 above the baseline levels documented prior to commencement of the project, in the project EIS (GHD 2009 e). The turbidity levels in May 2011 were often above the 99th centile of historical data, which were used to set the permit conditions for monitoring. These levels were unusually high, even after adjustment for tidal and rainfall influences, given that the median dry season baseline turbidity at sampling sites near Fishermans Landing (taken between June and October 2008 when dredging was not occurring) was between 5 and 16 NTU, with mean turbidity being 8.5 NTU on neap tides and 16 NTU on spring tides (GHD 2009e).

The timing of turbidity elevation is suggestive that harbour dredging and development on Curtis Island were the triggers resuspending sediments.

The widespread distribution of resuspended dredge sediment was widely reported by fishers and crabbers and observed first hand by FFVS. These observations were confirmed with further scientific evidence of a large dredge plume by JCU satellite studies (Petus and Devlin, 2012).

The Gladstone Area Water Board fish hatchery is located near the mouth of Auckland Creek, and Gladstone Harbour. Soon after dredging increased in May 2011, their incoming water filters blocked rapidly with fine sediment. The facility had to substantially increase its intake water filtration. This involved installation of several new filters and re-engineering water flows and settlement tanks. The frequency of filter changes required increased markedly. (K Hutchby (GAWB) hatchery manager pers. comm. 2012). Fouled filters were frozen and stored at the hatchery. FFVS examined black deposits on these filters in January 2012. Funds were unavailable to undertake further testing on the sediments which had blocked the filters.

FFVS recorded low secchi disc readings (<10 cm) in the Gladstone Western Basin area in January and July 2012. The July reading was surprisingly low, given the dry season conditions with absence of substantial rainfall immediately prior, and the moderate sized tides.

FFVS also recorded some low secchi readings (<10 cm) in the reference site where barramundi were captured. These barramundi did not have any skin disease, similar to that which was common in Gladstone barramundi. Finding healthy fish in turbid water demonstrates that not all forms of turbidity are toxic to estuarine species of fish.

It is not so much the increase in particles in the water that has an impact on aquatic animal health, more so, it is the contaminant loading on and/or in that sediment which can dissolve or be ingested that affects animal health (Weber, et al., 2012). DERM testing data from September 2011 onwards, demonstrates numerous dissolved metals exceeding the ecosystem ANZECC guidelines in Gladstone harbour.

On 18 June 2012 GPC reported[1] the bund wall of the reclamation area had been leaking. GPC acknowledged the leakage had contributed to increased turbidity in the Harbour. Many of the sediments which were pumped into the reclamation area had been blended with lime in attempts to buffer any acid formation in PASS as they oxidised in the reclamation area. The leakage of these dredge materials back into the Harbour, may provide a possible explanation for the elevations in Harbour pH (above normal marine pH) recorded by FFVS.

FFVS observed the absence of silt curtains around the dredging and disposal activities. Plumes of sediment were observed extending from the operating dredges which were clearly contributing to the increased Harbour turbidity. Silt curtains have the capacity to minimise the size of the resuspension plume and thereby minimise the impact/distribution of the resuspended sediments. DERM water quality reports from October 2011, December 2011 indicate a decline in oxygen saturation in the Western Basin compared to the baseline data in the EIS. Whilst the lowered levels were unlikely to be acutely lethal, they can cause sub-lethal stresses on aquatic animals and render them more susceptible to other diseases, such as parasites.

b) Algal analysis

Lyngbya sp. was identified in plankton samples collected by FFVS in Gladstone (Colosseum 23/2/2012), and in again in association with the nets of commercial fishermen (7 Mile April 2012). When 2 fishermen cleaned these nets, they suffered acute chest pain and respiratory distress and were rushed in an ambulance to hospital suffering an acute reaction to inhalation of algal toxins.

Lyngbya sp. was not identified at the reference site samples in the FFVS study.

The cyanobacterium *Trichodesmium* was identified in Colosseum, but not elsewhere in the FFVS study.

[1] GPC. (2012). Transitional Environmental Program, Western Basin Bund Sealing. Gladstone Ports Corporation. DA SPDE01611411. 18 June 2012.

Table 3: Algal analysis from Gladstone and reference site waters (Sample site maps- Figure 1, 2)

Sample	1. Graham's dredge	2. Harbour mouth Calliope	3. Benaraby Bridge (freshwater)	4. Colosseum	5. Gladstone	6. Gladstone	7. Auckland Harbour mooring	Stanage bay 1 (sandy bay)	Stanage bay 2	Stanage bay 4
Date	23/02/2012	23/02/2012	23/02/2012	23/02/2012	23/02/2012	23/02/2012	27/02/2012	19/04/2012	19/04/2012	20/04/2012
GPS	23.46.273	23.48.558	23.50.883	24.00.969	24.01.205	23.59.467	23.50.178	22.19.543	22.09.666	22.09.301
	151.11.391	151.13.189	151.12.647	151.26.688	151.27.330	151.26.112	151.15.147	150.00.450	150.04.433	150.04.433
	2 bottles	2 bottles	3 bottles	2 bottles	2 bottles	2 bottles	2 bottles	Net		
										sediment
Diatoms	Abundant					benthic				pennates
Actinoptychus				+		+				
Aulacoseira			+							
Bacillaria				+						
Bacteriastrum										+
Cerataulina		+								
Ceratoneis closterium	+				+		+			
Chaetoceros coarctatus					+					
Coscinodiscus	+					+				+
Hemiaulus		+								
Nitzschia sigmoides		+								
Odontella	+			+			+		+	
Paralia	+					+				
Pleurosigma	+							+		
Pseudo-nitzschia	+	+								
Rhizosolenia				+		+			+	+
Skeletonema	+						+			
Thalassionema	+				+					
Thalassiosira		++				+			+	+
Thalassiothrix	+			+						
Dinoflagellates										
Ceratium fusus				+						

Noctiluca		+							+	
Others										
Euglenoid								++		
Pediastrum **fresh**		+								
Spirogyra **fresh**								+		
Staurastrum **fresh**			+							
Cyanobacteria										
Filament fig.10								+		
Geitlerinema								+		
Cylindrospermopsis raciborskii Fresh			++							
Lyngbya				+						
Trichodesmium				++					++	

Examinations undertaken by Professor Gustaaf Hallegraef, University of Tasmania with contribution from A/Prof Larelle Fabbro, Central Queensland University

c) Gross pathology

Interim Reports 1 and 2, released earlier in 2012, contained descriptions of the aquatic animals examined in the Gladstone area and are attached as Appendices 1 and 2. FFVS identified grossly diseased tissues in a diverse range of aquatic animals from Gladstone waters in January and February 2012 including: fish (queenfish, sharks, blubberlip bream, jewfish, scaienids, mullet, catfish, barramundi, grinner, sole and rays); scallops; prawns; mud crabs; and a turtle. The prevalence was highest in gill net caught fishes, and in mud crabs, however common lesions of redness in the skin, fin erosion and inappetance were apparent broadly across all groups.

Gross lesions were virtually absent on all fish examined concurrently at the reference site.

A high percentage of wild oysters were observed to be recently dead at several inner Gladstone Harbour locations in January and February 2012, but not at the reference site in April 2012. There was strong coherence between commercial fishing descriptions of sick fish and 'rust spot' lesions in crabs and the field observations of the FFVS scientists and veterinarians.

d) Histopathology

Summary

Fish and sharks: Evidence of skin disease (frequently associated with elevated levels of parasitism) was common in Gladstone fishes, and virtually absent at the reference site. The intensity of parasitic infections in all tissues was generally higher for Gladstone based animals compared to the reference site. The range of diseased tissues within each Gladstone animal sampled, was substantially higher than those sampled at the reference site. A greater proportion of Gladstone fish had gill disease compared to the reference site.

Mud crabs: evidence of metals-related shell disease was common in Gladstone crabs and completely absent in the reference site crabs. The prevalence of shell disease in Gladstone crabs was markedly elevated compared to the reference site, and elevated compared to historical levels reported in Gladstone Harbour.

Gladstone Rough-shell scallops: High parasite loads, and bacterial infections in Gladstone Harbour scallops. Very low catch rates were observed with only 4 individuals being caught in large areas of sample trawling in January and February sampling efforts.

Gladstone Coral: evidence of bacterial disease and increased mucous cell proliferation, which are common sequelae to exposure to stresses such as exposure to increased organic sediment.

Full detailed histological reports are included in the Appendices.

Table 4: Summary of histological results by animal species sampled from Gladstone and reference site.

Sample Species	Gladstone	Reference Site (Stanage)
Queenfish	Gills: Mild proliferative branchitis with intralesional sanguinicolid eggs 4/7 fish Skin: Multifocal erosive to ulcerative dermatitis, acute in 3/7 fish No infectious agents were identified Gastrointestinal:	Gills: Normal 1/1 fish Skin: Normal 1/1 fish Gastrointestinal:

	Mild to moderate non-suppurative and necrotising enteritis with intra-lesional coccidia-like organisms 6/7 fish Severe eosinophilic mural gastritis with intralesional nematode larvae 4/7 fish Marked chronic active gastro-oesophagitis 1/7 <u>Other:</u> Granulomatous peritonitis with intralesional metazoan larvae Mild granulomatous myositis 1/7 Randomly distributed mononuclear leucocytes in liver 1/7 Encysted larval cestodes in one other liver 1/7	Marked eosinophilic infiltrate around blood vessels. No infectious agents seen. Low numbers of coccidia associated with little host response. <u>Other:</u> Liver regionally severe are of non-suppurative infiltrate. No infectious agents seen in this area.
Sharks	<u>Gill</u>: Lamellar distomiasis with mild proliferative branchitis 1/7 sharks <u>Skin:</u> Erosive dermatitis/ulcerative dermatitis 3/7 sharks; 1/3 with intralesional metazoan larvae Cutaneous distomiasis 2/7 sharks <u>Gastrointestinal:</u> Mild to moderate non-suppurative gastritis with intralesional coccidia +/- mucoso-flagellates 5/7 sharks Moderate non-suppurative enteritis 1/7 sharks Severe focal acute mural enteritis, aetiology unknown 1/7 sharks Mild to moderate non-suppurative pancreatitis 3/7 sharks No aetiological agent detected <u>Other:</u> Mild renal tubular protozoa 1/7 sharks Mild splenic metazoan larval cysts 1/7 sharks Mild intravenous branchial metazoan larvae 1/7 sharks	<u>Gill:</u> Mild lamellar epitheliocystis 1/5 fish. <u>Skin:</u> No changes to skin in any fish examined. <u>Gastrointestinal</u> Mild intestinal cestodiasis 2/5 fish Mild non-suppurative gastritis 3/5 fish with intralesional coccidia 1/3 fish Moderate- marked intestinal coccidiosis 2/5 fish Mild intestinal larval migrans 1/5 fish <u>Other:</u> Mild zonal non-suppurative hepatitis 1/5 fish
Ray	Shovelnose shark <u>Skin:</u> Many sub-cutaneous haemorrhages and marked oedema of tissue from vent <u>Oesophagus:</u> Severe mural oesophagitis associated with metazoan parasite. No lesions in spleen, or heart. Other tissues not examined.	Stingaree <u>Skin:</u> No lesions <u>Stomach:</u> Patchy mild gastritis- no infectious agents seen. <u>Pancreas:</u> Mild small foci of pancreatitis. No lesions in gill, spleen, skin, body wall, liver, kidney, intestine, spiral valve, heart, haematopoeitic tissue
Barramundi	<u>Gills:</u> Lamellar distomiasis 6/12 fish Lamellar encysted myxozoa (mild) 8/12 fish Mild to moderate-marked sanguinicolid granulomatous branchitis 4/12 fish Mild sanguinicolid branchitis 1/12 fish <u>Skin:</u>	<u>Gills:</u> Mild to marked multifocal branchitis with monogenean flukes and or myxosporidial cysts 4/5 fish <u>Skin:</u> No lesions observed in any of the

	Erosive to ulcerative dermatitis 6/12 fish <u>Gastrointestinal:</u> Mild-marked mixed gastritis and enteritis with focal granulomas and intralesional degenerative larvae 10/12 fish Marked non-suppurative enteritis with intralesional nematodes 1/12 fish Moderate coccidia associated enteritis 1/12 fish <u>Heart</u>: Mild to moderate sanguinocolid myocarditis 3/12 fish Granulomatous myocarditis with intralesional sanguinicolid eggs 4/12 fish <u>Other:</u> Granulomatous nephritis with intralesional sanguinicolid eggs, mild to moderate 3/12 fish Mild non-suppurative portal hepatitis 1/12 fish Mild non-suppurative portal hepatitis 2/12 fish Focal acute muscle necrosis (potentially ulcer related) 1/12 fish	fish sampled. <u>Gastrointestinal:</u> Mild mixed to granulomatous gastritis/enteritis with intralesional larval nematodes 4/5 fish Mild enteric coccidiosis 1/5 fish <u>Other</u> Mild to moderate visceral and lamellar granulomas within intralesional sanguinicolid eggs 4/5 fish
Catfish	<u>Gills:</u> Lamellar distomiasis 1/2 fish <u>Skin:</u> Erosive dermatitis 2/2 fish <u>Eyes:</u> Mild conjunctival distomiasis 1/2 fish	<u>Gills:</u> Mild lamellar distomiasis 1/3 fish <u>Skin:</u> No evidence of any skin disease <u>Gastrointestinal:</u> Moderate mixed gastritis 2/3 fish <u>Kidney:</u> Mild renal collecting duct ciliate protozoa 2/3 fish
Mullet	<u>Gills:</u> Proliferative branchitis, mild 2/4 fish; Mild Epitheliocystis 2/4 fish Monogenean fluke associated with branchitis 1/4 fish <u>Liver:</u> Focal non-suppurative hepatitis, mild to moderate 3/4 fish with intralesional nematode 1/3 fish <u>Kidney:</u> Encysted renal metaozoan larvae (suspect nematodes), mild to moderate 2/4 fish <u>Heart:</u> Encysted cardiac metazoan larvae (suspected cestode/trematode), moderate 2/4 fish <u>Multiple organs:</u> Visceral myxozoan cysts/granulomas, mild to moderate 4/4 fish Visceral microsporidial cysts/granulomas 1/4 fish	<u>Gills:</u> Mild lamellar myxozoal cysts 4/5 fish Mild lamellar distomiasis 1/5 fish <u>Multiple organs:</u> Mild visceral granulomatous disease with encysted unidentifiable metazoan larvae 1/5 fish Mild visceral myxozoan cysts 4/5 fish.
Mud crab	<u>Gills:</u> Mild to severe lamellar parasitism 13/15 crabs	<u>Gills:</u> Mild branchitis with intralesional sediment and nematodes 1/5 crabs Moderate lamellar copepodiasis

	  <u>Skin:</u> Multifocal shell ulcers 9/15 crabs with endocuticle separation consistent with elevated metal exposure induced disease in several crabs. <u>Hepatopancreas:</u> Bacculo-virus associated hepatopancreatic degeneration, mild 1/15 crabs <u>Multiple organs:</u> Mild to moderate chronic visceral granulomas, aetiology unknown 2/15 crabs	1/5 crabs <u>Skin:</u> External cuticle erosions 1/5 crabs. No endocuticle lesions. No evidence of pathologies related to elevated metals exposure <u>Hepatopancreas:</u> Mild hepatopancreatic bacculovirus-like inclusions 1/5 crabs
Prawn	<u>Skin:</u> Severe ulcerative dermatitis 2/5 prawns Lamellar distomiasis 1/5 prawns <u>Gut and viscera:</u> Hepatopancreatic cestodiasis 2/5 prawns Visceral larval metazoans (unidentified) mild to moderate 2/5 prawns <u>Other:</u> Encysted trematode larvae in skeletal muscle, nerve cords, and associated soft tissue 2/5 prawns	No samples available
Rough-shelled scallop	<u>Digestive gland and viscera:</u> Gregarine colonisation of digestive glands, severe 4/4 scallops. Inflammation of digestive gland with intra-lesional unidentified protozoa 2/4 scallops Encysted visceral juvenile metazoa moderate to severe 4/4 scallops <u>Mantle:</u> Encysted gram negative bacteria 3/4 scallops <u>Mouth:</u> Stomatitis, moderate 2/4 scallops	No samples available
Turtle	<u>Lung:</u> Multifocal trematodes eggs and chronic inflammation. Serous atrophy of peripheral pleura 1/1 <u>Heart:</u> Verminous granulomatous endocarditis (including valves) and myocarditis with adult trematodes within heart lumen, as well as egg granulomas 1/1 <u>Intestine:</u> Multifocal chronic verminous enteritis associated with trematode eggs 1/1 <u>Adipose tissue</u>: Serous atrophy, numerous melanomacrophages and oedema 1/1	No samples available

	Liver: Diffuse congestion. Fibrous and granulomatous hepatitis around trematode eggs. 1/1 turtle	
Grinner	Skin: Segmental loss of epidermis covering the fin; appears to be true necrosis in some exposed connective tissue elements 1/1 fish Intestines: Cestodes within intestinal lumen 1/1 fish	No samples available
Blubber Lip Bream	Gills: Moderate chronic branchitis 1/2 Intestines: Moderate intestinal coccidiosis 1/2 Moderate mixed enteritis 2/2 Mild intestinal cestode cysts 1/2 Mild intestinal distomiasis 1/2 Pyloric caecae: Moderate mixed leucocyte infiltration 1/2 Heart: Granulomatous epicarditis and myocarditis 2/2 with intralesional myxosporidia 1/2 and intralesional metazoan larvae 1/2 Eye: Marked keratitis and anterior uveitis ½	No samples available
Sole	Gills: Nematode larva encysted within the gill 1/2 fish Skin: Erosive dermatitis. No infectious agents are seen in these lesions 1/2 fish A Cryptocaryon-like ciliate is encysted within the skin 1/2 fish. Intestine: Granulomas within the wall of the intestine are centred on nematode larva. Multifocal mild granulomatous mural enteritis 2/2 fish Muscle: Several cestode larvae is encysted within the body wall 2/2 fish. Moderate to high numbers of myxozoan cysts are within skeletal muscle fibres of the body wall 1/2 fish. Spleen: Large microsporidial granuloma is within the spleen 1/2 fish	
Sciaenid	Intestines: Mild to moderate mixed enteritis with coccidia and distomiasis 4/4 fish Stomach: Mild to moderate multifocal granulomatous mural gastritis	

	with encysted metazoan larvae 2/4 fish Mild to moderate non-suppurative gastritis 1/4 fish Mild gastric protozoal parasitism (aetiology unknown) 1/4 fish <u>Abdominal cavity:</u> Encysted peritoneal cestode larva 1/4 fish <u>Kidney:</u> Renal tubular myxosporidiosis with glomerular injury 2/4 fish	
Herring	<u>Skin:</u> Focal acute ulcer 1/1 fish, no infectious agent observed	
Whiting	<u>Intestine:</u> Multifocal mural granulomatous enteritis, inactive and mild 1/1 <u>Brain:</u> Myxozoan encephalitis, mild to moderate 1/1 <u>Muscle:</u> Few myxozoan cysts 1/1	
Oyster cracker	<u>Gill:</u> Granulomatous branchitis 2/3 <u>Skin and fin:</u> Severe ulcerative dermatitis with intralesional copepods 3/3 fish <u>Intestine:</u> Mild to marked enteritis 3/3 fish with intralesional nematodes 1/3 fish, unidentified protozoa 3/3 fish, coccidia 1/3 fish and cestodes 1/3 fish <u>Heart:</u> Moderate to marked granulomatous myocarditis 3/3 <u>Kidney:</u> Moderate to marked granulomatous nephritis with intralesional organisms consistent with mesomycetozoea or fungus 3/3 fish	Dart (different species): <u>Gill:</u> No lesions 1/1 <u>Skin and fins</u> No lesions 1/1 <u>Intestine:</u> Mild enteritis associated with very low numbers of coccidia, moderate numbers of myxidia-like organisms. <u>Heart:</u> No lesions <u>Kidney:</u> No lesions
King Salmon	Samples not available	<u>Skin:</u> Patchy superficial dermatitis - no infectious agents observed. No other lesions observed in gill, stomach, intestine, kidney, spleen, pyloric cecae, body wall or heart.
	Pathologist Dr Michelle Dennis comment: The assortment of lesions is suggestive of immunosuppressive factors which seem to be impacting a population of fish as a whole.	

7. Discussion – weight of evidence assessment

The FFVS diagnostic investigation analysed multiple lines of evidence including:

-multiple different fish and shark species

-mud crabs

-turtles

-coral

-dolphins

-dugongs

-prawns

-oysters

-scallops

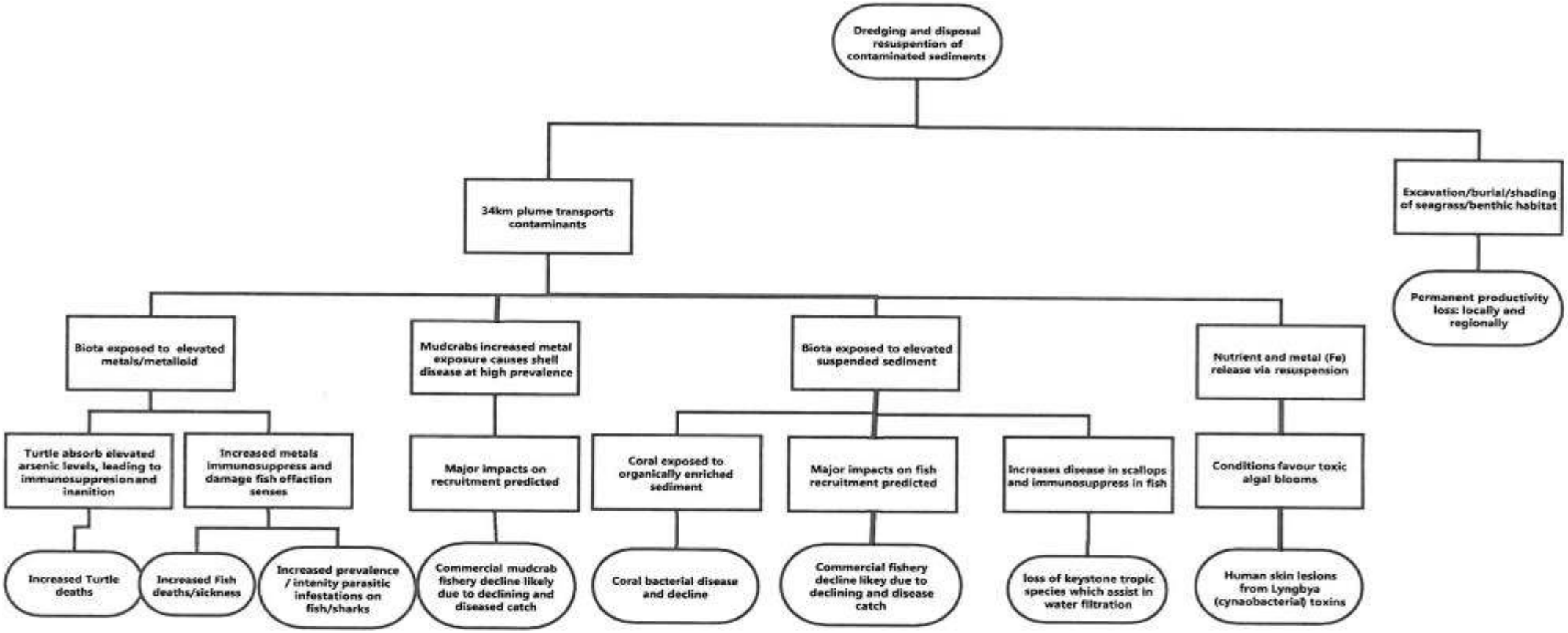
-algae

-water quality: metals/metalloids, dissolved oxygen, pH, ammonia, hydrogen sulfide

The data needs to be viewed in aggregate to attempt to explain the myriad of different expressions of disease across the Gladstone aquatic animals. After reviewing the data, I created the following working hypothesis, to demonstrate how the various data can be drawn together in a weight-of-evidence approach to demonstrate the likely cause.

Each line of evidence is discussed below with reference to likely causation of the observed clinical signs, lesions and changes.

Figure 8: FFVS Working Hypothesis of causal pathways for disease expression in Gladstone aquatic animals and people



a) Fish

i) Causes of skin disease- differential diagnosis

Many of the fish sampled by both DERM/DEEDI (Biosecurity Queensland, 2012a) and by FFVS around Gladstone Harbour exhibited a dermatitis; many with grossly visible ulceration in sharks, queenfish, barramundi and catfish. Images of affected animals were documented in FFVS Interim Report 1 (Appendix 1) and FFVS Interim Report 2 (Appendix 2). (Law, 2001) examined the myriad of mechanisms which can lead to ulcerative lesions on fish. The outcome of cell injury to superficial skin cells can be driven by:

a) damage to the cell membrane leading to associated fluid and ionic imbalances, or

b) inability of mitochondria, the powerhouse of the cell, to restart ATP synthesis.

Law highlights a range of potential infectious and non-infectious causes of skin ulcers including: toxins; physical causes; immunological causes; nutritional and metabolic perturbations.

The histological findings of FFVS and Biosecurity Queensland pathologists demonstrate that parasites and non-infectious causes were associated with the skin lesions. Udomkusonsri and Noga (2005) indicated that acute stress in fish can lead to an acute ulceration response in the skin. Once the surface of fish skin is damaged, the fish can suffer increased osmoregulatory stress, and increased vulnerability to infectious agents. This mechanism may explain some of the skin lesions observed grossly and histologically in fish sampled from the Gladstone area.

The skin lesions observed in Gladstone fish were not present in fish at the FFVS reference site, suggesting the cause is Gladstone-based.

The large changes to water quality in the Gladstone Harbour, may be a sufficient stress in some species to trigger this ulcerative response. The tested elevations in some dissolved metal levels (above ANZECC guidelines) are also likely to contribute to stress in fish (Poulsen and Escher, 2012; Zeilokoff, 1993), rendering them more susceptible to parasites. Both aluminium and arsenic are documented to increase secretion of skin mucous which may compromise mucosal immunity, and render the fish more susceptible to ectoparasitic infections (Poulsen and Escher, 2012; Zeilokoff, 1993). This is especially the case for certain species on monogeneans and copepod parasites which use external skin mucous as a food source and hence were provided with a large increase in food availability at the same time as their host was being stressed.

ii) Parasites

Neobenedenia melleni- this monogenean ectoparasite was observed and reported by DEEDI/Fisheries Queensland on large sick barramundi with skin and eye lesions in September 2011 in the South Trees Inlet and Calliope River and Gladstone Harbour. The affected fish were likely to be stock which had come over the spillway of Awoonga Dam 10 months prior. The onset of *Neobenedenia* disease was several months after the freshwater flow over the spillway into the Boyne River had ceased, as documented by the Gladstone Area Water Board. With the flow of freshwater ceasing, marine water from the harbour penetrates on the tide up the rivers and inlets, allowing obligate marine parasites such as *Neobenedenia* to access fish.

FFVS commenced the sampling for its investigation some 4 months after this earlier work by Fisheries Queensland.

Neobenedenia is an obligate marine organism and does not survive in freshwater. Freshwater is used in aquaculture as a bath to treat and kill *Neobenedenia* on barramundi. Hence the observation of *Neobenedenia* on the barramundi indicates that the fish had been in contact with marine (sea) water. This evidence suggests that the influx of freshwater into the marine environment after the flood was not the cause of fish health problems as was reported by Gladstone Ports Corporation, and DERM.

The Gladstone Fish Health Scientific Advisory Panel (2012) compared the Gladstone outbreak to an outbreak of *Neobenedenia* in the Hinchinbrook Channel that caused disease in barramundi. The authors of the panel report neglected to mention that the Hinchinbrook outbreak was within an aquaculture facility, where husbandry practices and seasonal conditions at the time were stressful to the fish, rendering them more susceptible to lethal levels of infestation (Deveney, Chisholm, and Whittington, 2001). Deveney, Chisholm, and Whittington (2001) correctly stated that the outbreak was in cultivated barramundi in sea cages, not wild fish as inferred by Queensland Government reports.

The inference of the panel report was that the Hinchinbrook barramundi were wild fish, like those in Gladstone. And therefore wild barramundi suffering disease due to *Neobenedenia*, was not without a precedent.

Such a comparison was invalid. There had never been a recorded outbreak of *Neobenedenia* associated disease of this severity in **wild** fish in Australia, prior to the Gladstone event.

The key link between the Hinchinbrook outbreak, and that observed in Gladstone, was the existence of substantial water quality based stress factors.

As water temperatures increased from their winter lows in August 2011, the parasitic infection resolved in the Gladstone barramundi, however the animals remained sick, as documented in FFVS sampling in January and February 2012. , None of the sick barramundi sampled by FFVS had any *Neobenedenia* detected on them, despite the fish being captured in near full strength seawater. This indicates that other factors were continuing to contribute to their morbidity at that time, a full year after the freshwater flow event had occurred in the Boyne River.

The histology of these fish indicates a range of internal disease processes that are unrelated to *Neobenedenia*. In the barramundi, FFVS also identified diplectanid monogeneans on the gills and *Lernanthropus latis* externally, as well as internal parasitism including blood fluke eggs in gill vessels, myxozoan cysts in gill, nematode worms in the gut and migrating through viscera.

Neobenedenia was not identified on other fish species in association with dermatitis by DEEDI/Fisheries Queensland in October 2011, nor by FFVS in January and February 2012.

Page 7 of the Fisheries Queensland December 2011 reported stated:

"*Skin abnormalities from non-barramundi fish species were generally mild and not due to Neobenedenia spp. Fin fish species examined included spangled emperor, spotted cod, whiting, and trevally. 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 which could explain the skin conditions on these other fin fish species."

Hence it is not likely that these naturally occurring monogeneans are the initiating cause of the overall pattern of ill health observed across the aquatic animal population. Rather their presence on the barramundi is more likely to be a consequence of an endemic parasite taking advantage of an immunosuppressed host. The primary causes of immunosuppression are likely to be a combination of elevated metals, metalloids, and toxic algae which were all reported in October 2011 in Gladstone Harbour.

Primary bacterial disease as a cause of the skin lesions was ruled out by Fisheries Queensland in their December 2012 report:

"*A range of bacteria were isolated from the fish. 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*."

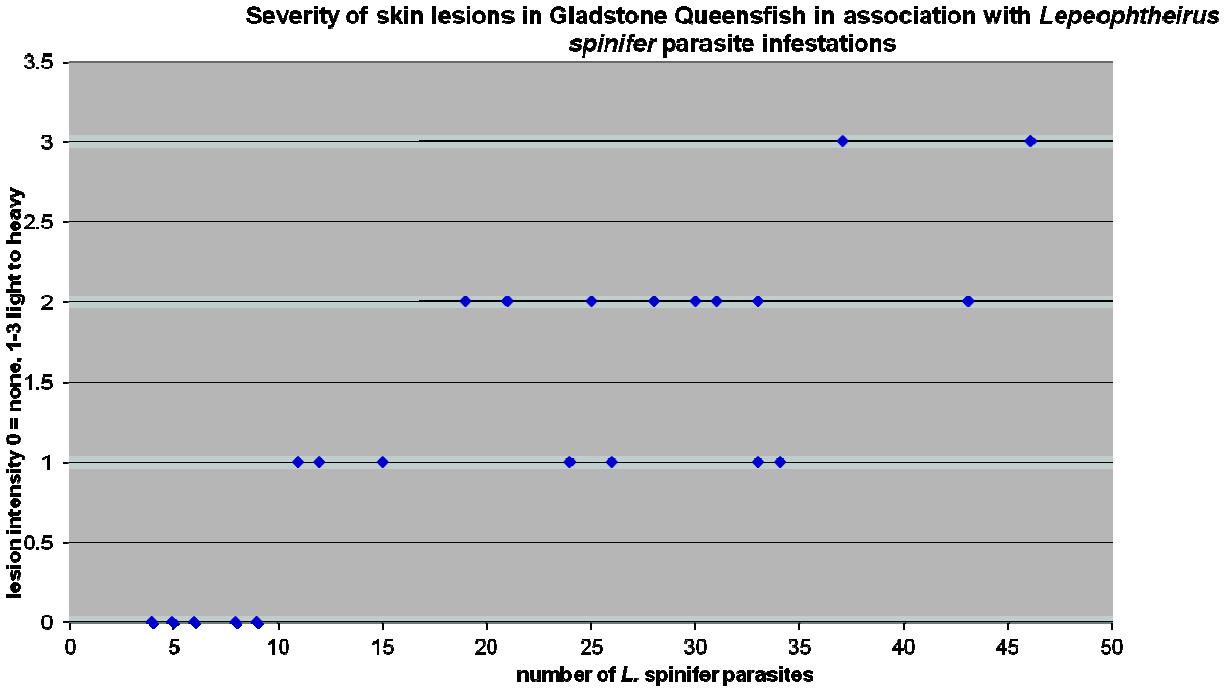
FFVS utilised the skills of a marine parasitologist and a veterinarian with further qualifications in aquatic animal health to assist in parasite identification on sampled fish in January 2012. The use of appropriately qualified personnel, for parasite investigation works, may explain the difference in Government observations and those of FFVS, which identified additional parasitic infestations and gross pathology changes in parasite attachment sites.

Figure 9: Location of diseased Queenfish capture (11-Queenies) at Gladstone

Queenfish (n = 27) sampled by FFVS near the ocean dredge spoil disposal site, exhibited a high prevalence of skin erythema, which was associated with high intensity infestations of *Lepeophtheirus spinifer* (sea lice) ectoparasites. These fish were alive at the time of capture, hence the erythema was not due to a post-mortem lividity artefact. Fisheries Queensland (2012) reported similar results. The parasites and the associated skin erythema lesions were located primarily on the pectoral girdle and rostro-ventrolateral surfaces of the body. This was apparently the first record of *L. spinifer* in Australia. Infection intensities of up to 46 copepods per fish were recorded (see Figure 2 below), with a mean intensity of 21.2 copepods/fish (range 4-46). This intensity of infestation is considered unusually high, particularly since these were wild fish (i.e. not living in high intensity aquaculture farming conditions). Queenfish examined from other locations in northern Australia have subsequently been found to be infected with *L. spinifer*, but only with less than 10 *L. spinifer* per fish (B Diggles, pers. comm. 2012). In the queenfish sampled from Gladstone Harbour, only fish with more than 10 *L. spinifer* exhibited skin erythema at the site of parasite attachment, and all fish infected with more than 35 parasites had very noticeable red skin lesions of moderate to severe intensity (Figure 2). Neither recreational nor commercial fishers, who frequently catch Queenfish, have reported similar red lesions before in Australia, highlighting the geographic and temporal specificity of this disease outbreak to the Gladstone region post-commencement of the Western Basin Dredging and Disposal Project.

Cruz-Lacierda, Pagador, Yamamoto, and Nagasawa (2011) identified up to 50 *L. spinifer* per Oyster Cracker Dart (*Trachinotus blochii*) in sea cages in the Philippines. These high intensities of *L. spinifer* were associated with erythematous superficial ulcerative lesions and loss of appetite. The description of the heavily infected cultured oyster crackers reported by Cruz-Lacierda et al (2011) thus closely matches that observed by FFVS in wild Queenfish captured in Gladstone sampling in January 2012.

Figure 10: Parasite infection intensity and severity of skin lesions in Queenfish from Gladstone



Bull sharks and some whaler sharks sampled by FFVS from Gladstone (Boyne river, 7 mile estuary, Seal Rocks, Facing Island and Sable Chief Rocks), exhibited a high prevalence of skin disease associated with

high intensities of infestation of *Dermophthirius* sp., *Pandarus* genus copepods. Fisheries Queensland (2012) found similar high rates of skin disease and discolouration in sharks associated with these parasites, which is a highly unusual situation (I. Whittington, pers. comm. to B. Diggles 2012), as these parasites usually cause lesions only on sharks held captive in aquaria (Cheung 1982, Thoney 1991).

In contrast, FFVS found very little skin disease in the fish and sharks sampled at the reference site (Stanage Bay site 3- see Figure 4). Fisheries Queensland (2012) also found little evidence of skin disease in their reference sites, apart from reporting some bull sharks in the Fitzroy River with pale skin and unspecified loads/types of parasites (Biosecurity Queensland, 2012a).

The intensity of parasitic infections, determined by gross and histological assessment, in the reference site was substantially lower than on similar species of Gladstone fish observed and sampled by FFVS.

The effects of metals on fishes have been the source of considerable study with many references in other chapters 10, 11, 12 13, 14, 15 of this report. Zeilokoff (1993) summarised the situation:

"*Contamination of aquatic habitats with metals from various industrial and mineral mining sources has been a problem for many years. The current interests in mineral mining, energy development and use, and dredging will undoubtedly result in further pollution of aquatic environments by such metals as arsenic, cadmium, lead, mercury, and zinc. The effects of metal pollution are measurable on both ecological and economic scales. Ecosystem impacts include contamination of sediments and the water column, accumulation of pollutants in biota over a wide area, and apparent increases in pollutant-related anomalies in the species that reside there. The biological effects of metallic pollutants in aquatic environments are significant. Documented effects include alterations in hematological parameters, homeostasis, carbohydrate metabolism, embryonic or ova development, and immunological competence. Effects on aquatic life may be surmised from these biological responses to heavy metal exposure as well as from the incidence of pollution-related disease in these organisms.*"

Zeilokoff (1993) mentions dredging as an activity which is highly disturbing to the aquatic environment, due to the remobilisation of legacy pollutants (see also Rice, 1987; Bonnet, 2000; Nayar, 2004; Esslemont, Russell, and Maher, 2004; Burton, 2010; and Hedge, Knott, and Johnston, 2009). The resuspension of sediment increases the exposure of aquatic life to mixtures of bound pollutants such as polycyclic hydrocarbons and metals by altering their bioavailability. This alteration in bioavailability of toxicants in turn has detrimental effects on aquatic animal health (Wilber and Clarke, 2001; Millward, 2004; and Eggleton and Thomas, 2004).

Exposure of fish to pollutants is known to generate an increased infection pressure of monoxenous parasites (species with only one host, such as monogeneans like *Dermophthirius* sp. and copepods like *L. spinifer*), and a decreased infection pressure of heteroxenous parasites with complex life cycles (Khan, 1991; Diamant, 1999). Increased susceptibility to these parasites occurs after chronic exposure to pollutants, especially for ciliates and monogeneans for which intensity of infection tends to increase significantly as they become associated with lesions caused by contaminants such as metals and eutrophication (Khan, 1991; Overstreet, 1993). This is likely to be due to increased stress or exposure to toxic substances, both of which can cause immunosuppression; and other toxicant induced alterations in external mucous production (Ellis, 1981; Bols, 2001).

In captivity, higher infection pressures are generally noted, due to stressors such as high population densities, reduced water quality, sub-optimal nutrition and handling. Under these conditions increased intensities of infestation and accompanying disease are more likely to occur (Thoney, 1991). Only under exceptional circumstances do monogeneans proliferate on wild fish (Hendrix, 1985). Hendrix (1985) examined flounder around dredge spoil disposal sites in the New York Bight and found twice the

intensity of the monogenean *Bothitrema bothi* infestation (mean of 16.4 monogeneans per fish), compared to reference sites. Overstreet (1993) and Hendrix (1985) suggest that monoxeneous macroparasites, particularly monogenea, are a better indicators of environmental health than use of the entire parasite community, as quantification of monoxenous macroparasites produces more accurate data and better differentiates between sites (Diamant, 1999).

Monogenean ectoparasites such as the *Dermophthirius* spp. found within dermal lesions on sharks throughout the Gladstone region are usually associated with lesions only in sharks held captive in aquaria (Cheung, 1982; Thoney, 1991). Parasitic infestations are mediated in these situations by intensive housing of animals and a combination of other stress factors such as husbandry and nutrition. Levels of these parasites in Gladstone were substantially higher than the reference site. The elevated intensity of infections was associated with increased severity of ulcerative skin lesions on sharks, which was not detected at the reference site with histological examination.

It is extremely unusual for both monogeneans and copepods to be associated with external lesions on wild fish. Given that both monogeneans and copepods are monoxenous parasites (species with only one host), that are known to proliferate when their hosts are stressed. The evidence of several marine fish species (i.e. species that have not entered Gladstone Harbour from Awoonga Dam) with lesions associated with heavy infections of copepods and monogeneans suggests their hosts are being exposed to a common stressor in the form of adverse environmental conditions which is causing the immunosuppression. As detailed in the other chapters there is considerable evidence that water quality conditions have deteriorated since the commencement of the Western Basin Dredging and Dsposal project.

Exposure to metals can also increase skin mucus secretion, encouraging growth of ectoparasitic monogeneans and copepods, which feed on mucous (Bols, 2001).

FFVS concludes that the increased rates of disease and lesions observed in Gladstone fish, in comparison with the reference site, are highly likely to be due to exposure to sub-optimal environmental conditions in Gladstone Harbour including: increased exposure to toxicants (primarily metals/metalloids); elevated suspended sediment loads; and increased noise based stressors which are primarily the result of the Western Basin Dredging and Disposal project. The parasitic infestations observed are likely to be a secondary sequelae to the stress and immunosuppression of the host from exposure to poor water quality.

b) Mud Crabs – evidence of impacts

FFVS survey of mud crabs around Gladstone showed an elevated prevalence of shell lesions (rust spots) compared to the reference site using the same scoring system as described in Andersen (2003). In addition to the histological sampling, many of these mud crab lesions were photographed for evidence of the accuracy of lesion detection.

Benthic (bottom dwelling) animals are the most exposed to contaminated sediments (Gerbersdorf, et al., 2011). As such, and given the conditions described in Gladstone Harbour, it is not surprising that mud crabs were suffering from serious metals related diseases after the commencement of dredging. (Andersen, et al., 2005) demonstrated that mud crabs take up metals both through the dissolved metals in water, and through ingestion of food and sediment. Hedge, Knott, and Johnston (2009) demonstrated that the activity of dredging re-suspends metal contaminants, and that they become more bioavailable for uptake, as a consequence.

A chi-square test was used to examine whether the number of mud crabs observed with disease in the Gladstone and the reference sites (Table 5) differed significantly from the number of mud crabs that would be expected if there was no difference between disease prevalence in the two sites. There was a significant difference identified ($\chi^2 > 24.37$, d.f.= 1, $P < 0.001$), with mud crabs from the Gladstone site being significantly more likely to have the disease than those from the reference site.

Table 5: Summary of shell disease prevalence data in Gladstone and reference site mud crabs

	Gladstone	**Reference site**
Total number examined	716	153
Percentage with shell lesions	37.8%	13.7%

The rates of rust spot were highest in crabs sampled in closer proximity to the Western Basin Dredging and Disposal project, with a decreasing rate of shell disease at Turkey Beach more than 50 km from the dredging activities. A linear regression was performed on the data resulting in a statistically significant (at 0.05 level) relationship between distance from dredging grounds and spoil sites and prevalence of disease (df = 5, $F = 20.008$, $P = 0.011$, Figure 3).

Table 6: Linear regression analysis of crabs with shell disease and proximity to dredging and disposal in Gladstone

Site	**Distance from closest Western Basin dredging or ocean spoil disposal site**	**Percentage of mud crabs with shell disease**	**Number of crabs examined**
Narrows, Badgers Creek, Mosquito Creek	11 km (Western Basin)	50%	70
Enfield, barge landing, Nutmeg creek,	0 km (Western Basin)	61%	122
Graham's Creek, Flying fox river, adjacent dredges opposite Curtis Is	0 km (Western Basin)	44%	87
Colosseum	14 km (ocean disposal)	34%	139
7 Mile	23 km (ocean disposal)	28%	155
Turkey Beach	32 km (ocean disposal)	7%	45
		Total examined (n)	618

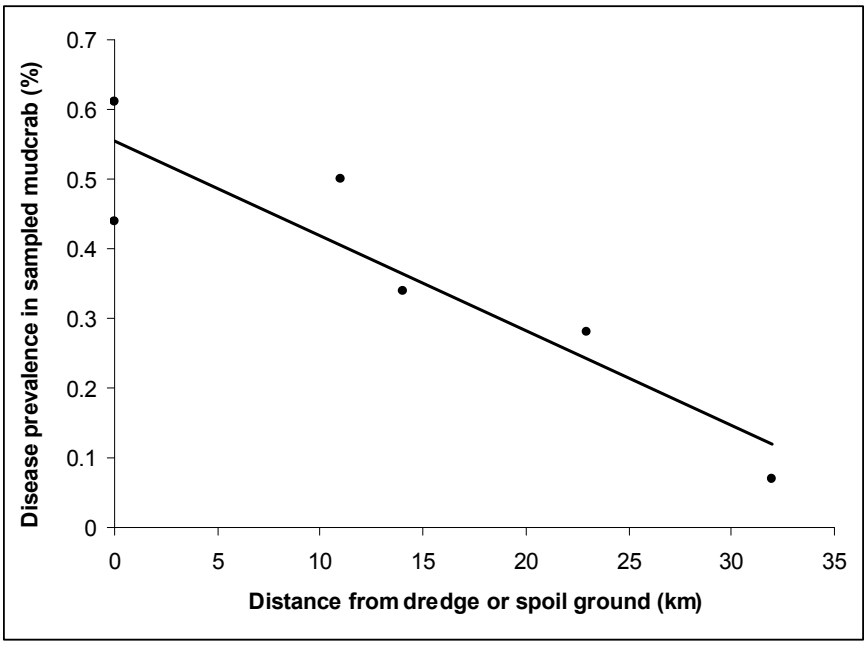


Figure 11: Relationship between prevalence of diseased mud crabs (% sampled) and distance from the dredging or spoil dumping ground (r^2 = 0.833, n = 618 crabs).

From the visual observations of the presence of gross shell lesions on the carapace of mud crabs it cannot be easily determined by eye, if the cause is traumatic, infectious or non-infectious disease. FFVS histological analysis of crab shell lesions (Appendix 8 Report 12-5311480) demonstrated that Gladstone crabs have unique shell lesions which were identical to those described in Andersen, Norton, and Levy (2000). These unique shell lesions were not present in any of the samples from the reference site. This distinct lesion in Gladstone mud crabs, highlights that an additional cause of shell disease is present in Gladstone Harbour, which was not evident in sampled crabs from the reference site. The increased metals exposures demonstrated in DERM water quality report 6, particularly to copper and zinc, are likely to be the causal factor, as originally described by Andersen (2003).

Under suitable conditions the rust spots may progress to full shell thickness ulcerations with secondary bacterial infections (Andersen et al. 2000, Andersen 2003). Such severe lesions are likely to seriously compromise the health and reproductive capacity of affected animals. The physiological effects of the elevated metals exposures are not known, but may also be potentially deleterious to mud crab health and reproduction as described in other chapters of this report.

Fisheries Queensland (2012) published conflicting results on the prevalence of Gladstone mud crab shell disease suggesting only 5.3 % prevalence of lesions on crabs sampled in December 2011 and January 2012. In January 2012 the Fisheries Queensland survey design excluded the highest prevalence inner Harbour areas (detected in FFVS survey) and focused only on the narrows in the north and turkey beach to the south, and detected only 5% of crabs with shell lesions. Such limited coverage of the crab population, limits the power of the Fisheries Queensland study to determine the prevalence across the exposed population.

This may also provide an explanation for the difference in QLD Fisheries results compared to the FFVS data collected in February 2012. FFVS's study design provided more spatial coverage of crab populations over a shorter timeframe. The details were presented in Interim Report 2 (Appendix 2).

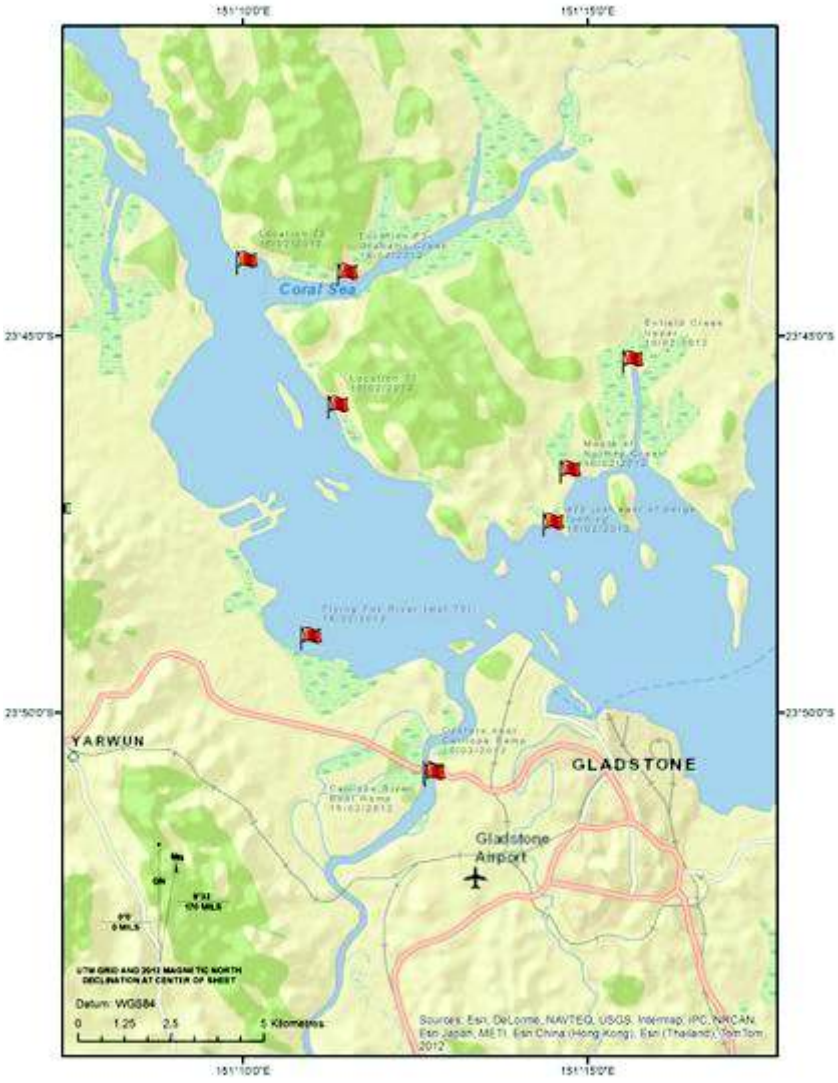


Figure 12: Crab sample sites, Gladstone inner harbour.

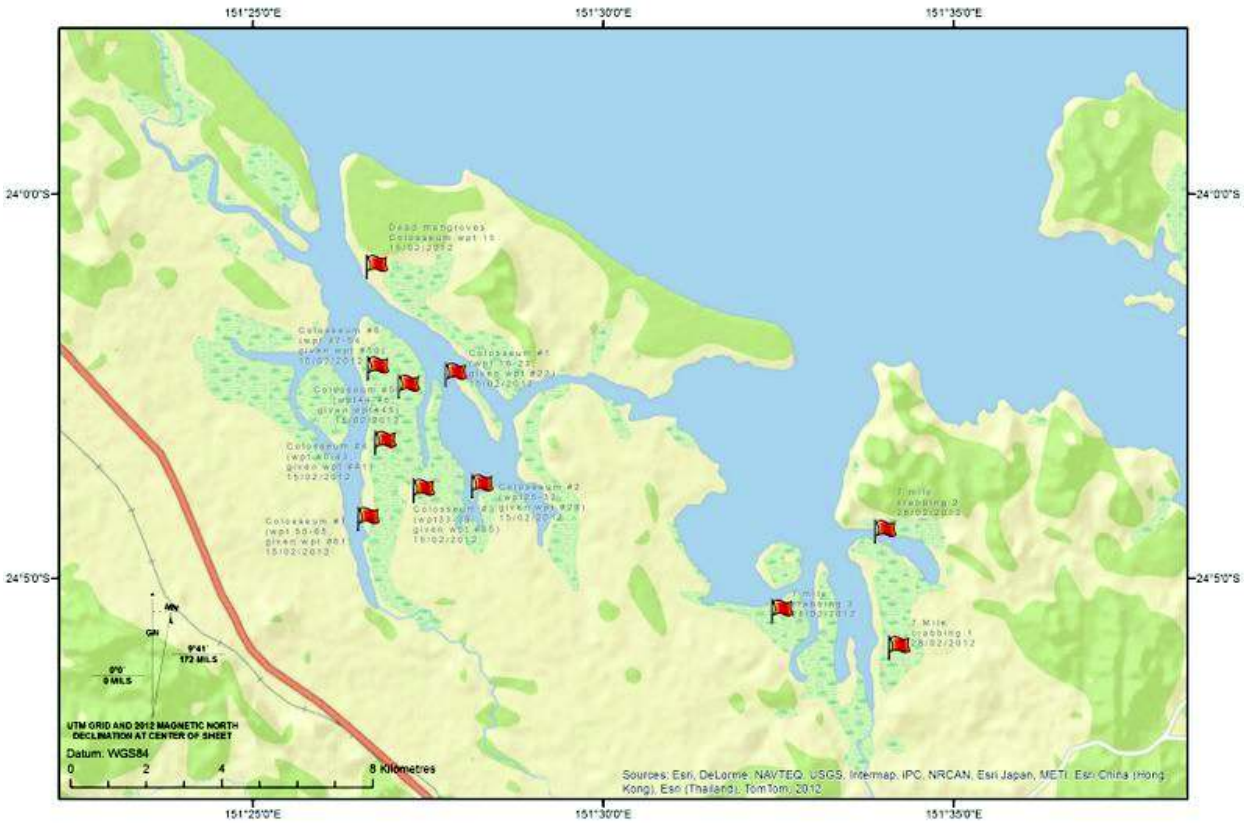


Figure 13: Crab sample sites: Colosseum and 7 mile estuary, Gladstone.

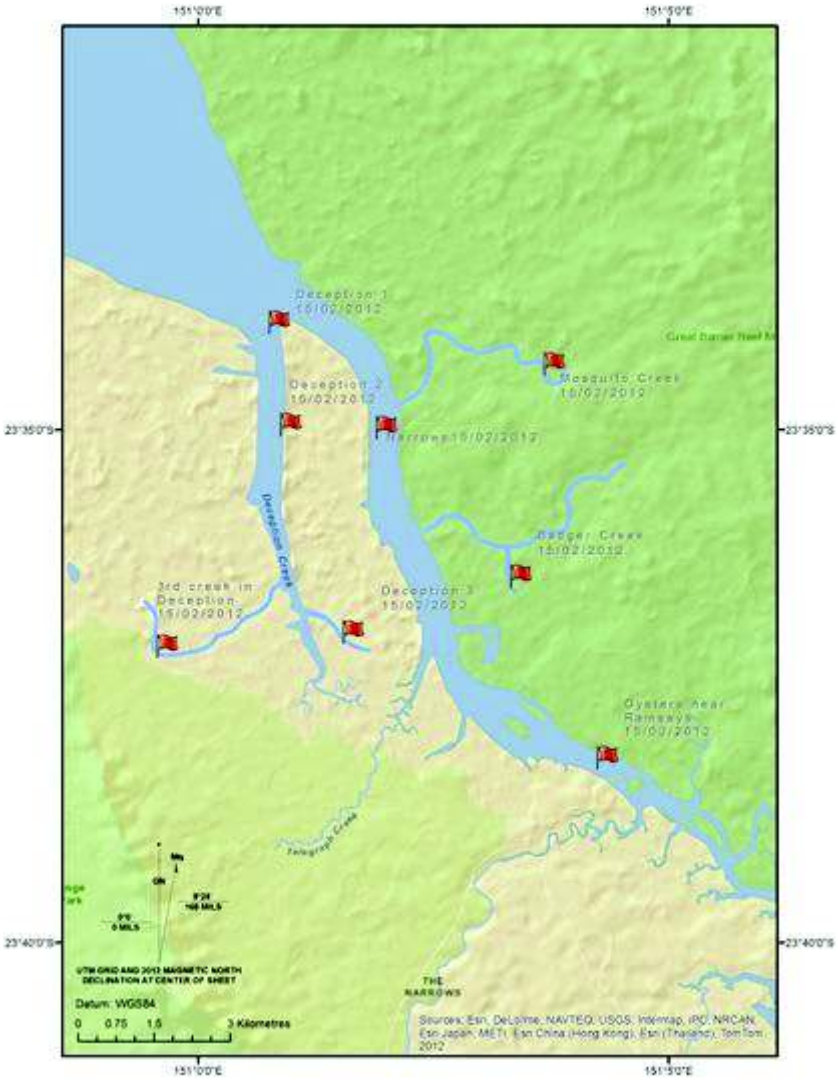


Figure 14: Narrows and Deception Creek: Gladstone.

In April/May 2012 and June/July 2012 Fisheries Queensland undertook further crab surveys. 1559 mud crabs were reported to be examined- 640 from reference site (Bundaberg) and 959 within Gladstone. Shell abnormalities were reported in 3.2% of reference site crabs and 3.9% of Gladstone mud crabs. . The levels of shell disease quoted by Fisheries Queensland are below the historical background levels of 5% in mud crabs where there is no metal-induced shell disease as reported in Andersen (2003).

Trauma is reported to be the cause of the background level of shell disease reported in mud crabs populations, as distinct from the metal-induced endocuticle lesion, in rust spot affected Gladstone crabs. The rate of fighting seems unlikely to have altered over time. The lower than historical average figures quoted by Fisheries Queensland in their survey, suggest there may be an issue with the sensitivity of their detection techniques.

Whilst some variability in crab catch is expected, with tide/lunar cycles, which may alter the proportions of affected mud crabs that are captured, the difference between FFVS and Fisheries Queensland results requires an explanation.

The FFVS data definitively demonstrates that high levels of shell disease were present in February 2012 in Gladstone compared to the FFVS reference site and compared to historical data (Andersen, Norton, and Levy, 2000) using the same monitoring techniques.

In the late 1990's a rust spot disease similar to that identified in 2011-12 in Gladstone Harbour was detected by Andersen (2003) and Andersen, et al. (2000). These authors attributed the shell disease to increased exposure to copper and zinc levels in Gladstone, creating unique lesions in the endocuticle of the carapace of the affected mud crabs.

Above ANZECC Guideline levels of copper were reported in January 2012 by DERM from December 2011 sampling at monitoring site BG10 which near to the area on the southern end of Curtis Island, where FFVS detected the highet prevalence of shell disease in its study in February 2012. In September 2011 sampling the dissolved Zinc concentrations peaked at this same monitoring site. Levels reported by DERM in their January 2012 report were at the trigger value level of 15 μg/L. BG10 is directly adjacent the dredging operations on Curtis Island.

Fisheries Queensland data suggests that the rust spot rate in December 2011, January, April/May and June/July 2012 was lower than the late 1990's. The data, photographs in FFVS interim reports and histology demonstrate that shell disease was occurring in mud crabs in Gladstone HarbourJanuary and February 2012.

For it to be occurring in January and February 2012, requires the affected animals to have been exposed to increased metal concentrations. Dredging and disposal of metal enriched sediments is the most likely activity happening at the right time, in the right place, to be the driver to increasing metals bio-availability.

A similar outbreak of rust spot disease from 1996-97 was reported to be associated with major capital dredging works (B Appo and C Dale pers. comm.) The activity of dredges is annotated in Queensland Notices to Mariners. The research reported by Andersen, (2003) and Andersen et al. (2000) was done at the time, because it was recognised that the condition being reported by commercial fishers was novel, and abnormal. The fishers were again the first point of alert in this rust spot outbreak. The history of the events is remarkably similar with the same process of dredging and resuspending metal enriched sediments, increasing bioavailability. This is likely to have taken place back then, similar to what is being documented in this present case.

Dredging is known to remobilise sediment and its contaminants. The water quality data from DERM, though inadequate, confirms this occurred with numerous exceedances of total metal levels and dissolved metal levels being recorded after (but not before) dredging. Dissolved metals are considered more bioavailable and can move into the food webs through absorption into algae and biota. The massive resuspension of material has also brought substantial volumes of metals to the surface interface of benthic sediments, where they are now more available for consumption by biota.

The effects of sustained elevated suspended sediment and contaminants loads on mud crabs, due to the dredging and disposal project, are likely to be highly significant for future recruitment into this mud crab fishery. The catches of juvenile crabs (<20mm carapace width) in Gladstone were reported by fishers to be very low. This report was corroborated by FFVS sampling, where these juvenile crabs were only reported at the reference site. None were observed in Gladstone sampling.

FFVS remains concerned about the safety of mud crabs for human consumption from Gladstone waters, given the significant elevations of a range of metals including arsenic reported in water testing, and the evidence of mud crabs exhibiting lesions that are likely to have been caused by increased metals exposure.

It is the opinion of FFVS that the FFVS data collected demonstrates a statistically significant increase in shell disease in Gladstone Harbour post-dredging compared to the reference site, and to long term data from Gladstone Harbour and other reference sites. This is consistent with the reports of Gladstone commercial crab fishermen (Bob Appo, Gary Otto, Alan Holland, Des Mercer, Sam Roberts and Jeff Robson).

The increased exposure to metals including copper and zinc are likely to be responsible for the increase in this disease. The same exposures are likely to be deleterious to crab reproduction and it is highly likely that future production will be seriously impacted by the sustained poor water quality in the Harbour, generated by the dredging and disposal project.

c) Shellfish – scallops and oysters

FFVS sampling for scallops was undertaken on board a trawler. FFVS spent more than one hour trawling around the ocean spoil disposal area to the South East of Facing Island on 20 January 2012 (see Figure 3 for sample trawl sites) in an attempt to sample an area previously known in 2010 (D Wise pers.comm.2012) to contain saucer scallops. No saucer scallops were caught, and virtually no benthic fish or prawns were caught either, which, according to the fishers, was unusual for the transects trawled. Only four live rough shelled scallops were caught within Gladstone Harbour, again in an area where catches had been reported to be common in previous seasons. The animals were preserved for histology. Histology revealed bacterial disease in the mantle of the scallops and heavy parasitic fluke infestations. There was 100% prevalence of these lesions in limited number of samples which were able to be caught (Pathology Report 12-3951675 within Appendix 2). The decline in the presence of catch and identification of disease suggests there is a problem with benthic shellfish health within the Harbour.

Work done at the Queensland Government, Bribie Island Aquaculture Research Station, on spawning scallops for ocean ranching projects identified that scallops were acutely sensitive to increases in suspended sediment, with secondary infections commonly reported soon after increased sediment exposure. Given the disposal of large volumes of heavily contaminated sediments at the ocean dump site, into high current areas, the potential for exposure of a large area of historical scallop bed is considerable. And it is likely that such exposures would be deleterious to the health and reproduction of adult and juvenile scallop. Wilber and Clarke (2001) provide numerous references to corroborate the deleterious impacts of suspended sediment on molluscs. Effects are particularly severe on larval shellfish (although wide species variation is observed), potentially affecting future recruitment.

Commercial fishers subsequently collected some saucer scallop material from commercial trawls (see Figure 7) under instruction from FFVS.

Histological examination of convenience sampled saucer scallop offshore from the ocean spoil disposal site off Gladstone, did not reveal any significant histological lesions in the ten animals examined (Appendix 3: Pathology Report 12-5795283). Fishers again reported very few scallops were caught on the tows closest to the ocean dredge spoil dumping ground, which had historically been productive ground. FFVS documented negligible aquatic animal life in trawls around the ocean spoil disposal area.

Department of Agriculture, Fisheries and Forestry, Biosecurity Queensland (2012) reported on saucer scallop sampling undertaken by DAFF, Biosecurity Queensland. Samples from the Gladstone area had higher levels of inorganic arsenic (1.9 mg/kg wet weight of adductor muscle only) compared to a reference site at Bundaberg (1.5 mg/kg wet weight). Levels detected at both sites were above the Australia New Zealand Food Standards Code of 1 mg/kg. Similar results were reported from sampling of scallops and prawns in 2011 by Biosecurity Queensland. The impact, if any, of these levels on the health of the host scallop is not known. As viscera were not tested, it is unclear what the total levels of Arsenic were in these biota. As gills are often the acute phase site of uptake of metals and metalloids, it is unfortunate they were not tested for levels.

Oysters were reported to be dying and falling off pylons with much dead shell observed at the Calliope boat ramp, Auckland River, around Ramsay's Crossing boat ramp at the head of the Narrows and at Canoe Point, time coincident with elevations in turbidity in the Harbour in mid-late 2011 and early 2012. Knott (2009) demonstrated that shellfish will bioaccumulate metals during dredging operations and that such accumulations, can be lethal. Bioassay studies using transplanted oysters (*Saccostrea glomerata*) that were conducted in Gladstone Harbour during previous dredging events in 2002 (Andersen et al. 2002, GHD 2009e) concluded that increased metal concentrations in oysters at the site closest to the dredging activity were likely due to dredging, but not all metals were accumulated at the same rate.

FFVS is aware that bioassay work using oysters was commissioned by Rio Tinto in Gladstone Harbour after the dredging commenced. The results of these studies have not been released.

PCIMP has in past years placed bags of oysters as biomonitors in Colosseum Inlet. A commercial crabber reports that the oysters were not deployed in 2012 (J Robson pers. comm. 2012).

FFVS has not been able to access PCIMP oyster sampling results from 2011.

It is the opinion of FFVS that shellfish populations exposed to the increased sediment load generated by dredging are likely to have been significantly impacted. The timing of mortality of oysters (and other aquatic biota) suggests that sediment resuspension and associated increased metal exposures from the dredging and disposal project has contributed to oyster mortalities.

d) Coral

Esslemont, Russell, and Maher (2004) noted the negative effects of metal rich fine sediment from dredging activities on coral exposed to suspended sediment plumes from dredging activities in Townsville. FFVS remarked on the poor health of examined coral on reefs on the oceanic side of Facing Island in Interim Report 2 (Appendix 2).

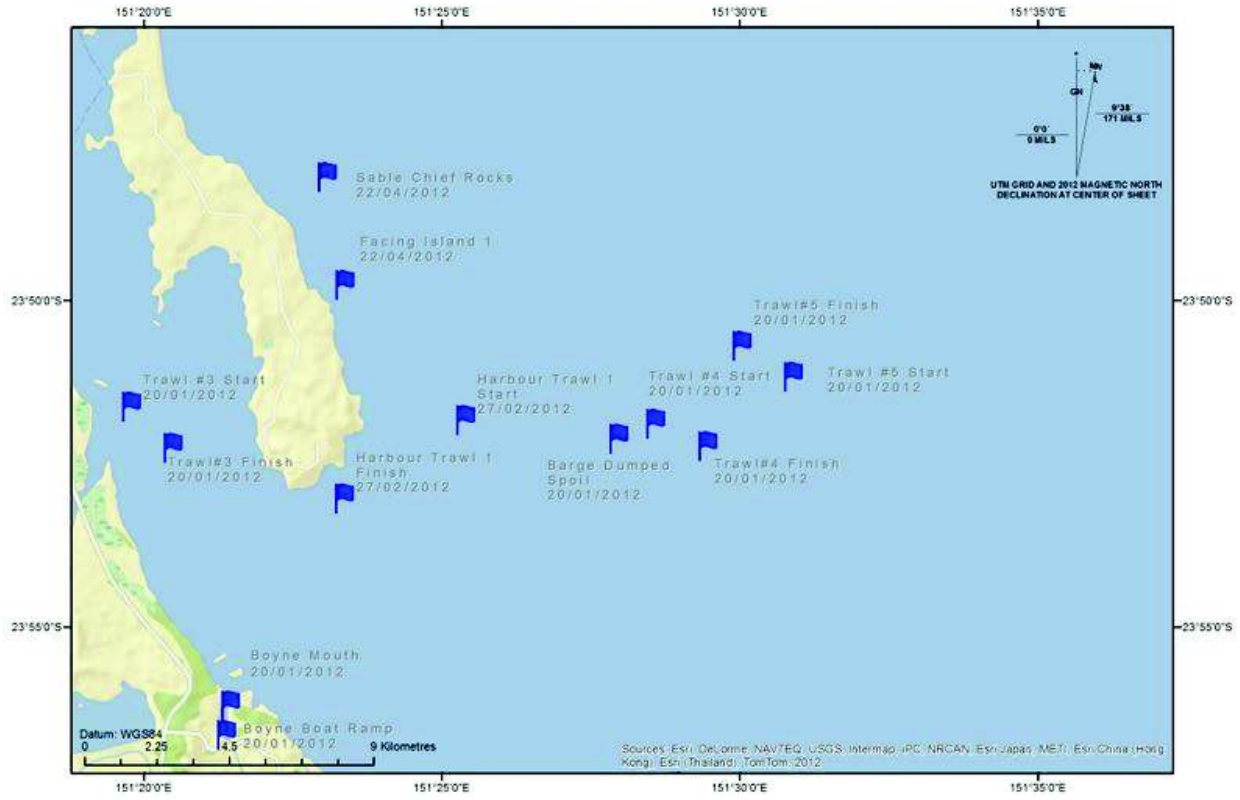


Figure 15: Facing Island 1 and Sable Chief Rocks coral sample sites: Gladstone.

This coral had been surveyed, after the freshwater influx in Jan 2011 and prior to the substantial increase in ocean dredge spoil dumping in May 2011. The coral communities on Facing Island are in direct line of sight to the ocean dredge spoil disposal area, approximately 5 to 7 nautical miles away (a similar distance observed between the coral and the dredging site in the study by Esslemont, Russell, and Maher (2004)).

Photographs of some affected coral were included in Interim Report 2, with material sent to the Australian Institute of Marine Science for histological analysis. The findings included an increase in mucous cells on the surface of the affected coral, in addition to bacterial infections. Such findings are morphologically consistent with the hypothesis that the coral had been exposed to dredge spoil from resuspension from the ocean disposal site, and from movement from the Western Basin dredging activities.

Weber, et al. (2012) studied the factors affecting coastal coral deaths associated with organic-rich and organic-poor sediments. Corals appeared to tolerate organic-poor sediments well. In distinct contrast, the deposition of organic-rich sediments on corals led to declines in pH and dissolved oxygen leading to tissue degradation.

FFVS observed that the proximity of the coral communities on the oceanic side of Facing Island were likely to be exposed to resuspension of dumped dredge spoil and movement of dredge plumes through the channel at the Northern end of Facing Island from the Western Basin Dredging and Disposal operation. Mapping of the satellite dredge plume by Petus and Devlin (2012) demonstrates movement of the dredge plume around the front of Facing Island. A paper modelling the hydrodynamics of the area around Facing Island by Herzfeld et al. (2004) states that:

"*Release at the dredging spoil site results in distributions of dissolved material in the form of a plume originating from the source and directed north-westwards along the seaward coast of Facing Island*."

The material which was selected to be disposed of at the Ocean Spoil Dumping site was high Potential Acid Sulphate Soil (PASS) risk. Vincente-Beckett et al. (2006) acknowledge that disturbance of PASS can result in release of iron, aluminium and other heavy metals.

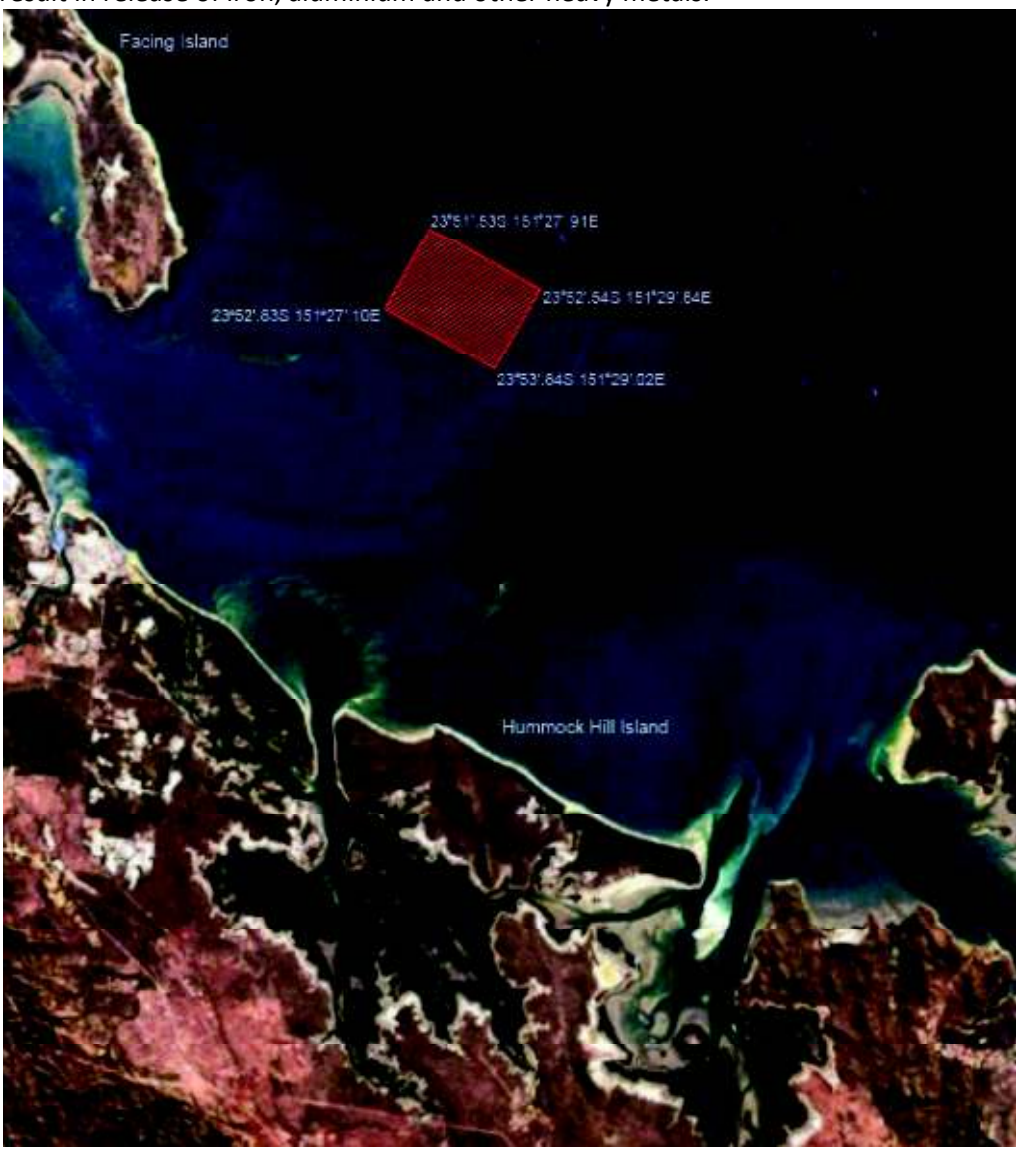


Figure 16: Red area denotes the Ocean spoil dumping site: note proximity to Facing Island to the north, and colosseum and seven mile estuaries to the south.

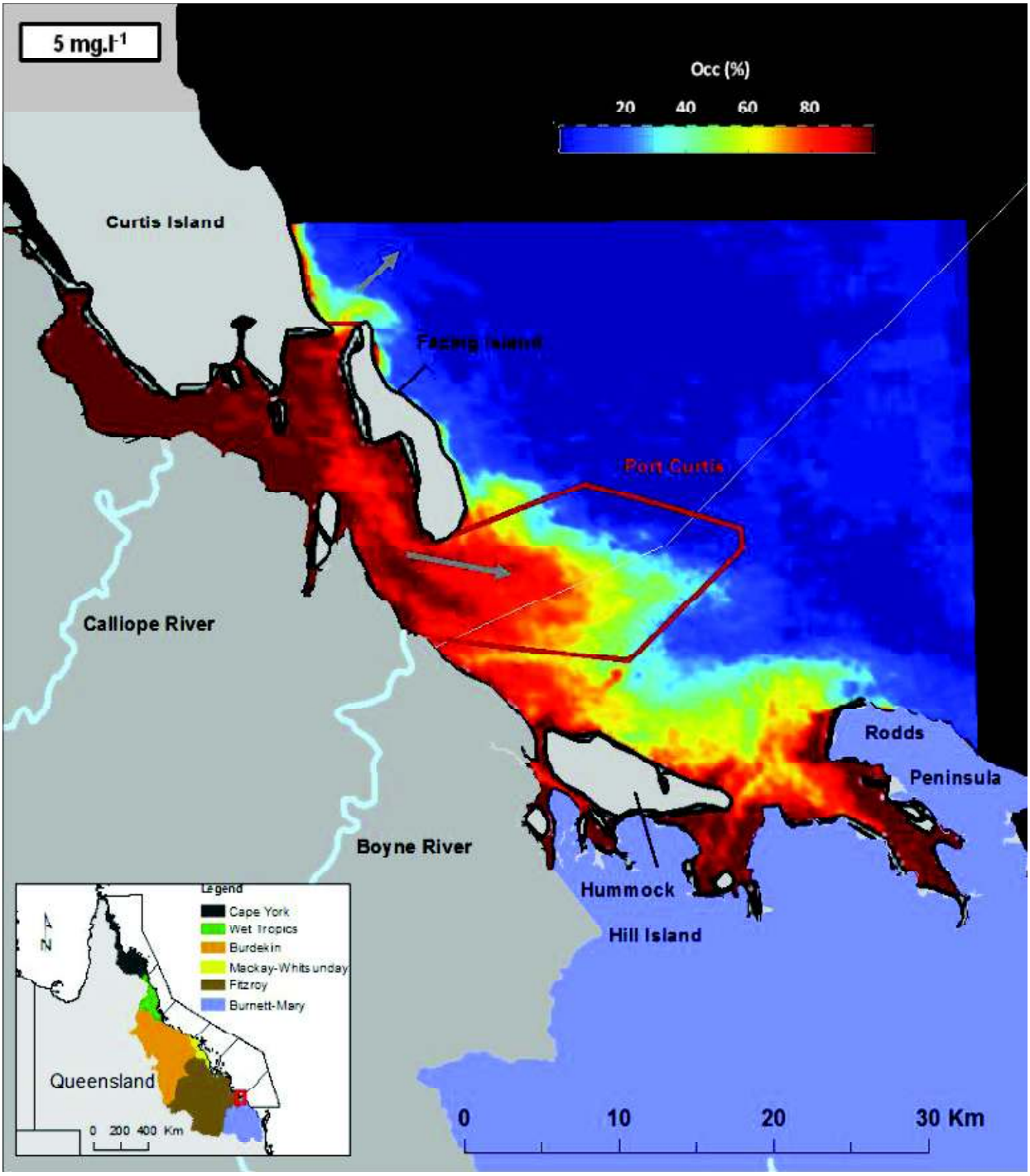


Figure 17: Occurrence maps corresponding to the number of times (%) any given pixel of the GBR was covered by values higher than ~ 5mg.L^{-1} during the Spring of 2011 (Petus and Devlin, 2012).

"*Coastal corals are pretty good at coping with naturally-occurring sediment and the microorganisms that it contains – but if that sediment is enriched with even a small amount of organic matter it can cause sudden coral death*." (Fabricius, 2012)

It is the opinion of FFVS that the ocean disposal of fine sediments from the Western Basin is likely to have contributed to disease of corals along Facing Island, through deposition of organically enriched sediments onto coral, in addition to the toxicological effects of the metals and metalloids released from resuspended dredged and disposal material. Examination of the Facing Island corals using the methods employed by Esslemont, Russell, and Maher (2004) would inform the extent of exposure of these corals to trace metal remobilisation from dredged sediment resuspension plumes.

e) Turtles, Dolphins and Dugongs

The rate of turtle, dolphin and dugong mortality in Gladstone was dramatically elevated above annual averages and significantly different to other parts of the Queensland coast, which also received substantial freshwater inflows. The deaths were occurring synchronously after the commencement of the Western Basin Dredging and Disposal project (Figures 7, 8 and 9) and are still occurring according to media reports in Queensland Telegraph by John Mikkelsen in August and September 2012. No sampling has been undertaken to ascertain the cause of these recent deaths.

No histopathological or toxicological data are publicly available for the dugongs and turtles which were processed by the University of Queensland and EnTox for GPC, in mid-late 2011.

The turtle deaths were initially attributed to activities with commercial fishers. Subsequent Fisheries Queensland Observer monitoring of fishers, absolved the fishers of responsibility.

The Queensland Minister for the Environment, Vicky Darling, then announced that the turtle deaths were due to starvation due to a lack of seagrass, as turtles were described to be thin.

Weight loss in animals leading to them becoming thin, is not always due to insufficient feed availability. It can also be due to inadequate intake in animals which are diseased or suffering from toxicosis.

The seagrass monitoring does not support the contention that the animals starved to death because there was an absence of food available. The necropsies of dugongs, performed around the same time as the turtles, identified ~ 10 kg of seagrass in the gut of each dugong (S. McCauley pers. comm.), indicating there was still seagrass available in Gladstone.

Toxicology and pathology was performed on several turtles during the mortality event from April 2011. Sources advised FFVS that arsenic was a compound of concern identified in turtle blood, however results have not been able to be obtained (Source name with held). The Queensland Department of Environment and Heritage Protection (2012c) water quality results recently released demonstrating elevations of dissolved arsenic in Harbour waters. Hence it would be expected that exposed animals are likely to have elevated arsenic levels. Arsenic is known to be a highly toxic element, depending on the form it is in. Arsenicosis (arsenic poisoning) is documented to cause appetite suppression in humans (Hossain, et al. 2005).

FFVS is unaware whether any turtles were tested for algal toxins in 2011 or in further strandings as recently as August, 2012 (Mikkelsen, 2012). Sampling of recent turtle strandings in the Gladstone region has not been undertaken to try and ascertain the cause of death. Considerable expertise is available through the Australian Wildlife Health Network to assist with such investigations including American Board certified veterinary pathologists.

FFVS considers metals and algal toxicosis, in addition to secondary intense parasitic infestations, are a more likely explanation for Gladstone turtle deaths, rather than acute starvation from inadequate feed availability, given available evidence. FFVS documents the evidence for exposures to toxic algae and metals/ metalloids which took place around the time of turtles becoming sick, through water and contaminated feed ingestion in chapters 7, 10, 11, 12, 13 of this report.

Given the detection of *Lyngbya* blooms in Gladstone Harbour, concern should exist for the potential effects on turtles given publications by Arthur, Limpus, and Whittier (2008) and Arthur, et al. (2008) indicating turtles can consume and absorb the toxin, which has been linked to blood chemistry changes and potential induction of the disease, fibropapillomatosis.

FFVS examined one thin hawksbill turtle which had signs of pericardial oedema. Histopathology confirmed the animal was suffering from heart disease and elevated levels of parasitism. There was ingesta (primarily mangroves) throughout the digestive tract. Unfortunately no funds were available to undertake toxicology tests on this animal. The animal was offered to Qld Fisheries officers for testing, they declined, and requested that it be disposed of it into municipal waste. The high intensity of parasitic disease in the turtle is a common thread with the Gladstone fishes, and adds to the weight of evidence linking aquatic animal sicknesses the Western Basin Dredging and Disposal Project.

There were reports of snub-fin and bottlenose dolphins with skin lesions similar to those described in fish – red and ulcerated concurrent with elevated mortalities reported in the Gladstone area, based on DERM stranding data. Other scientists have been undertaking research documenting dolphin behaviour responses since ~2005. Mortality rates have been recorded on the DERM marine strandings database from prior to the Harbour development through to the present (D Cagnazzi pers. comm. 2012). The results of these researchers, document changes in dolphin behaviour and survival around the time of dredging. The details of the data should be assessed when available in peer reviewed literature, as they may add another line to the weight of evidence approach of this report, specifically implicating the role of the Western Basin Dredging and Disposal Project in the health of another aquatic animals species.

Figure 18 Photograph of a dead dugong, by Clive Last. Image from: http://www.brisbanetimes.com.au/queensland/Harbour-deaths-still-unexplained-20111223-1p8em.html

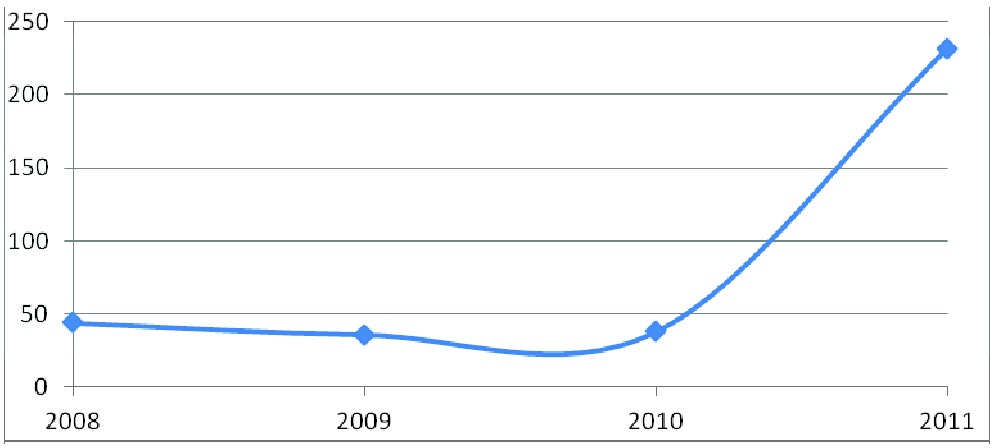


Figure 19:. Turtle Strandings to September 2011 in Gladstone area (DERM marine strandings data).

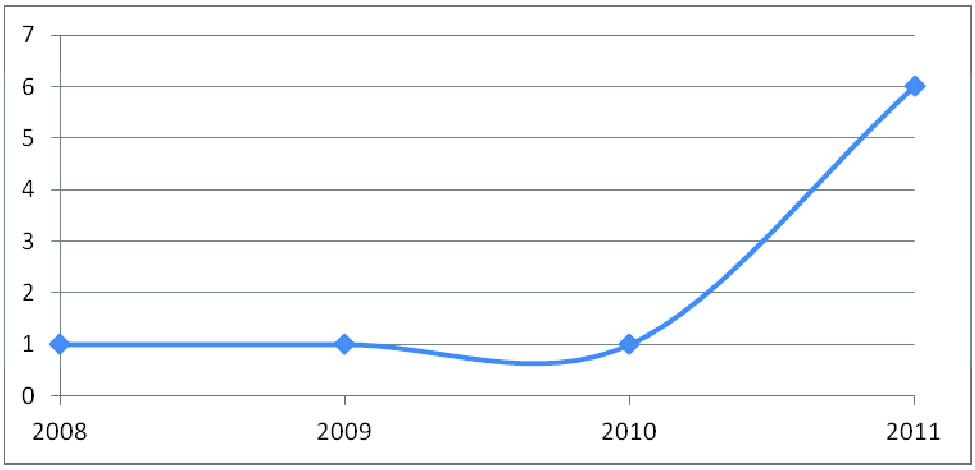


Figure 20: Dugong strandings to September 2011 in Gladstone Area (DERM marine strandings data). Four dugong mortalities were from May-September 2011.

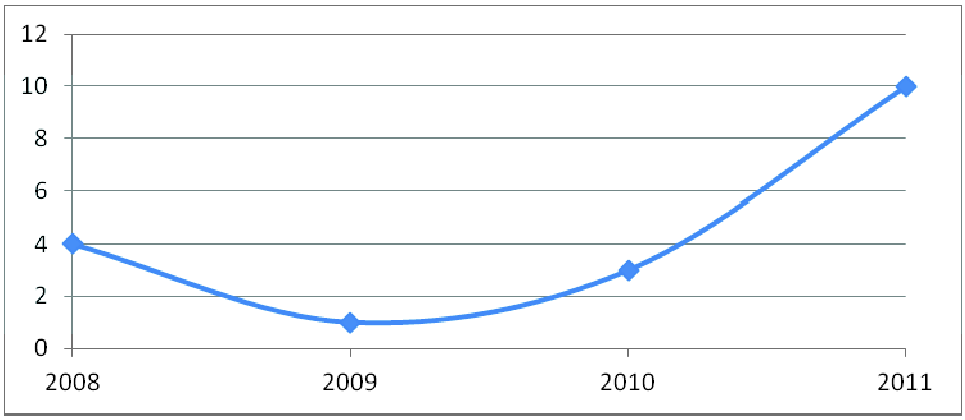


Figure 21: Dolphin strandings to September 2011 in Gladstone Area (DERM marine stranding data).

It is the opinion of FFVS that the sharp increase in turtle, dolphin and dugong mortalities was a direct consequence of the Western Basin Dredging and Disposal project through toxicosis and secondary disease generated by resuspension of contaminated sediments. Pathways of exposure include contaminated food/water ingestion and skin absorption. It is likely that direct toxicosis and secondary immunological compromise and secondary diseases were involved. This conclusion is supported by the limited FFVS histological and limited DERM water toxicological data available.

f) Algae

i) Aquatic Animal Effects

There is international consensus that blooms of harmful algae, including cyanobacteria, are favoured in water with elevated nutrient sources (Heisler, et al., 2008), elevated iron sources (Ahern, Ahern, and Udy, 2008), and disturbances to the natural balance of zooplankton and phytoplankton.

Monitoring performed by DERM post-dredging, documented intermittent increases in nitrogen levels in the Harbour and concomitant elevations in Chlorophyll a readings indicating elevations in algal levels. The Courier Mail (Williams, 2011) reported that monitoring undertaken for GPC had identified blooms of other toxic algae including the dinoflagellate *Chatonella*, the diatom *Chaetoceros*, and a cyanobacterial bloom of *Trichodesmium* (see results chapter). Official results from this monitoring to confirm the densities or identities of the algae detected have not been made available. Vision Environment Queensland also are believed to have tested for the presence of toxic algae blooms. Whilst toxic algal

blooms may have contributed to the onset of the disease in fishes as identified below, they cannot readily explain the shell lesions in crabs.

Limited information is available through the Port Curtis Ecosystem Health Report 2008 to 2010 (Vision Environment Queensland, 2011) on historical algal blooms, as indicated by elevations in Chlorophyll a. Speciation of algae was not available to determine if these post-dredging blooms are a new phenomenon in the Harbour, and around the dredge spoil ocean disposal site. However the following comment from p20 of the Port Curtis Integrated Monitoring Program (PCIMP) suggests that toxic blooms had not been a problem historically.

"*Many of the parameters not examined in PCIMP but examined in other programs are related to nutrient inputs, such as sewage N isotopes, POC, DOC, phytoplankton and cyanobacteria concentration. As nutrient input through sewage discharge is not a major concern for Port Curtis (treated sewage water is recycled in Gladstone), the parameters have been given less emphasis than contaminants that have been identified as being of potential concern to Port Curtis, particularly metals.*"

FFVS sampling in February 2012 identified *Lyngbya* and *Trichodesmium* in 25μm plankton net samples in Colosseum, but not elsewhere. All algae species identified are documented in Table 3. The failure to identify significant toxic algae blooms in the same areas as FFVS captured sick fish suggests that other factors are contributing to the sickness.

Blooms of *Trichodesmium* have been associated elsewhere with detrimental effects on benthic shellfish (Negri et al. 2004). In Gladstone, oysters were reported to be dying and falling off pylons with much dead shell observed at the Calliope boat ramp, Auckland River, and around Ramsay's Crossing boat ramp at the head of the Narrows and at Canoe Point. Such observations were time coincident with elevations in turbidity in the Harbour in mid-late 2011 and early 2012. Sick scallops were also documented in histological samples collected by FFVS in January 2012 from Gladstone Harbour in Appendix 1.

Fishermen reported large amounts of slime exuding from the skin of affected fish, sharks and rays around the ocean dredge spoil dump site in April and May 2012, and elsewhere in the Harbour as the aquatic animal health problems worsened. The increased secretion of mucous is a common response of fish to an external irritant or skin damage. Histology of Gladstone fish and sharks sampled by FFVS, revealed the presence of dermatitis in 20/42 fish sampled, with 8/20 associated with ectoparasites. The remaining 12/20 cases of dermatitis have no evidence of an infectious cause.

Some non-infectious causes of increased secretion of mucous:

Non-infectious causes include:

-toxic levels of metals and metalloids (see metals Chapters of this report),

-toxic levels of ammonia and

-toxic or dense algal blooms.

Leibovitz and Lebouitz (1985), Yanong, et al., (2002), Vasas, et al. (2012) and Graneli, et al. (2012) have reported multiple different algal species generating toxins that can lead to skin damage.

Pfiesteria is an example of a dinoflagellate which can produce toxins. Patterson, Noga, and Germolec (2007) identified that the toxins could damage and kill skin cells. Whilst *Pfiesteria* has not been identified in samples collected from Gladstone, the poor timing and spatial profile of sampling could

have missed blooms of this, or other potentially toxic algae which may be acting on fish skin through similar mechanisms.

It is plausible that toxic algal blooms were a component stressor of Gladstone Harbour aquatic animals around the onset of observed sick fish, turtles, dugongs, dolphins and people. There is however no clear aetiological link with toxic algae with the induction of the rust spot shell lesions in mud crabs. This suggests that additional factors are involved, and the entire event cannot be explained by algal blooms alone.

Lyngbya was not mentioned in the early algal sampling media reports (Williams, 2011), yet the lesions in fishermen provide a high index of suspicion that it may have been present, but not detected, or not reported from DERM/Vision/GPC sampling.

ii) Food safety risks of algal blooms:

Some cyanotoxins and algal toxins are known to accumulate in seafood. Prawns are known to consume *Lyngbya* in Gladstone Harbour (Andersen, et al., 2005), however it is not known if they bioaccumulate the algal toxins. The blooms may represent a risk to human health from consumption of the local seafood, should the biota be absorbing elevated levels of toxins. Recently, a bream fishery in Victoria's Gippsland Lakes was closed in December 2011, due to food safety concerns over bioaccumulation of nodularin toxin in seafood from waters where a toxic algal bloom was present.

Dietrich, Fischer, Michel, and Hoeger (2007) noted that human health risk assessment of cyanotoxins has several deficiencies. FFVS considers algal toxins, metals and metalloids to be contaminants of concern in relation to human health for consumption of Gladstone Harbour seafood. The sediment sampling undertaken for the project EIS identified some highly contaminated areas of the Harbour, which have been dredged. This activity resulted in substantial sediment resuspension as documented by numerous turbidity exceedances and the JCU satellite mapping of the dredge plume. The uptake of these compounds in aquatic animals will vary with exposure, but is likely to be elevated, due to the increased bioavailability created through the resuspension process. The presence of toxic algal blooms in the area where seafood is being harvested poses significant risks. The oyster industries elsewhere in Australia undertake substantial monitoring programs to ensure their products do not contain algal toxins which pose risks to human health. The same level of responsible monitoring for algal toxins is not evident for seafood being sold from Gladstone Harbour.

iii) Human effects of algal exposure

In May 2012 two fishermen were hospitalised after cleaning an unusual slime off their nets which had been soaking in 7 mile estuary, approximately 50 km south the Curtis Island developments, and around 19 km from the ocean spoil disposal ground. The fishermen have been fishing the area for more than 25 years and have not experienced any symptoms similar to these before. Results from the Queensland Government revealed the presence of *Lyngbya majuscula*. This cyanobacteria has been reported to cause this kind of problem and skin lesions in humans (Osborne, Shaw, and Webb, 2007; Osborne, Webb, and Shaw, 2001; Osbourne, 2012; Rzymski and Poniedzialek, 2012), with large blooms reported in Moreton Bay and Hervey Bay.

Whilst the organism has been reported in Gladstone previously it has not been associated with numerous human health issues. The organism is reported to produce more than 70 biologically active substances, many of which are highly toxic, and others are poorly studied at this time. Osborne, Webb, and Shaw (2001) also report that the bacteria, *Vibrio alginolyticus* and *Vibrio parahaemolyticus* have been cultured from *Lyngbya*, hence can become involved in skin lesions initiated by the algal toxins.

Elevated loads of potentially pathogenic bacteria have been associated with other cyanobacterial blooms (Berg, et al., 2009), and it is likely that this was also the case within Gladstone Harbour, given the substantial volume of resuspended organic sediments.

From August to November 2011, more than 40 people (the majority being commercial fishers) reported unusual skin disease issues in Gladstone. The timing of the emergence of the skin lesions correlated strongly with contacting slime from affected fishes, or from water in areas where affected fishes were being captured. Osborne, Seawright, and Shaw (2008) demonstrated that *Lyngbya* generated toxins from crude extracts were capable of causing a rapid severe inflammatory response (within 1 to 3 days) with acute swelling on contact to skin.

The effects of *Lyngbya* blooms on the sediment meifaunal assemblages and effects on oxygen availability in sediments have been documented in Moreton Bay by Garcia and Johnstone (2006). The net effect is likely to be reduced fishery productivity. *Lyngbya* has not been reported to cause skin lesions in fish, so is not likely to explain all of the aquatic animal health disorders observed.

The conditions favouring *Lyngbya majuscula* blooms have been studied in the USA (Gross and Martin, 1996) and Australia (Albert et al. 2005). The authors suggested that blooms were promoted by availability of iron, phosphorus and organic carbon. Further work in Moreton Bay by Ahern, Ahern, and Udy (2007; 2008) demonstrated that organically chelated iron, phosphorus and nitrogen can all promote prolific growth of a bloom. The authors further recommended limiting the release of such nutrients to avoid stimulating more severe blooms. Measures such as silt curtains and dredging only during low velocity tides, can reduce distribution of resuspended sediments, and thereby reduce nutrient being distributed. The Western Basin Dredging and Disposal Project did not employ these measures.

g) Seagrass – evidence of impacts

The seagrass meadows have been studied in Port Curtis since the 1990's because their importance to the productivity of marine ecosystems to provide food, habitat and support biodiversity is well understood (Connolly, et al., 2006). These authors noted that much of the seagrass was close to existing port infrastructure and dredged channels and was:

"*vulnerable to direct impacts of future port infrastructure developments such as wharves, breakwaters and reclamation. The seagrass is of regional importance as one of the few large areas of seagrass in central Queensland, with the closest other areas more than 170 km to the north and south*."

Seagrass provides critical habitat as a nursery areas to support recruitment of new year classes of aquatic animals for important commercial and recreational species of fish, prawns and mud crabs. Prior to the Western Basin Dredging and Disposal Project, the Western Basin was a closed area for prawn trawling. This Queensland Fisheries management zoning measure was in place to protect the seagrass and critical nursery habitats.

Seagrass is also an essential food source for turtles and dugongs known to live in Gladstone Harbour.

The impact of the Dec 2010 to Jan 2011 freshwater inflows on seagrass beds appears to be variable within surveyed areas of Gladstone Harbour (McCormack and Rasheed, 2012a). Redcliffe area declined in November 2010, before the major freshwater influx, but after the commencement of early dredging works in October 2010.

It was apparent that seagrass cover failed to stage a substantial recovery at the Wiggins Island site in Gladstone Harbour. McCormack and Rasheed (2012a) noted that:

"*Some meadows have shown good recovery following 2010/11 floods however seagrass percent cover at some inner Harbour locations remains at very low levels*".

It is likely that a combination of turbidity (light restriction), eutrophication and sediment deposition from Harbour development activities are contributing to the poor state of seagrass meadows in the inner Harbour areas and their failure to recover to previous levels.

Further surveys by McCormack and Rasheed (2012b) documented significant declines in seagrass cover at numerous monitoring sites including Wiggins Island, Fishermans Landing, Redcliffe and Facing Island since the commencement of the Harbour development. The authors' state that:

"*These changes are consistent with seasonal declines as a result of seagrasses entering the "senescent" season. If weather conditions continue to be favourable for seagrass growth and providing there is sufficient seed bank for re-colonisation, we would expect to see the beginnings of recovery at inner Harbour locations as early as July 2012, similar to what occurred in 2011. However, given that seagrasses did not return to historical peak levels over the 2011 growing season, the seed bank from which re-colonisation should occur may also be at a low level, which could potentially restrict the extent of recovery*."

The most recent transect study of inner harbour seagrass beds in Gladstone in August 2012, identifies that there has been a near complete failure of seagrass to recover. Authors had predicted recovery in July and August 2012. The September 2012 seagrass survey data demonstrated that recovery was not evident. Seagrass has been reported in areas remote to Gladstone (eg Tin Can Bay) to have now fully recovered from flood damage in January 2011, back to pre-flood levels. This is not the case inside Gladstone Harbour as documented by McCormack, Sankey, Davies, Carter and Rasheed (2012).

Seagrass has critical light requirements that can be impaired by excessive turbidity in the water. FFVS notes that under the Transitional Environmental Program (Gladstone Ports Corporation, 2012b) dredging was permitted to continue operating, even when turbidity exceeded the original project conditions for more than 48 hours. Turbidity exceeded the limits at monitoring stations throughout a period of six weeks through July and early August as GPC tried to seal the leaking bund wall.

Deterioration in water quality due to sediment re-suspension, from the Western Basin Dredging and Disposal Project is likely to be a significant factor in causing the decline, and hindering recovery of seagrasses, within Gladstone Harbour. The flow-on negative effects to the productivity of the fishery are likely to be substantial.

8. Foreseeable effects of dredging and Harbour development

a) Physical Loss of habitat-impact and costs

A substantial area (see Table 7) of the Western Basin has been excavated by dredging operations. The ecological significance of the Basin as a nursery area for prawns and fish; key foraging habitat for turtles and dugongs has ensured it was protected from harbour trawling activities. Large swathes of seagrass, mangroves and inter-tidal mud flats have been lost through dredging, thus impacting fisheries sustainability (Connolly, et al., 2006). Estuarine benthos provides habitat, food and key ecosystem services facilitating maintenance of water quality and productivity. Protecting seagrass meadows is therefore inextricably linked to waterway health and fishery productivity.

Coastal ecosystems are interconnected, as evidenced by tagging studies of fish showing migration of hundreds of kilometres from source estuaries (Infofish, 2012). When activities occur that degrade habitat connectivity and quality, loss of fishery productivity should be expected. Gladstone Harbour has shown a decline in fishery productivity, which must follow such a substantial loss of habitat, an effect that, is likely to impact areas well outside of Gladstone Harbour. The decline in fish catch as far south as Turkey's Beach and 7 Mile Estuary has already been documented and reported by commercial fishers.

Figure 22: *Example of mangrove loss associated with LNG development on Curtis Island*

GHD (2009a) determined that the reclamation of land provided a high risk to reduce biodiversity, remove seagrass, benthic species, fish and crabs. The control strategy listed was:

"*No ability to control impact. Habitat and communities represented elsewhere in region* ***except for seagrass complex****. Offsets to be implemented*".

It is the view of FFVS that this control strategy would in no way offset the loss.

FFVS concludes that the loss of habitat and its associated food sources and shelter are likely to have generated stress in aquatic biota which relied upon those areas. Permanent loss of fishery productivity will predictably follow this loss of habitat.

b) Water quality

i) Sediment resuspension and impacts – turbidity and dredging

See Appendix 10 for a sample of the data logs from the GPC website demonstrating the numerous exceedances of the permit conditions for turbidity. Gladstone Area Water Board (2011a) documents elevated suspended sediment and turbidity values in mid-May 2011 which coincides with the major increase in dredging activity. Seasonal and rainfall adjusted data shows large increases in NTU compared to baselines documented by GHD in the EIS (GHD2009e). GHD (2009a) acknowledged in their risk assessment, that substantial dredge plumes were likely.

Eggleton and Thomas (2004) stated the obvious:

"*maintenance and capital dredging, and the disposal of historically contaminated sediments, result in major sediment disturbances*."

Knott (2009) state what happens to this disturbed sediment:

"*Dredging activities resulted in the large-scale resuspension of contaminated sediments*",

and go on to allude to the evidence-based impacts;

"*The severe decrease in recruitment of sessile invertebrates within an estuary exposed to dredging and the deposition of contaminated sediments indicates that the resuspension of these sediments pose a real ecological threat to organisms in contact with the contaminated water column".*

The Western Basin Dredging and Disposal Project results in major sediment disturbance. There is also evidence that the Brisbane dredge was undertaking maintenance dredging and disposal at the East Banks Ocean disposal site on 2-11 February 2011 and 14-22 February 2011 from Mariners notices. No data was available to report volumes dredged or disposed. No data was available on the toxicological composition of this material prior to dredging and disposal, because it was not included in the Western Basin project as such. FFVS notes that it did take place immediately prior to the first reports of sick fish at the ocean disposal site, and with reports of material fouling the nets of fishermen, which is likely to have been algal blooms. In April 2012, similar slimy material fouled fishers nets in 7 mile, and was tested and found to be *Lyngbya* algae.

Due to the hydrodynamics of Port Curtis the Harbour is slow to flush suspended sediments (Herzfeld, et al., 2004). Given the slow flushing of Port Curtis, resuspended dredge sediment will tend to linger, rather than rapidly disperse, thereby enhancing the risk of toxicity and ecological harm. The hydrology of Port Curtis does generate high current velocities up to 2 ms^{-1} , due to large tidal variations up to 4 m. Such velocities will have the effect of increasing the risk of resuspension of dredged sediments, during dredging, ocean disposal, leakage from the reclamation area and into the future with ongoing resuspension. The lack of use of measures to control the resuspension, such as silt curtains, is highly likely to result in widespread redistribution of contaminated sediments. Herzfeld, et al., (2004) suggest particle movements of 15 km are likely on a single tidal movement in Gladstone Harbour. This hypothesis is confirmed with evidence by Petus and Devlin (2012) suggesting a 34 km dredge plume during the period of their observation of satellite images mid 2011.

Bolam, et al. (2011) found minimal ecological changes after disposal of some maintenance dredged contaminated sediments in SW United Kingdom. The authors suggested this was likely due to the dispersive nature of the disposal site. This is the opposite of the situation in Port Curtis/Gladstone Harbour. The construction of the bund wall was acknowledged by the engineers to permanently change the hydrology of the Western Basin area and further restrict the flushing and dispersal of the resuspended dredged/prop wash/leaking bund sediments (LNG Port Dredging and Gladstone Ports Corporation, 2012), exacerbating the likelihood of continued resuspension of sediments.

ii) Risks of suspended sediment (without assessing contaminants)

Wilber and Clarke (2001) reviewed the effects of suspended sediments from estuary dredging and found a wide range of impacts on health and behaviour of fishes. The impacts varied widely with the species and nature of the exposure. Effects were particularly dire on eggs and larvae (early life stages) with fertilisation and hatching failure and direct mortality of larvae common at modest sediment increases.

Increased sediment also inhibited feeding in many studies. As fish/crab/prawn larvae only have very small body reserves it is imperative that they feed frequently to survive. A prolonged period of reduced feeding is likely to substantially reduce survival. Should sediments contain contaminants, further deleterious effects on reproduction and early life stages can occur with increasing deformity rates reported.

The effects of uncontaminated suspended sediment on fish vary with species adaptation – with estuarine species more adapted, compared to reef species. Au et al. (2004) noted that chronic exposure to elevated suspended sediment can result in damage to gills, which is likely to lead to sub-lethal stress (osmoregulatory and respiratory function disturbance) and compromise fish health.

It has been documented that very small particles (nanoparticles) can be absorbed by fish from their environment. Subtle effects on fish behaviour (reduced swimming velocity) have been reported by Chen, Lin, and Tseng (2011) with exposure of larval fish to nanoparticles of titanium dioxide. It is uncertain how this may translate into larval survival and fitness, but it is unlikely to be beneficial.

Reid et al. (2003) documented that fish exposed to increased suspended sediment increased their respiration rates, and experienced changes to the haematocrit (% of red blood cells: plasma). The gills remained normal histologically. Such changes are consistent with low level stressors. Redding, Schreck, and Everest (1987) documented similar effects, but additionally documented an increase in plasma cortisol. Elevations in cortisol are considered an indication of stress, and when they remain high, immunosuppression is a common consequence. The authors observed that fish became more susceptible to bacterial infections when exposed to elevated suspended sediment loads.

FFVS and DERM documented elevated suspended sediment levels in the Harbour post-dredging. Whilst cortisol levels have not been measured in Gladstone fishes by FFVS or Government Authorities, secondary indicators of immune status, such as parasite loads, are suggestive that fish are immunosuppressed. The Gladstone Panel (Gladstone Fish Health Scientific Advisory Panel, 2012) also suggested that immunosuppression appeared to be present in Gladstone fishes from their investigation.

FFVS observed increased intensity of parasitic infections on sampled Gladstone fish compared to reference site fish. Palm (2011), Khan (1990; 1991; 2011), Mackenzie (1999), Marcogliese (2005) and Palm and Ruckert (2009) discuss and demonstrate that in wild fish the normal host-parasite relationship can be disturbed by exposure to contaminants. The result is increased prevalence and increased intensity of parasitic infections. This is precisely what FFVS has documented in Gladstone fishes compared to the reference site.

FFVS also observed signs of chronic inanition in Gladstone fishes. . Inanition in fishes may be a response to stress but can also be due to impairment of olfactory (smell) receptors by sediment and/or metals which has been documented in various fish species (Norrgren, Wicklund Glynn, and Malmborg, 1991; Klaprat, Evans, and Hara, 1992; Mirza, et al. 2009; Scott and Sloman, 2004; Tierney, et al., 2010).

Partridge and Michael (2010) demonstrated substantial impacts on larval survival with exposure to elevated calcareous suspended sediments which can be resuspended by dredging. For larvae prior to opening their mouth the 12 hr LC-50 (the lethal concentration at which 50% of the population die within 12 hours of exposure) was 2020 $mg.L^{-1}$. Once larvae opened their mouths this 12 hr LC-50 declined to 157 $mg.L^{-1}$. This level of suspended sediment was exceeded in sampling undertaken by Gladstone Area Water Board at their hatchery intake in September 2011, indicating that impacts on recruitment may be occurring in the areas of the harbour affected by the dredge plume.

The sediment also had an effect on visually mediated feeding behaviour of certain life stages of the larvae leading to reduced zooplankton ingestion.

The opinion of FFVS is that these mechanisms are likely to result in reduced larval survival, thereby impacting on subsequent recruitment into the fishery, and productivity in the coming years. The prolonged periods of elevated suspended solids over a large areas of Gladstone are likely to be deleterious to the success of fish and crab reproduction during these periods.

Drabble (2012a) recorded declines in several species of fish in association with dredging activities. For benthic fish, he implicated recuitment failure as a component of the cause. The lack of abundance of benthic fish, prawns and scallops observed when FFVS undertook sampling trawls around the spoil dumping ground was remarkable. The fisheries catch data also demonstrates this similar dredge related decline. The declines noted by Drabble (2012a) were considered too large to be due to natural variation alone, thereby implicating the dredging with a causal role in the decline of fish populations. Drabble (2012b) also identified entrainment in dredges as a potential cause of population decline for susceptible species.

FFVS considers that the already observed declines in fish, prawns and crabs observed in Gladstone, are due to a combination of increased disease and mortality rates, in part recruitment failure, also entrainment in hydraulic dredging activities, and movement away from the noise and poor water quality. Given the overlay of the dredging plume over key nursery area of the Western Basin, the Narrows, South Trees Inlet, Colosseum Inlet, 7 mile inlet and Rodds Bay and its extended duration, FFVS expects serious deleterious impacts on fishery recruitment as a consequence of sediment resuspension.

iii) Ammonia/nitrogen release and impacts

Ammonia is considered a highly toxic naturally-occurring constituent of sediment pore waters. The compound is derived from the microbial breakdown of organic nitrogenous compounds by microplankton and microphytobenthos (Batley, 2009).

Ammonia toxicity is well documented in fish (Noga, 2000), with clinical signs ranging from hyperexcitability and death in acute cases, hyperplasia and hypertrophy of gill tissue, hypersecretion of mucous from gill and skin and immunosuppression in cases of chronic exposure. High aqueous ammonia is known to increase blood and tissue ammonia levels, the secondary effects of this being increased blood pH, osmoregulatory disturbances, decreased blood oxygen transport and increased oxygen consumption (Noga, 2000). Such disturbances are likely to compromise the immune function of fish and rend them at greater risk of disease from infectious agents, such as parasites and bacteria.

The presence of ammonia exists in its ionised and unionised form, illustrated in the below equilibrium equation:

$$NH_3(aq) + H_2O(l) \Leftrightarrow NH_4^+(aq) + OH^-(aq)$$

Unionised ammonia is recognised to be more toxic to fish in comparison to ionised ammonia. The proportion present in each form is dependent on the pH, temperature and salinity. Higher pH, higher temperatures and lower salinities favour the presence of the unionised compound (Noga, 2000).

The pH levels detected by FFVS during sampling in January and February 2012 were ~8.3 in the Western Basin at water temperatures of 28.6^{o}C. At these levels Total Ammonia Nitrogen levels would only need to be above 0.18mg.L^{-1} to exceed the ANZECC 99% ecosystem protection guidelines.

GHD (2009b) reported in sediment sampling in the EIS that levels of ammonia, exceeded the limit of reporting in 244 of 377 samples. Average concentration was 2.2 mg/kg, with a maximum of 10 mg/kg detected. Hence, upon dredging and ocean disposal, resuspension could release substantial amounts of ammonia. Acutely ammonia can increase skin mucous production as has been documented in numerous species (Kalogianni, et al., 2011; Caglan, Benli, and Koksal, 2005). This symptom was observed by commercial fishers (T Falzon, G Otto pers comm. 2012) early in the disease outbreak in fishes both within the Harbour and around the ocean disposal site.

Mahmood, Kovacs, Gibbons, and Paradis (2010) demonstrated that ammonia and hydrogen sulfide were released through dredging at levels that were toxic to exposed fishes in their study.

DERM monitoring did not detect levels of ammonia nitrogen above 0.017 $mg.L^{-1}$ at any monitoring sites from Sept 2011 to Jan 2012 (Department of Environment and Resource Management, 2011; 2012). However, as with comments on pH and hydrogen sulphide, the monitoring sites may not have been located sufficiently close to the points of discharge to detect significant elevations. The available data does provide evidence that there was no widespread increase in Total Ammonia Nitrogen on the dates tested from the locations sampled. It is possible that benthic animals were exposed to increased ammonia levels in association with recently dumped dredge spoil, which testing would not have detected. Images of sting rays provided by commercial fishers (G. Otto, T. Falzon, and D. Wise), and flat fish examined by FFVS demonstrated that there was more severe gross signs of skin lesions on the ventral (lower) surface of these animals. These animals were caught from inner harbour trawl sites, documented in pathology reports, and on Figure 3.

GHD (2009d) acknowledge that "ammonia can be readily released from sediment" particularly in association with the operation of the trailer suction hopper dredges, where they anticipated levels would exceed Queensland Water Quality Guidelines of 0.01$mg.L^{-1}$ by 3-4 fold. Exposed fish could incur significant stress with such levels.

DERM did document significant increases in nitrogen, and chlorophyll, after the commencement of dredging, at times unrelated to rainfall influxes (Department of Environment and Resource Management, 2012a).

FFVS considers there to be a high risk of ammonia release from the dredging activities in the absence of mitigation measures. As such FFVS considers it likely that water quality due to unionised ammonia in this area may have been stressful to exposed biota.

iv) Dissolved oxygen

Oxygen enters the water column by diffusion through atmospheric air and photosynthesis. It is then consumed by aquatic organisms e.g. bacteria, fish, molluscs and zooplankton. The saturation of oxygen is dependent on temperature and to a lesser extent, salinity (Noga, 2000). Department of Environment and Heritage Protection (2012c) have described decreased levels of oxygen saturation compared to pre-dredging data in some areas of the Harbour.

Should fish gills be exposed to elevated unionised ammonia, or suffer deposition of aluminium, then it is likely that their capacity to perform all their physiological roles (oxygen uptake, carbon dioxide and ammonia elimination and osmoregulation) will be impaired. This impairment leads to physiological stress. The capacity of affected fish to handle lowered oxygen saturations will be reduced. The very high levels of aluminium reported on the gills of some fish (Fisheries Queensland, 2012), suggests that deposition is occurring and will be compromising gill function, as has been documented in the peer

reviewed scientific literature (Genemer and Playle 1999). Similar levels of aluminium deposition were not reported in reference site fish. The effect will be compounded by the lowered oxygen tension in the water, which is likely the result of increased turbidity from ongoing dredging operations, prop wash from large increase of boat traffic on Harbour and the leaking bund wall of the reclamation area.

It is unlikely that the amount of atmospheric air being diffused through surface water or the amount of photosynthesis has been altered dramatically in the region, nor has a population boom of higher marine vertebrates been recorded that could explain the decrease in oxygen saturation. The only major change still being encountered is dredging activity. It is known that dredging and disposal of dredged material releases ammonia and organic matter into the water column from the resuspension of sediment. It is therefore postulated that an increase in biological activity following dredging is causing an increase in consumption of oxygen due to bacterial nitrification, consequently lowering dissolved oxygen levels in the Harbour. The release of anoxic pore water, and oxidation of ASS sediments is also likely to be a significant draw down on the dissolved oxygen levels.

It is the opinion of FFVS that even the mild reductions in dissolved oxygen observed post-dredging may be biologically significant to exposed biota, as gill function may be compromised from increased suspended sediments and compounding impacts of contaminants such as aluminium, arsenic, zinc and copper.

v) Hydrogen sulphide and other toxicants

Many estuarine sediments contain reduced forms or inorganic sulfur, existing mostly as pyrite, as well as hydrogen sulfide which exists as a by-product of bacterial breakdown of organic matter in an anoxic environment (Clark, 2004). Hydrogen sulfide acts as a weak acid and reacts with both water and metals to form sulfhydric acid and metal sulfides respectively, and can act as a transitory toxin upon release as a gas through water. The main effect is on respiration of fish, leading to hypoxia (Schwedler, Tucker, and Beleau, 1985). It is very difficult to test for due to its volatility and ANZECC Guidelines do not provide a safe exposure limit for hydrogen sulphide in marine waters.

With the dredging of large areas of shallow organically enriched mudflat and seagrass, it is likely that there were transitory releases of hydrogen sulphide which could be acutely stressful to exposed fish and other biota in the immediate area.

DERM reported in March 2012 that Orica had exceeded by more than double, their discharge licence for cyanide (1 mg/L) release into Gladstone Harbour in January and February 2012 (Stitt, 2012). The effect of this release is expected to be localised and temporary, and appears insufficient to explain the wide geographic area where sick aquatic animals were recorded around Gladstone, unless cyanide contaminated sediments were resuspended from dredge and disposal activities. The Orica plant is located adjacent the fisherman's landing area in the Western Basin. It may have contributed to local impacts on aquatic animal health. Prashanth (2012) reported an LC-50 for the freshwater fish, tilapia, of 44.3 μg/L. The author also noted that hypersecretion of mucous in fish was a clinical sign that can be associated with toxicity.

The cyanide spill is highly unlikely to be causal of fish health problems observed on the oceanic side of Facing Island, and >30km to the south in 7 mile estuary, where FFVS sampled diseased fish, due to the substantial dilution effects. Hence the cyanide spill is insufficient on its own to be the factor responsible for the common disease issues across the wider Gladstone area.

It is the opinion of FFVS that biota in the local area of dredging may have been exposed to stressful or lethal levels of hydrogen sulfide released from dredged sediments, and potentially toxic levels of cyanide from Orica releases above their licence limits.

vi) Petroleum Hydrocarbons (PAH's) and Polychlorinated biphenyls (PCB's)

Elevated naphthalene concentrations in Gladstone Harbour can be attributed to oil shale deposits underlying the area. Jones, et al. (2005) suggested that:

"exposure of natural minerology through dredging of the shipping channels"

may have contributed to contamination of the Harbour. Given the proximity of the current Western Basin dredging operations to these deposits, it is a substantial risk that further contamination could have occurred, and not been detected by the monitoring regime.

There are other substantial anthropogenic sources of polyaromatic hydrocarbons (PAH's) to this estuary from a history of 45 oil spills prior to 2000, aluminium smelters emissions of 23000 $kg.yr^{-1}$, and dust and run-off from coal stockpiles and loading wharves. Aside from the human health impacts of PAHs that are well known, there is substantial scientific evidence to support their role in environmental toxicities. Due to their hydrophobic nature, shortly after release, PAHs rapidly bind with deposited sediment (Sun, 2012). Hence resuspension through dredging and Harbour development can lead to remobilisation of these contaminants, and exposure of biota.

Jones, et al. (2005) identified elevated levels of PAH's in the Fisherman's Landings area, a site which was part of the early dredging program, time coincident with sickness in aquatic animals in the Harbour. (GHD, 2009b) detected 64% of samples from Stage 2 (p67) and 88% of samples from Stage 3 (p67) above the Limit of Reporting (LOR) for the total petroleum hydrocarbons (TPH, +C10 to C36). Low concentrations of benzene, toluene, ethylbenzene and xylene (BTEX) were detected in these sites also. Total levels were below the guidelines levels of concern used in the EIS.

The EIS considered dioxins to be below levels of concerns, but at the same time urged:

"*Caution needs to be applied to the laboratory results for PCB's within the Stage 1B and Stage 2 areas due to a discrepancy in the data resulting in some samples being reported with a LOR concentration of <1000µg/kg which is equal to the NAGD Screening Level*"

The Limit of Reporting of the tests used to determine levels of PCB's in some areas which were to be dredged, was high. Should levels of PCB's have been present, below this LOR, they would not have been detected in this laboratory analysis. Importantly, levels of PCB's below this LOR, are documented to be significant for aquatic animal health.

It is the opinion of FFVS that there is insufficient evidence to implicate PAH's and PCB's with a primary role in observed aquatic animal diseases.

c) Impacts of contaminants in sediments

i) Resuspension of sediment role in altering bioavailability and toxicity of metals

It is not so much the increase in particles in the water that has an impact on aquatic animal health, more so, it is the contaminant loading on and/or in that sediment (Weber, et al., 2012).

The effect of suspended sediment on fish varies with both the contaminants on the sediment, particle size, age of exposed fish and other environmental factors. Eggleton and Thomas (2004) and Berry and

Hill (2003) discuss the factors which influence the release and bioavailability of contaminants during sediment disturbance events. Importantly, they note gaps in knowledge to predict the behaviour of contaminants in disturbed sediments, noting that changes in redox potential and pH can alter desorption, partitioning and bacterial affinity of contaminants to sediments (Forstner, Ahlf, and Calmano, 1989). These authors demonstrated mechanisms by which metals such as copper can be transferred into the aquatic food chain. They concluded:

"*It is particularly problematic to disperse waste materials in ecologically productive, high energy environments such as estuaries*".

It is with this in mind, that FFVS views the ANZECC ecosystem protection guideline levels of contaminants with caution. There is potential to underestimate the impacts of contaminants as they occur in mixtures, rather than individual one-by-one exposures (Poulsen and Escher, 2012). Furthermore, the ANZECC guidelines do not consider other potentially significant sublethal impacts, such as olfactory impairment in fishes, even though this may result in significant ecological damage over the long term (Scott and Sloman 2004; Mirza et al. 2009; Tierney et al. 2010).

Chapman, Wang, Janssen, Persoone, and Allen (1998) highlight that to determine the bioavailability and toxicity of a particular sediment, bioassays are necessary, as the complexity of interactions is considerable. The limitation of bioassays, is that the toxicity result will only be relevant to the test organism, and other organisms may have differing levels of sensitivity. FFVS has not seen any data demonstrating the use of any bioassay methods, used prospectively or retrospectively, by Government Authorities to ascertain the actual risks of contaminated Gladstone sediments to aquatic biota.

Historically the PCIMP utilised oysters as biomonitors in Gladstone Harbour (Andersen et al. 2002). Oysters have great utility as biological monitors of toxicity and for detection of resuspension and distribution of metals (Hedge, Knott, and Johnston, 2009; Knott, 2009). However there appears to have been no such use of oysters, in this project. And results from more recent PCIMP work (i.e. post 2010) are currently unavailable.

The resuspension of deeper anoxic sulfide containing sediments in Gladstone Harbour, and exposure of them to oxygen in the water, is likely to have increased the redox potential (Chapman, Wang, Janssen, Persoone, and Allen, 1998), increased the microbial activity and decreased the sediment pH. Where sediments are already partially oxidised, the release of metals may be negligible. The detections of elevated metals in Gladstone Harbour by DERM, GAWB (Gladstone Area Water Board, 2011b) and Diggles (2011b) post-dredging indicate that some metals have been released in sufficient quantities to elevate measurements of dissolved and total metals. These elevations took place even though the lowest recorded pH at monitoring stations was 7.1. Monitoring stations may not have been close enough to the sources of potential acid release to detect it prior to marine water buffering it up: e.g. from the leaking bund; pore water from dredging; construction on curtis island; and PASS on barges.

The bioavailability of those metals is largely dependent on how strongly they are bound to particulate matter, which is reflective of the species state the metal is in. Dissolved metals are the most bioavailable to aquatic animals, with bound minerals only becoming more available upon ingestion (eating or drinking). Peng, Hung, and Hwang (2011) highlight the potential for the submarine hydrothermal vent crab (*Xenograpsus testudinarus*) to bioaccumulate a range of metals. The authors suggest the primary mode of uptake may be via gills through detecting higher levels of many metals (Al, Cd, Co, Fe, Cu, Ni and Zn) in the gills of the sampled crabs. Mn was highest in the exoskeleton and Cr and Pb were similar across the range of tissues tested. It is likely that the combination uproutes of ingestion and absorption

across the gill have been responsible in mud crabs for increasing their metals exposures leading to increased rates of rust spot shell disease (see Crab Chapter) in Gladstone Harbour compared to the reference site.

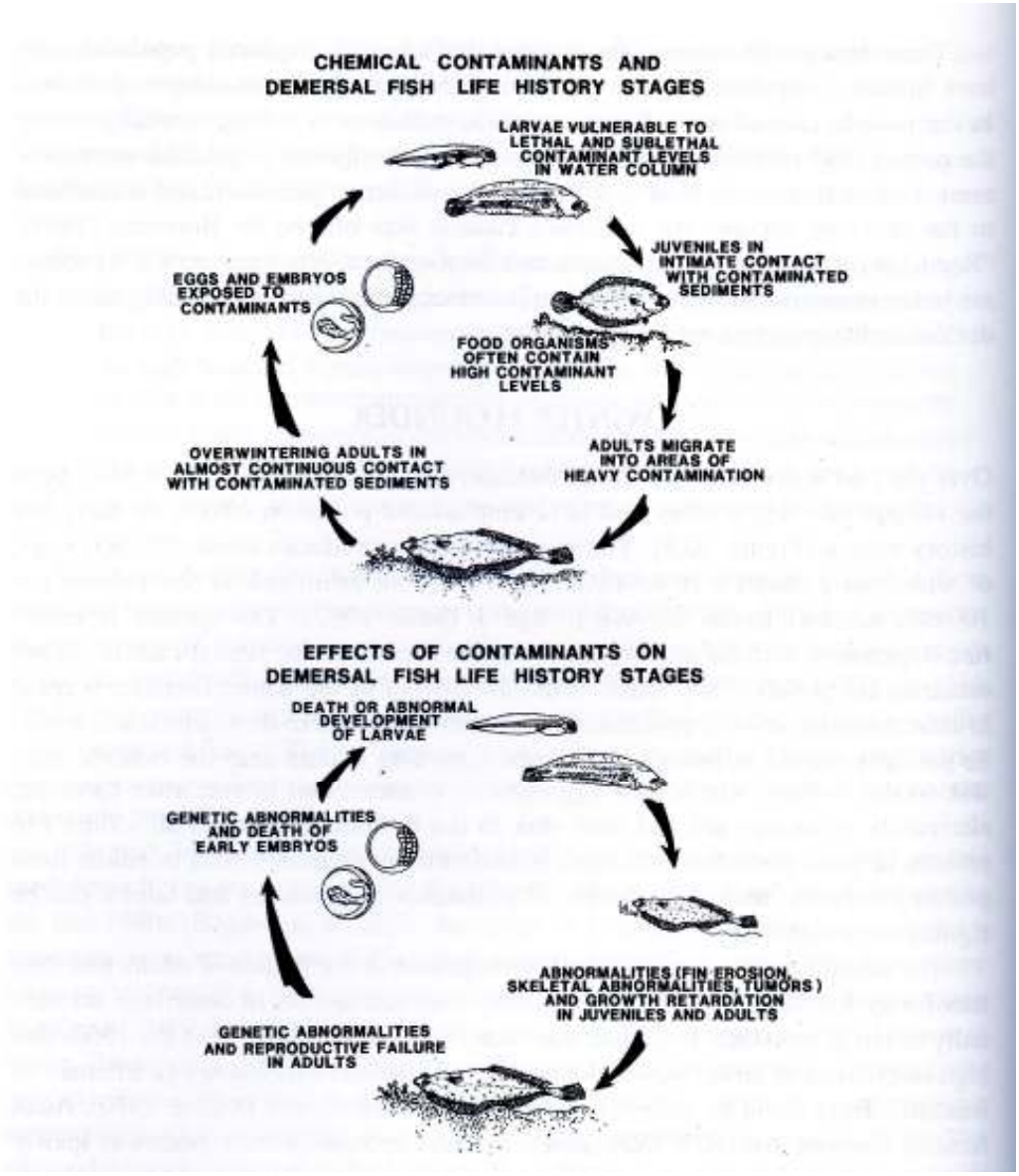


Figure 23: Lifecycle of the winter flounder, *Pleuronectes americanus,* with potential pollutant impact points and effects of pollutants. Illustration from Sindermann 2006 p 154

It is the opinion of FFVS that the resuspension of sediments, in particular PASS, which was present in large areas of dredged sediments in Gladstone Harbour, has facilitated the release of large loads of metals and metalloid compounds into more bioavailable dissolved forms. The measured elevations of metals in late 2011 (including zinc, copper and aluminium) by DERM, compared to historical levels, provide evidence of these elevations taking place after the commencement of dredging.

9. Historical contaminants in Gladstone Harbour

The sediments of Gladstone Harbour have been the subject of study prior to this Harbour expansion. (Jones, et al., 2005; Vincente-Beckett, Shearer, Munksgaard, Hancock, and Morrison, 2006; Apte, et al., 2005). These authors noted TBT, arsenic and naphthalene were all contaminants of potential ecological concern, based on their risk assessment. The concentration of metals in the sediments was generally

below levels of regulatory concern prior to the dredging activity. However, the levels of arsenic, chromium and nickel were consistently above the ANZECC low interim sediment quality guidelines. The levels of copper, nickel, lead and zinc were all elevated relative to a "pristine" reference site. These authors flagged the need for more studies to determine the origin of elevations of particulate arsenic.

Recent sediment studies also demonstrated above guideline elevations in arsenic (Department of Environment and Heritage Protection, 2012c).

Apte, et al. (2005) also flagged that concentrations of aluminium, arsenic, copper, chromium, iron, mercury, nickel, selenium and zinc were significantly enriched in marine biota within Port Curtis compared to a reference site. They were not able to comment as to whether the levels detected were deleterious to animal health, and suggested that bioassays were necessary. Uptake may have bio-accumulated through the food chain via ingestion. Despite this recommendation, the bioassay method of testing was not utilised in the EIS, and no results of bioassays have been published by DERM, Fisheries Queensland, Rio Tinto or Biosecurity Queensland since the onset of fish disease in the Harbour.

Simon Apte from CSIRO recommended metals testing in biota in the recent ABC Catalyst program on 20 September 2012 (http://www.abc.net.au/catalyst/stories/3593812.htm). He noted that this work had not been undertaken in Gladstone at the time of the interview.

Additionally, GHD identified issues associated with the bund wall development, including:

"*The majority of sediments underlying the proposed Western Basin Reclamation Area contain excess sulphur acidity and net acidity at varying depths. It has been assumed that a maximum of 2 m may be disturbed during the construction of the bund wall. Based on laboratory testing, the majority of the samples from the Reclamation Area do not appear to contain enough buffering capacity to self-neutralise.*" (GHD, 2009d)

In Angel et al (2012), the authors claimed that elevated arsenic levels in Gladstone Harbour were of geological origin. They referenced the work of Apte et al (2005). However upon closer reading of the 2005 document, there was no data presented which could assess the origin of the arsenic with forensic accuracy. Hence the origin of the detected arsenic remains untested. FFVS agrees with earlier recommendations by Jones, et al. (2005), Vincente-Beckett, Shearer, Munksgaard, Hancock, and Morrison (2006) and Apte, et al. (2005) that more work is required to identify the cause of elevated arsenic levels, including the use of forensic trace element analysis, given the nature of adjacent industry and likely emissions profiles of arsenic and other contaminants.

In the Western Basin Dredging and Disposal Project sediment assessment EIS GHD (2009b) documented that some areas of the Harbour sediments had 20 600 mg/kg of Aluminium, 76 000 mg/kg of iron, 7 680 mg/kg manganese, 171 mg/kg copper, 49 mg/kg of nickel, 49.5 mg/kg of arsenic, 0.5 mg/kg benzene, 0.5 mg/kg ethylbenzene, 0.3 mg/kg toluene and 412 mg/kg PAH's (sum of total). Through the use of averaging all samples across a wide area of the harbour and shipping channel, the maximum levels of compounds such as manganese were brought under the Environmental Investigation Level (EIL) (GHD 2009b). This does not however reduce the hotspots of metals which clearly existed well over the sediment guidelines in areas which were subsequently dredged immediately prior to the onset of sick aquatic animals in Gladstone Harbour. The DERM data of metal levels in the Harbour, demonstrates that levels went up and down for a range of elements.

The use of averaging has also been a feature of the Vision Environment monitoring, which has the effect of systematically under-estimating the peak impacts of the elevations recorded in water quality. The responses of biota, are related to the actual level experienced, rather than averaged levels.

It is notable that the more contaminated sediments of the Western Basin were being dredged at the time of onset of aquatic animal sicknesses. Many of the highest concentrations of metals/metalloids were detected in the surface sediments to 1.12 m depth, making them the first and most likely sediments to be resuspended into the water column.

Esslemont, Russell and Maher (2004) noted that dredging activities in Townsville harbour resuspended fine sediments, creating plumes that extended to local coral communities in the Townsville area. Testing revealed elevated copper and zinc concentrations within the coral, demonstrating that metals were taken up by the coral and accumulated within the exposed biota. The authors noted the negative sub-lethal effects of excessive metals exposure on corals including impairment of reproduction.

FFVS concludes that in some areas of the Harbour very substantial concentrations of metals and metalloids were available to be resuspended and depending on a range of other conditions, leading to mobilisation in association with dredge plumes (Petus and Devlin, 2012). DERM/GPC monitoring for metals was infrequent. Hence numerous time points were not sampled. As such, peak values around the times when aquatic animals were reported to be sick, may not have been documented. The DERM monitoring did not use the appropriate metal analysis method for dissolved metals, until after aquatic animals were already being reported to be sick. As soon as monitoring commenced, elevations over ANZECC guidelines in several dissolved metals were reported.

FFVS notes that there is substantial risk, and demonstrated spills (Andersen, Melville, and Jolley, 2008) of industrial contamination of the Harbour in Gladstone. Historically, Gladstone has had significant air pollution issues from local industry, which all eventually follow gravity, with the assistance of water, into the Harbour. Queensland's largest coal fired power station is in Gladstone. Coal fired power stations are known to be emitters of arsenic, mercury and other contaminants, at varying levels depending on the feed source of coal and the level of emissions control. The emissions of the alumina refineries and aluminium smelter are also likely to be substantial contributors to pollutants in the Harbour.

The dust from coal and alumina waiting to be shipped and cyanide spills from Orica (Stitt, 2012) are amongst other known contaminant sources. On page iv of the DERM 2011 water quality report the authors do not mention these known major emitters, and label the origin of Harbour contaminants as "due to natural sources" without any analysis of contaminant origin. This appears to lack supportive evidence, and fails to acknowledge the likely substantial contribution from these pollution sources.

The major local industries have operated a monitoring program in the harbour, the PCIMP. Its focus has been to a large extent on metals. This is because of concern surrounding the potential for metal emissions from these industries which line Gladstone Harbour to affect the local ecosystem. If all the sources of metals were simply "natural sources" as DERM describes them, then it would be unclear why industry would run the PCIMP to monitor them, and their potential environmental impacts.

FFVS observes that there were significant levels of contamination of metals and metalloids detected in the Western Basin Dredging and Disposal EIS sediment assessment in some areas of the harbour. Substantial historical test data is available to compare results from sampling after September 2011 when aquatic animal disease events were occurring.

It is the opinion of FFVS that the origin of some metals and metalloids has not been adequately studied to determine the contribution, if any, from local industry compared to natural geological sources.

10. Predictable acid sulphate soil (ASS) disturbance consequences

a) Presence of ASS and PASS in dredging and disposal area in association with metals

GHD, (2009b) detected ASS in 96% of bore holes in the reclamation area. Historical studies of ASS and PASS in the region demonstrate its widespread occurrence. Undoubtedly significant volumes of PASS have been disturbed by dredging in this project and the resuspension of ASS has led to oxidation of sediments. This oxidation generates acid production, which can mobilise metals through increasing their solubility, and changing their speciation, into more toxic and more bioavailable forms.

Prior to dredging, GHD (2009d) found dissolved oxygen levels were 90–100%; post-dredging, oxygen levels were monitored to be mostly >80% saturation in the Western Basin and FFVS dissolved oxygen data found similar levels of oxygen saturation, indicating that some decline of oxygen levels has occurred post-dredging. This is not surprising given the increase in turbidity, and resuspension of organic matter.

DERM acknowledge in publications that disturbance of ASS was likely to result in release of iron, aluminium, acid and hydrogen sulphide amongst a suite of other contaminants. The DERM fact sheet published in July 2011 (Department of Environment and Resource Management, 2011) states:

"Fish kills may be associated with acid sulfate soil or monosulfide exposure. Sudden changes in acid, aluminium, iron or oxygen levels in the aquatic environment can kill fish and may also kill less obvious aquatic organisms, including crustaceans and oysters. Diseases such as red spot may also be triggered."

b) Impact of release of pore water

The pore water beneath and within ASS sediments can already be acidified and then released upon dredging (Chapman, Wang, Janssen, Persoone, and Allen, 1998; Eggleton and Thomas, 2004). The pore water can mobilise contaminants from surface sediments (recently deposited pollutants) and spread them through the deeper sediments (D Weeks pers.comm. 2012). FFVS observed some areas where a depth of more than 5 metres of material had been removed from mud flats. This can add to the metals released from sediment resuspension and oxidation of sulfide. The pore water may contain high levels of metals, due to the acidity changing their solubility, and their species into potentially more toxic forms. This source of metals/metalloid release may have contributed to the elevations detected in the Harbour. There is insufficient data and monitoring available to quantify this area.

11. Post-dredging changes to metals levels in Gladstone Harbour

In the third water quality report released by DERM in January 2012, p 28, they acknowledged that the dissolved metal concentrations reported by DERM in post-dredging sampling are generally higher than those reported by Angel et al. (2010) from historical testing, prior to the Western Basin Dredging and Disposal Project.

Increases in total aluminium, copper, chromium, zinc, iron and arsenic measured by the Department of Environment and Heritage Protection (2012b, c) and Vision Environment (2012) after the commencement of the dredging project demonstrate that resuspension of sediments and release of associated metals and metalloids took place, and continues to occur.

Table 7: DERM water quality date of ANZECC Water Quality Exceedances from Gladstone Harbour

Date	Metal	Proportion of sites sample that exceeed	Peak Levels in Gladstone testing	ANZECC 95% ecosystem

		ANZECC guideline		protection level
Sept 2011	Al (dissolved)	6/20 sites	80 μg/L	0.5 μg/L*
Oct 2011	Al (dissolved)	n/a	20 μg/L	0.5 μg/L*
Nov 2011	Al (dissolved)	18/19 sites	50 μg/L	0.5 μg/L*
Dec 2011	Al (dissolved)	2 sites	30 μg/L	0.5 μg/L*
Jan 2012	Al (dissolved)	3/19	16 μg/L	0.5 μg/L*
Mar 2012	Al (dissolved)	10/36	21 μg/L	0.5 μg/L*
Feb 2012	Al (dissolved)	1 site	19 μg/L	0.5 μg/L*
Sept 2011	Cu (dissolved)	3/20 sites	2.0 μg/L	1.3 μg/L
Oct 2011	Cu (dissolved)	7 sites	n/a	1.3 μg/L
Dec 2011	Cu (dissolved)	4/8 sites	3 μg/L	1.3 μg/L
Mar 2012	Cu (dissolved)	4/36	3 μg/L	1.3 μg/L
Sep 2012	Cr (dissolved)	2/20 sites	6.2 μg/L	VI: 4.4 μg/L III: 10 μg/L
Nov 2011	Zn(total)	1 site	15 μg/L	15 μg/L
Mar 2012	Zn (dissolved)	1 site	25 μg/L	15 μg/L
Sep 2011	As (dissolved)	1 site	2.3 μg/L (adjacent bund wall)	III: 2.3 μg/L (marine low reliability) V: 4.5 μg/L (marine low reliability)
Feb 2012	As (dissolved)	1 site	2.4 μg/L	III: 2.3 μg/L (marine low reliability) V: 4.5 μg/L (marine low reliability)
Mar 2012	As (dissolved)	1/36 sites	2.6 μg/L	III: 2.3 μg/L (marine low reliability) V: 4.5 μg/L

				(marine low reliability)
Aug 2011	Cr (total)	n/a	22 μg/L	
Aug 2011	As (total)	n/a	2.3 μg/L	
Aug 2011	Al (total)	n/a	2000 μg/L	
Aug 2011	Cu (total)	n/a	2μg/L	
Aug 2011	Mn (total)	n/a	27 μg/L	
Aug 2011	Ni (total)	n/a	3.3 μg/L	
Aug 2011	Iron (total)	n/a	2300μg/L	

* Note the LOR for the aluminium test was 10 μg/L. So exceedances of the Trigger Value (TV) may have occurred but were not able to be detected.

Critical data was not presented by DERM for zinc in October 2011 and November 2011, and copper in November 2011 and January 2012, as DERM reported,

"*the samples were found to have been possibly contaminated due to the presence of zinc/copper in blank samples*"

Chapman, Wang, Janssen, Persoone and and Allen (1998) indicated that total metals do not provide a good indication of potential toxicity. Free metal ion activity is a better measure, however it may not correlate with toxicity in all cases. Complex toxicity can be associated with other labile metal species, organometallic substances, oxyanions, humic substances and biological processes in the fish gill and/or gut. Such examinations were not undertaken prior to fish becoming sick. Water quality experts, Jon Brodie (pers. comm. 2012), Wilkinson and Hichens (2011) and Barry Hart (Hart, 2011) have made this criticism (amongst others) of the initial monitoring program.

Since the commencement of analysing the dissolved metals, detections over ANZECC guidelines have been frequently reported (Department of Environment and Resource Management, 2012; 2012b; 2012c).

In the EIS GHD (2009d), suggested that the contaminants detected in sediments would not be desorbed into the water column of the Harbour. Post-dredging DERM water quality monitoring results have consistently identified levels of various metals elevated above pre-dredging levels and in many cases above the ANZECC Guideline levels in all of their sampling exercises. These data demonstrate that GHD's assumption was incorrect.

DERM have dismissed the exceedances as being non-significant for fish health.

"However, at the reported levels it is unlikely this would affect fish health. There are some consistently high metal analysis results from South Trees Inlet, which are being investigated to determine whether there may be some localised impacts on ecosystem health." (Department of Environment and Heritage Protection, 2012)

CSIRO reported in Angel, et al. (2012) a 3-day sampling of the water in Gladstone Harbour from 6–8 December 2012. FFVS notes that this sampling coincided with one of the smallest tidal variations of the

year, which results in slower water velocities, and potentially could reduce suspended sediment loads. They compared metals values to other ports in the world suggesting Gladstone compares "favourably". The levels identified by CSIRO, and thence compared to international ports, from their 3 day study were well below the peak levels reported by DERM and GAWB. The pre-existing productive commercial fish, prawn, scallop and crab fishery, turtle and dugong grounds, are features of Gladstone Harbour which should also have been compared to these international harbours to validate the comparison.

The authors did identify an increase in copper and zinc levels in Gladstone Harbour in December 2011, compared to pre-dredging data.

FFVS does not agree with the DERM/DEHP interpretation that the recorded exceedances are unimportant in terms of causation of observation aquatic animal health problems. Poulsen and Escher (2012) and Berntssen, Kroglund, Rosseland andand Wendelaar Bonga (1997) demonstrate that the levels of metals observed in Gladstone Harbour are sufficient to be biologically active and stressful to fish. The increased general disease rate, and increased parasitic intensities observed by FFVS and Fisheries Queensland provide substantial evidence to support this hypothesis.

12. Specific metal elevations in Gladstone Harbour: potential impacts

a) Copper

DERM water quality monitoring reported dissolved copper levels above ANZECC 95% ecosystem protection level in September 2011, October 2011, December 2011 and March 2012. The levels recorded are above those reported in the pre-dredging sampling and include areas outside of the marina, and adjacent to dredging activities.

Copper was measured in water samples collected by local seafood processors in Gladstone Harbour in October 2011, at levels 7 times above the ANZECC 2000 95% ecosystem protection level (Diggles, 2011b). Around the same time the Gladstone Area Water Board (2011b) tested water at its intake in Auckland Creek on 20/09/12, also detecting dissolved copper levels 9.2 times the 95% ANZECC guideline, or 40 times the 99% ecosystem protection level. Gladstone Area Water Board, (2011a) also detected 10$\mu g.L^{-1}$ of dissolved copper on 26/9/11;

Around this time Fisheries Queensland detected the *Neobenedenia* ectoparasite on barramundi in the Boyne River and GAWB fish succumbed to the same parasitic infections with *Neobenedenia* around this time. Baker, Knittel andand Fryer (1983), Zeilokoff (1993), Poulsen and Escher (2012), Heterick, Knittel, and Fryer (1979), Knittel (1981) and Rodsaether, Olafsen, Rea, Myhre and Steen (1977) have demonstrated that sub-lethal copper exposure has led to immunosuppression in fish, and thereby increased susceptibility to disease. Copper induced olfactory toxicity is also known to occur in fishes at levels similar to those detected in Gladstone Harbour (Mirza et al. 2009; Tierney et al. 2010).

The increased dissolved copper could also be involved with triggering the increase in prevalence of rust spot disease seen in Gladstone mud crabs compared to the reference site, based on the work of Andersen L (2003). The highest levels recorded in the harbour, are spatial located around the area of the highest prevalence of shell disease reported in the FFVS shell disease survey.

It is the opinion of FFVS that animals exposed to elevations in dissolved copper levels, particularly those above the ANZECC guideline, are likely to have incurred a physiological stress. In combination with other stressors outlined, this is likely to have led to immunosuppression, and can explain the

increased intensity of parasitic infestations observed. It is also likely to have contributed (in addition to other metals like zinc) to the increased rust spot disease observed in mud crabs.

b) Aluminium

Aluminium has frequently been detected in high levels in both total metal and dissolved metal analysis (see Table 9) reported by DERM/DEHP (Department of Environment and Heritage Protection, 2012b,c; Department of Environment and Resource Management, 2012) and GAWB (Gladstone Area Water Board, 2011a). Aluminium is relatively insoluble at pH 6.0 to 8.0, however solubility increases in more acidic or more alkaline conditions. DERM monitoring has not detected pH levels lower than 7.1, however frequently pH levels have exceeded 8.0. FFVS water quality data collected during fish sampling confirmed pH levels were frequently above 8.0, with a maximum recorded of 8.86 in Colosseum Inlet using a hand-held TPS WP-81 calibrated digital pH meter. Dissolved aluminium can exist in various hydroxyl species, which have varying toxicities. It is not evident from DERM water quality results that such speciation has been undertaken.

Department of Environment and Heritage Protection (2012b) reported that dissolved levels of aluminium peaked in September at 70–80 $\mu g.L^{-1}$ reducing to 30 $\mu g.L^{-1}$ in December 2011. These levels exceed that reported to cause severe stress in fish by Berntssen, Kroglund, Rosselandand and Wendelaar Bonga (1997). FFVS observes that the dissolved aluminium concentrations were highest when fish health was observed to be at its poorest in the Harbour.

Once aluminium is solubilised, it can take a while to come back out of solution. This could lead to considerable movement throughout the Harbour in the dredge plume of this contaminant. Aluminium will be prone to forming colloids in marine water and potentially being organically complexed, which keeps it in solution. Colloids are acknowledged in Genemer and Playle (1999) to be hazardous to aquatic animals with gills. The organic complexation gives the toxin another avenue to enter the animals. Recent data trends reported in Department of Environment and Heritage Protection (2012c) have indicated sustained elevations of dissolved aluminium in some areas of the Harbour.

Large volumes of lime were reported to be used to mitigate acidity generated from ASS disturbance (GHD 2009c, p58), in addition to other measures in reclaimed sediments, which are acknowledged to have substantially leaked through the reclamation bund wall. The risk of over-liming is that pH will exceed normal marine levels of 8.2, thereby slightly increasing the solubility of aluminium. pH was frequently measured above 8.2 by DERM, GAWB and FFVS. Increasing the pH also increases the toxicity of releases of ammonia (see ammonia section).

As ANZECC guidelines do not have an ecosystem protection value in place for aluminium, it is uncertain what level is considered safe. There is also limited literature on the toxicity of Aluminium to aquatic organisms. Bjerknes, Fyllingen, Holtet, Teien, Rosseland and Kroglund (2003) noted that solubilised aluminium can move from a freshwater source, into seawater and be deposited on the gills of fish and be detected within cells and result in mortality. DERM testing of some fish, reported in Biosecurity Queensland (2012a, 2012d) has demonstrated some extremely high levels in the gill tissue of barramundi and catfish, indicating that biological uptake is occurring. It is unclear what effect this may be having on the physiological function of the gills. The valency of Aluminium has not been reported from water samples in Gladstone, however the levels ($130\mu g.L^{-1}$ of dissolved aluminium on 26/9/11; Gladstone Area Water Board, 2011a) have been well above those reported by Bjerknes, Fyllingen, Holtet, Teien, Rosseland and Kroglund (2003), which were associated with stress and mortality in Norwegian salmon. Norrgren, Wicklund Glynnand and Malmborg (1991) also recorded that the gills

were the main site of uptake of aluminium in minnows, and found that exposure to dissolved aluminium resulted in lesions of the olfactory organs and gills. Loss of olfaction due to aluminium toxicity (Tierney et al. 2010) may explain the inanition in some barramundi and catfish observed in Gladstone samples. It is also plausible that the levels of aluminium, being released in Gladstone, through resuspension of contaminated sediments by Harbour development activities, may be interfering with osmoregulatory functions of the gill.

An extensive review of the bioavailability and toxicity of aluminium in aquatic environments (Genemer and Playle, 1999) noted two pathways of toxicity: (1) ionoregulatory, involving losses of plasma chloride and (2) respiratory, shown by lower plasma oxygen tension, due to clogging of gills by mucous at high aluminium (Al) concentrations. The later mechanism is more prevalent in high hardness waters, such as marine water. Some studies have illustrated that early larval life stages are more sensitive to the toxic effects of aluminium. Given the reported decline in mud crab juveniles (<2 cm crabs) by Gladstone fishers, aluminium toxicity (in addition to other toxic exposures to metals and metalloids and suspended sediments) may represent another mechanism by which recruitment has been seriously impacted.

Images provided by commercial fishers (G. Otto and T. Falzon, 2011) from early in the fish disease outbreak illustrated increased skin mucous production in a range of fish species including: barramundi, cobia, mackerel and sharks. This is a critical clinical sign across multiple species which would have been exposed resuspended sediment from dredging and disposal. It was observed around the dredge spoil ground where toxic dredge spoil was being disposed. Nets were reported to become fouled in slime also at this time. Exposures to toxic levels of aluminium have been demonstrated to elicit prolific mucous secretion in salmon (Berntssen, Kroglund, Rosseland, and Wendelaar Bonga, 1997). However, FFVS considers the slime to most likely be due to a toxic algal bloom (see Algae chapter), triggered by the increased availability of nutrient and metals (particularly iron) due to sediment resuspension from the dredging and disposal of Western Basin sediments.

The United States Environmental Protection Agency (2012) highlighted the difficulty in interpreting either total or dissolved aluminium test results.

"*In tests with the brook trout at low pH and hardness, effects increased with increasing concentrations of total aluminum even though the concentration of dissolved aluminum was constant, indicating that total recoverable is a more appropriate measurement than dissolved, at least when particulate aluminum is primarily aluminum hydroxide particles. In surface waters, however, the total recoverable procedure might measure aluminum associated with clay particles, which might be less toxic than aluminum associated with aluminum hydroxide*."

It is the opinion of FFVS that substantial elevations in dissolved aluminium are likely to be the result of resuspension and release of aluminium from Harbour sediments due primarily to dredging and spoil disposal, with contributions also from local industry. This has likely resulted in significant impacts on the skin and gills of affected fish, causing direct effects such as increased mucous secretion, olfactory cell damage, as well as osmoregulatory and respiratory stress. This stress has contributed to immunosuppression of fish, leading to increased intensities of parasitism, and may also be contributing to failure of some fish to feed.

c) Arsenic

Gladstone Area Water Board, (2011a) reported 16μg.$L^{-1}$of arsenic on 12/10/11 from intake water sampling from Auckland creek.

(GHD 2009b) detected elevations of arsenic above Queensland EPA, EIL in 12 samples. Bhattacharya, Bhattacharya and Roy (2007) described effects of arsenic on freshwater fish to include:- immediate death from suffocation of gills through excessive mucous production, or direct detrimental effects on the gill; chronic exposure may cause liver neoplasia; bizarre morphological alterations; reduced growth; avoidance behaviour; reduced fertilisation and hatching of eggs; degeneration of testis and ovary in high doses.

Early in the Gladstone disease outbreak at the spoil dumping ground and subsequently closer inshore, fish were reported to be exuding large volumes of mucous. This may be in part due to the elevated arsenic exposures, because of the increased mobilisation of arsenic from sediments by dredging resuspension.

The physiological pathways which may be involved include: cellular thiol, generation of free radicals or reactive oxygen species, oxidative stress, antioxidant enzymes, induction of stress proteins (e.g. metallothioneins) and activation of mitogen activated protein kinase (MAPK) pathways in the carcinogenic and anticarcinogenic action of Arsenic. Arsenic has been documented to affect the nervous system through interference with the neurotransmitter, acetylcholine, production (Bhattacharya, Bhattacharya, and Roy, 2007) Lipid peroxidation has been reported in some species of fish after exposure to elevated levels of arsenic (Schlenk, Wolford, Chelius, Steevens and Chan, 1997). This particular symptom was not observed in histological samples of Gladstone fish by FFVS.

Fish exposed to elevated levels of arsenic are likely to experience sub-lethal stress, which in turn elicits increases in oxidative stress, rendering the exposed animals less capable of defending themselves against pathogens such as parasites. Exposure to arsenic may also have contributed to the increased mucous production on the fish, in addition to elevated aluminium and toxic algal blooms.

d) Zinc

The Department of Environment and Heritage Protection (2012c) reported increased levels of dissolved zinc in September 2011 compared to pre-dredging water analysis. From around this time, commercial crab fishers in the Harbour began reporting an increased incidence of shell lesions on crabs. Anderson (2003) suspected that increased exposure to copper and zinc were causally associated with increased shell disease in Gladstone Harbour crabs.

A subsequent survey by FFVS in February 2012 identified a significant increase in shell disease in Gladstone crabs, with increasing prevalence with proximity to the Western Basin dredging (see Figure 3, Table 5). The disease was found to be absent in the reference site crabs based on histological assessment.

High zinc levels (100mg/kg) have been detected by Biosecurity Queensland (2012d) in catfish gills from a fish kill in the Boyne River.

Increased exposure of mud crabs to dissolved zinc, which has been mobilised by dredging and resuspension of sediment, is likely to have contributed, in combination with other stressors, to the increased rates of shell disease (rust spot) in Gladstone crabs.

e) Iron

Elevated iron levels were detected by DERM (2012a) in the harbour in August 2011. This was time coincident with the increase in sick aquatic animals being reported throughout the harbour. It was also time co-incident with fishers and other members of the public reporting skin lesions that were

morphologically consistent with those caused by Lyngbya toxin exposure. Ahern et al (2007, 2008) identified that *Lyngbya* blooms were promoted by increased availability of iron. The presence of elevated nutrient levels in the harbour, at the same time, was likely to also promote algal blooms.

High iron levels (315mg/kg) were documented by Biosecurity Queensland (2012d) in the gills of catfish from a fish kill in the Boyne River in April 2012.

Increased availability of iron, together with elevated nutrient levels in the harbour, are likely to have provided conditions suitable for blooms of the cyanobacterium *Lyngbya*, which has been associated with skin lesions on fishermen (Figure 10), and other toxic phytoplankton blooms.

f) Manganese

Whilst levels of dissolved manganese from DERM water quality analysis in 2011 and 2012 were not reported to exceed ANZECC guidelines, the GHD sediment quality report in 2009 identified that high levels of manganese were present in some areas of Gladstone Harbour. Weinstein, West and Bray (1992) identified that elevated manganese were consistently associated with blue crabs with shell disease. Hence it is possible that manganese was contributing, in a cumulative fashion with copper and zinc, to the development of elevated rates of shell disease in exposed Gladstone mud crabs.

13. Aggregate effects of some metals

a) Physiological impacts

Forstner, Ahlf and Calmano (1989) reported that some metal toxicity mechanisms include inactivation of enzymes by binding with amino, imino and sulfhydryl proteins; or through competing for uptake with other essential elements such as Na^{+} and Ca^{2+}; or may damage cells by acting as anti-metabolites, resulting in disruption of metabolic pathways. Such physiological changes will not be detected on histological sections, hence FFVS sampling is not able to determine the extent, if at all, such conditions were active in the sampled fish.

b) Immunological impacts

The review of Poulsen and Escher (2012) determined that metals (including copper, chromium, zinc), metalloids (aluminium) and organic chemicals may have suppressive effects on the immune system of marine wildlife, which consequently can increase their susceptibility to disease. The authors acknowledged that there was also limited literature suggesting that mixture effects may be important, in that multiple exposures may have additive effects. Further, they identified that in reality the safe limits were not known, as it depended to some extent on the environmental context of the exposures, where other concurrent stressors could contribute to an observed negative outcome. Benedetti, et al. (2012) pointed out that a more accurate assessment of potential sediment toxicity is gained from a multidisciplinary approach, incorporating biological assays, in addition to sediment chemistry. Such bioassay methods appear to have been completely excluded from the original project EIS and in all subsequent Government monitoring programs. The biota of Gladstone Harbour, are in effect providing the very large experimental bioassay, and are illustrating that the sediment cocktail of contaminants exceeds their biological tolerance.

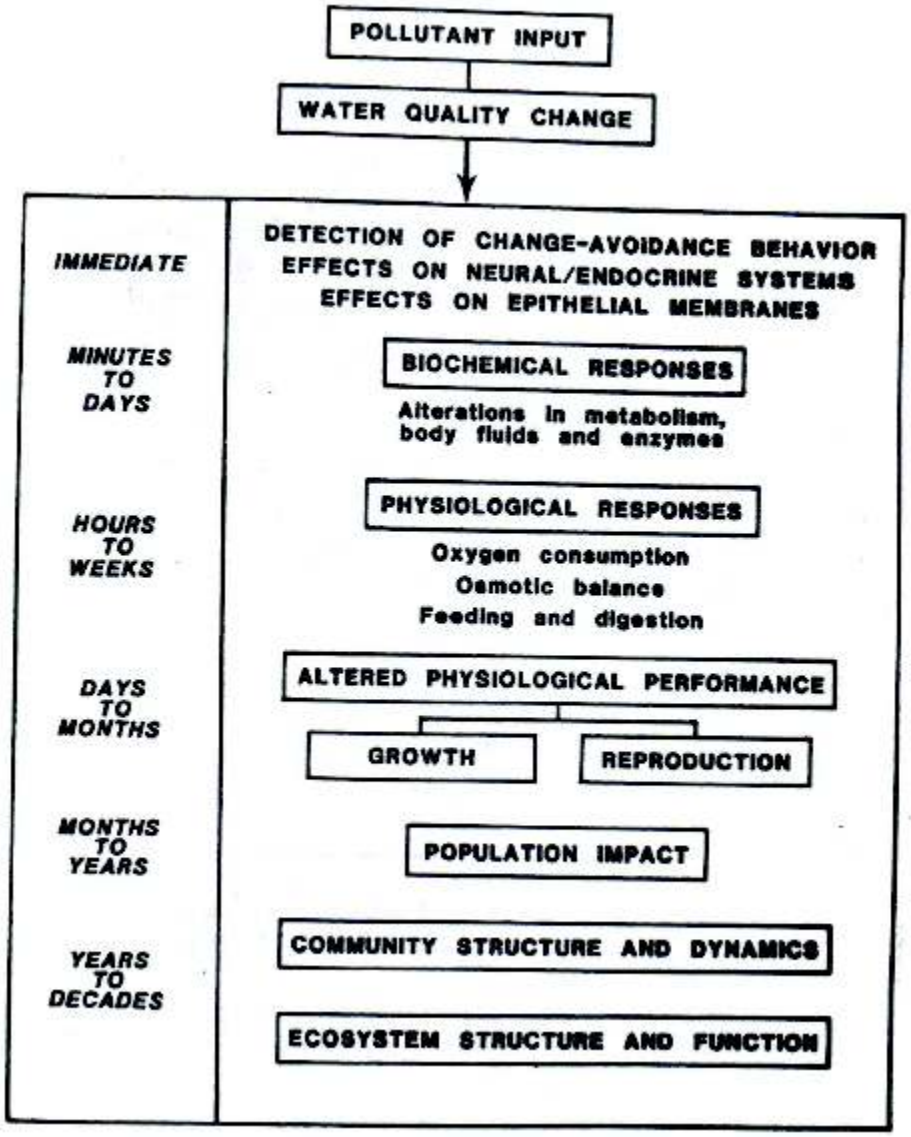


Figure 24: Temporal sequence of stress effects after pollution: Illustration from Sindermann 2006 p 154

As highlighted in this FFVS report, a multiplicity of stressors have been generated in Gladstone Harbour, secondary to this Harbour development project which are likely to have a cumulative impact on the biota. One of the potential consequences of immunosuppression is increased intensities of parasitic infections, with concomitant increased pathological impacts. Such changes were observed in samples collected and examined by FFVS from Gladstone compared to the reference site.

c) Olfactory impacts

Metal contaminant exposure may include chronic long term impairment of olfaction (ability to smell) (Tierney, Baldwin, Hara, Ross, Scholz, and Kennedy, 2010). Mirza, Green, Connor, Weeks, Wood and Pyle (2009) documented changes in anti-predator behaviour and electrophysiological responses to alarm cues in fish from metal-contaminated lakes compared to fish from clean lakes. Metals including aluminium, silver, cadmium, mercury, copper, manganese, nickel and zinc can disrupt olfactory receptors or olfactory-mediated behaviours. Copper exposures of 10–25 $\mu g.L^{-1}$ are documented to have caused olfactory cell death. Baldwin, Tatara and Scholz (2011) made similar findings with dose related impacts from 3-hour exposures of 5–20 $\mu g.L^{-1}$ in salmon and steelhead.

As noted in 13a, 13b, 13d above, elevated levels of dissolved copper, aluminium and zinc have all been detected after the commencement of the dredging.

FFVS documented numerous inappetant fish in its January and February 2012 study in Gladstone Harbour. Biosecurity Queensland (2012d) provided further evidence that many Gladstone fish were not eating, observing that catfish which died in a fish kill in the Boyne River:

"*The initial observation that the fish were in poor condition was confirmed with the examinations revealing fish with reduced muscle mass and mostly empty stomachs, indicating a possible absence of normal feeding.*"

No infectious disease cause was identified for this fish kill. Hence non-infectious causes should be considered more likely.

FFVS noted that many barramundi and some catfish sampled were in poor body condition, and had not been feeding, despite the presence of abundant feed items. In addition to the other stress factors highlighted in this report, an impairment of the sense of smell may be compounding the other stresses negatively affecting feeding. In general, sick fish have reduced, or no, appetite, irrespective of the cause of sickness.

d) Fish behaviour impacts

Scott and Sloman (2004) documents that metals, amongst other contaminants that are known to be present in the sediments of Gladstone Harbour, can have disruptive effects on complex fish behaviours such as: predator avoidance; reproduction; and social behaviour. These kinds of subtle impacts are unlikely to be observed by monitoring regimes employed in this dredging project. The consequences are more likely to be seen with a reduction in fish populations, through stock moving away, reduced growth, and reduced recruitment (less reproductive success).

The aggregate effect of metals, contaminants, turbidity and noise are likely to have contributed to the alterations in fish/other aquatic animals (cetaceans) behaviour in the Gladstone area, with animals either moving away, or not entering many areas they usually inhabit.

14. Noise- Underwater Acoustic Impacts

a) Background

Sound is a crucial component for communication, navigation, foraging and possibly orientation for marine organisms (Gotz, et al., 2009). Sound sources audible in the ocean come from both environmental (ambient) sources as well as anthropogenic sources. The effects of sound on marine organisms has been well documented in a number of studies, describing negative effects of noise pollution on the behaviour and physiologic parameters of affected animals.

"*Without any question, sound is critically important to aquatic animals for all aspects of their lives. Anything that interferes with the detection of sound has the potential to have a significant impact on the lives of these organisms and affects not only individual animals but also reproduction and the survival of species*." (Popper, 2009)**.**

"Noise" is defined here, as any adverse or unwanted sound.

The sound pressure levels of an array of anthropogenic causes of underwater noise, including many which are associated with the Western Basin Dredging and Disposal project (pile driving, dredging, drilling, small boats, large boats) are detailed in Table 10 below.

Table 8: Overview of the acoustic properties of some anthropogenic sounds from (OSPAR commission, 2009)

Sound	Source level (dB re 1 µPa-m)*	Bandwidth (Hz)	Major amplitude (Hz)	Duration (ms)	Directionality	Source citations in module
Offshore construction						
TNT (1-100 lbs)	272 - 287 Peak	2 - 1000	6 - 21	~ 1 - 10	Omnidirectional	1
Pile driving	228 Peak; 241 - 257 Pk-to-Pk	20 ->20 000	100-500	50	Omnidirectional	1
Offshore industrial activities						
Dredging	178 - 186 rms	30 - > 20 000	100 - 500	Continuous	Omnidirectional	1
Drilling	145 - 190 rms**	10 – 10 000	< 100	Continuous	Omnidirectional	1
Wind turbine	142 rms	16 - 20 000	30 - 200	Continuous	Omnidirectional	1
Shipping						
Small boats and ships	150 - 180 rms	20 - >10 00	> 1000	Continuous	Omnidirectional	5
Large vessels	180 - 190 rms	6 - > 30 000	> 200	Continuous	Omnidirectional	5
Sonar						
Military sonar low-frequency	215 Peak	100 - 500	-	600 - 1000	Horizontally focused	6
Military sonar mid-frequency	223 - 235 Peak	2800 - 8200	3.500	500 - 2000	Horizontally focused	6
Echosounders	235 Peak	Variable	Variable 1.500-36.000	5 - 10 ms	Vertically focused	6
Seismic surveys						
Airgun array	250 - 252 Pk-to-Pk	10 – 100 000	10 - 120	30 - 80	Vertically focused	7
Other activities						
Acoustic deterrent / harassment devices	132 - 200 Peak	5000 -30 000	8000 – 30 000	Variable 15 – 500 ms	Omnidirectional	8
Tidal and wave energy devices***	165 - 175 rms***	10 - 50 000	-	Continuous	Omnidirectional	8

* Nominal source; **Higher source levels from drill ships use of bow thrusters; *** Projection data based on literature data levels back calculated at 1m

Under water, sound will travel spherically around the source. The further the sound wave travels, the more energy is lost and the acoustic power of the sound wave is dampened (Gotz, et al., 2009). In shallow water, transmission loss begins to be more complex as sound is reflected from the water surface and the sea-floor. Other factors that we must consider include the frequency of sound (low frequencies

can travel hundreds of kilometres with little loss in energy); water depth; and density differences within the water column which varies with temperature and pressure (Nowacek, 2007). Within Gladstone Harbour and nearby waters the sound arriving to an animal will vary subject to these conditions. A significant proportion of a fishes sensory perception is facilitated by its lateral line, while some fish also have specialist hearing organs. Sensory perception is dependent on the organism's sensitivity to particular frequencies.

b) Likely effects of increased noise on Marine Organisms in Gladstone

It has been demonstrated that marine organisms (cetaceans, cephalopods, pinnipeds, fish and crustaceans) have a varied spectrum in hearing sensitivity ranging from values as low as <10 Hz frequencies of blue whales through to ultrasonic clicks of >200 kHz in off-shore dolphins (Gotz, et al., 2009). Southall, et al. (2007), Gotz, et al. (2009) and Popper (2009) expressed concern that the increase in background noise to which animals are exposed to (primarily due to increases in boat traffic and shipping); as well as intermittent increases in cumulative noise from mining activity such as seismic testing, drilling, and dredging may have deleterious effects on aquatic animals. Possible effects of underwater sound on animals include masking, behavioural changes, and hearing loss (Temporary Threshold Shift and Permanent Threshold Shift (explained below)).

Potential effects from intense sound sources such as pile driving, includes immediate death. Other effects include soft tissue damage, which may significantly lower the chances of survival in affected animals (Popper, 2009). FFVS were undertaking fish sampling during pile driving in the Western Basin. The impulses were palpable through the floor of a prawn trawler at a distance of approximately 1.5 km from the pile driving.

It is evident that the amount of anthropogenic noise generation has increased dramatically in Gladstone Harbour. The RHM Captain Mike Lutze recently advised that there are now 700 boat movements to and from Curtis Island to Auckland Harbour each day, that is >20 000 per month. Large vessel movements have also increased since 2008 as illustrated in Figure 12 below, and will increase dramatically once new coal and gas terminals begin operation from around 2014.

CQG Consulting (2011) made the following observations of noise from their monitoring program of the Western Basin Dredging in Gladstone:

"*Anthropogenic noises (ie pile driving and vessel movements) were notably higher than background noise for all areas and at all times of the day. The anthropogenic noise levels measured during the surveys were considered to be audible by all species.*"

"Sound Pressure Levels (SPL) recorded in the second winter survey were higher at most sites than those recorded during survey one. The increased levels were identified as likely caused by an increased level of boat traffic noted in June 2011 compared to the earlier survey."

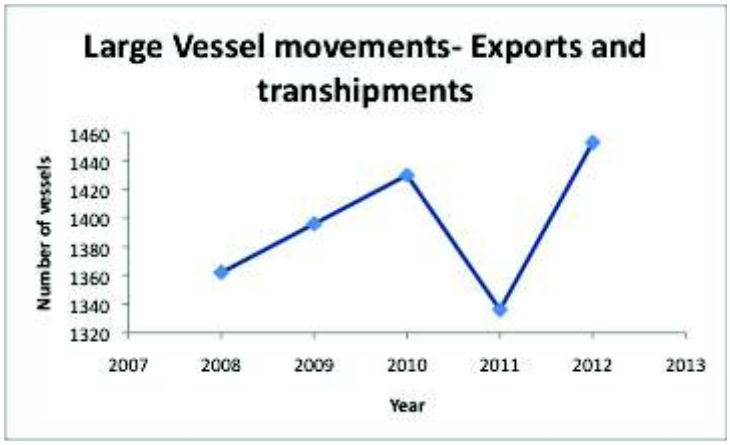


Figure 25: Large vessel movements in Gladstone Harbour 2008-August 2012. Source: (Gladstone Ports Corporation, 2012).

It is generally accepted that stressors (chemical, physical, and perceived) elicit physiological and related effects in all animals. In fish, these responses include primary responses of increases in plasma corticosteroids and catecholamines, as well as alterations in neurotransmitter activity. Secondary responses include metabolic changes, cellular changes, osmoregulatory disturbances, haematological alterations, and changes in immunity. Tertiary responses include changes in whole animal performance characteristics such as swimming, growth, and disease resistance (Barton, 2002). The available evidence suggests that the fishes of Gladstone have been exhibiting signs of stress and immunosuppression.

Buscaino et al. (2010) conducted an experiment using sea bass and sea bream, exposing test groups to low level frequency sounds of short duration in a continuous cycle (0.1–1.0 kHz in a 1 second linear sweep repeated for 10 minutes without pause). It was observed that test groups of fish had increased swimming activity, as well as changes in their blood parameters (blood glucose and lactate) as a result. It was concluded, that even at these low frequency sounds, fish exhibited behavioural and haematological signs of stress.

Shipping noise is considered to be low frequency chronic with a low sound pressure level. It is likely to be increasing in Gladstone Harbour in line with increased vessel movements, hence there is a high likelihood of it exerting a negative effect on exposed biota based on available evidence.

Wysocki (2006) demonstrated that exposure to shipping noise elicits a cortisol stress response observed in different species of fish, regardless of their hearing sensitivities. It was shown in this experiment that fluctuations in amplitude and frequency of sound waves were more likely to activate the hypothalamic pituitary adrenal-axis (Wysocki, 2006).

c) Noise impacts on crustacean and molluscs

Simpson et al. (2011) conducted a study which illustrated that crustaceans actively avoided reef noise, and sought refuge in areas with less noise pollution. Changes in behaviour of mussel larvae have been experimentally documented in both high and low intensity noise exposure. Wilkens (2012) exposed mussel larvae to 126 dB and 100 dB re 1µPa RMS in high and low intensity vessel noise treatment groups. Frequency in experimental groups varied between 100–1000 Hz. It was observed that sound primarily influenced settlement behaviour and mussel larvae gravitated towards areas with higher noise intensity (Wilkens, 2012). This abnormal behaviour was considered the result of exposure to the primary stimulus of sound.

d) Sound impacts on cetaceans

It is acknowledged that the increases in noise may interfere with necessary biological functions of marine mammals (cetaceans) including changes in behaviour and social interactions, foraging and predator avoidance, temporary or permanent compromise to auditory pathways, habitat avoidance and in extreme cases, death (Gotz, et al., 2009; Southall, et al., 2007; Richardson, Greene, Malme, and and Thomson, 1995). DERM marine strandings data demonstrated an increased rate of strandings soon after the major increase of the dredging in the winter of 2011.

When FFVS was undertaking sampling onboard a Gladstone prawn trawler, the long term operator, D Wise, reported changes in dolphin behaviour after the commencement of the major dredging in May 2011. Prior to the major dredging, dolphins would consistently be observed coming behind the nets as they were pulled up, to pick fish out of the nets. After the increase in turbidity in May 2011, the dolphins were not observed.

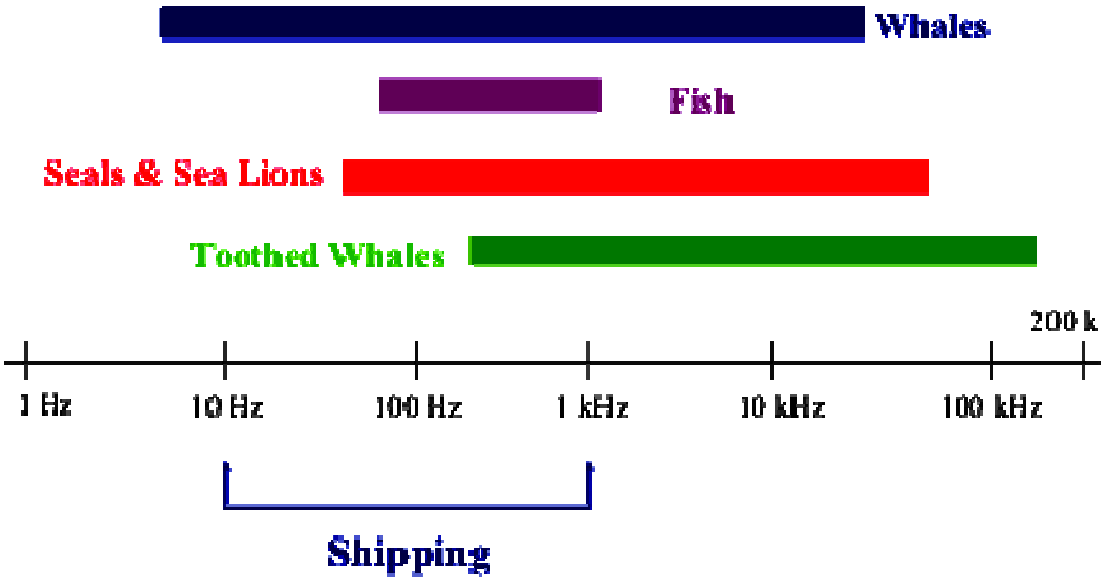


Figure 26: Frequency bands of sounds produced by marine mammals and fish compared with nominal low frequency sounds associated with commercial shipping (Gotz, et al., 2009).

Figure 27, illustrates the typical frequency bands of sound produced by marine mammals and fish compared to lower frequency sounds created by commercial shipping. There is an obvious overlap, which illustrates that all species noted could potentially be affected by the increased noise which has been created in Gladstone Harbour

Auditory masking is observed in marine mammals, when exposure to noise (background noise) partially or entirely reduces the audibility of signals. This directly interferes with communication systems, echolocation signals and passive listening capabilities (for orientation and predator avoidance) (Southall, et al., 2007). These changes can expose marine mammals to predation, interrupt their mating behaviour, and orientation. Because of the logarithmic nature of sound and our current knowledge of hearing systems of animals, small changes in background sound can possibly result in large reductions in communication ranges (Gotz, et al., 2009). A summary of noise and its detrimental effects on marine life is provided in Table 10 below.

IMPACT	TYPE OF IMPACT
Physiological *non auditory* *Auditory* ("Sound Induced Hearing Loss/SIHL")	• Damage to body tissue: e.g. massive internal haemorrhages with secondary lesions, ossicular fractures or dislocation, leakage of cerebro-spinal liquid into the middle ear, rupture of lung tissue • Induction of gas embolism (Gas Embolic Syndrome, Decompression Sickness/DCS, "the bends". Caisson syndrome) • Induction of fat embolism • Gross damage to the auditory system – e.g. resulting in: rupture of the oval or round window or rupture of the eardrum • Vestibular trauma – e.g. resulting in : vertigo, dysfunction of co-ordination, and equilibrium • Permanent hearing threshold shift (TTS) – e.g., a temporary elevation of the level at which a sound can be detected
perceptual	• masking if communication with con-specifics • masking of other biologically important sounds
behavioural	• stranding and beaching • interruption of normal behaviour such as feeding, breeding and nursing • behaviour modified (less effective/efficient) • adaptive shifting of vocalisation intensity and/or frequency • displacement from area (short or long term)

Table 9: Overview of the observed effects of underwater noise on marine life (OPSAR Commission 2009).

Observational data from groups of marine animals exposed to seismic surveys showed that there was a variation in behavioural changes ranging from no observable change to avoidance behaviour, cessation of vocalisation, startle responses from pinnipeds, and reduction in grouping of dolphins (Gotz, et al., 2009). It is therefore apparent that sound propagation through water should be considered prior to any activity producing potential noise.

Noise is only one of the stressors highlighted by FFVS, which in combination with metals and metalloids, and toxic algal blooms, may explain the sudden increase in cetacean deaths in Gladstone Harbour (refer to Figures 7, 8, 9), and the change in movement patterns observed by commercial fishermen (D. Wise pers. comm. 2012).

Based on published findings, the cumulative noise impact of Harbour activities including dredging, drilling, pile driving and vessel movements appear likely to reach levels sufficient to trigger behaviour change and stress responses in fish within a certain acoustic transmission zone. The associated stress may result in a cascade of events (Barton 2002).

The proximity of this development to the nursery grounds of aquatic animals will lead inevitably to exposure of these sensitive early life stages, with potentially negative reproductive outcomes.

15. Conclusion

In considering the weight of available evidence on the potential causes of observed aquatic animal health disorders in Gladstone, FFVS considers these disorders are most likely to be a direct consequence of the Western Basin Dredging and Disposal Project. Specific mechanisms include:

- resuspension and mobilisation of contaminants (metals and metalloids) from sediments;

- increased parasitism due to stress, immunosuppression and external irritation from poor water quality and toxicosis;

- increased boat traffic vessel strike risk;

- noise and

- generation of toxic algal blooms due to disturbance of sediments and release of nitrogen, iron and other nutrients.

16. Why freshwater and/or the release of barramundi from Awoonga Dam are not the primary cause of stress, or the observed aquatic animal diseases

Connolly, et al. (2006) provides evidence that “freshwater flows are important to the productivity of Port Curtis. Years of large flow tend to have higher benthic invertebrate productivity, resulting in higher growth rates in fish such as whiting”. This was also confirmed in the September 2012 Fisheries Research and Development Corporation News which reported on the project "River Flows benefit estuarine fish 2007/002" which stated:

"*This project provides quantitative evidence that freshwater flows to estuaries are crucial in supporting fisheries production*"

So, quite apart from triggering the diseases observed in crabs, fish, rays, turtles, dolphins and humans from May 2011 to the present – the freshwater influx should have supported increased productivity. Figure 14, 15 and 16 present data suggesting that productivity has suffered a measurable decline.

Major freshwater inflows have occurred before, without major aquatic animal health incidents including Dec 1962, Dec 1969, Jan 1970, Dec 1970, Jan 1971, Dec 1973, Jan 1974, Dec 1988 and Jun 1991 (Bureau of Meteorology rainfall data). In January 2011, the freshwater discharge down the Fitzroy River was even greater than that from the Boyne River in the same rainfall event, and no aquatic animal disease outbreaks of similar severity were reported there.

This pattern held true in Gladstone Harbour prior to the major dredging operations with increases in mud crab and prawn catches after major freshwater inflows in the years prior to 2010. However, after the major inflow in December 2010 and January 2011, the prawn and mud crab catches show signs of decline in fisheries data for the first 8 months of 2011 based on Fisheries Queensland data.

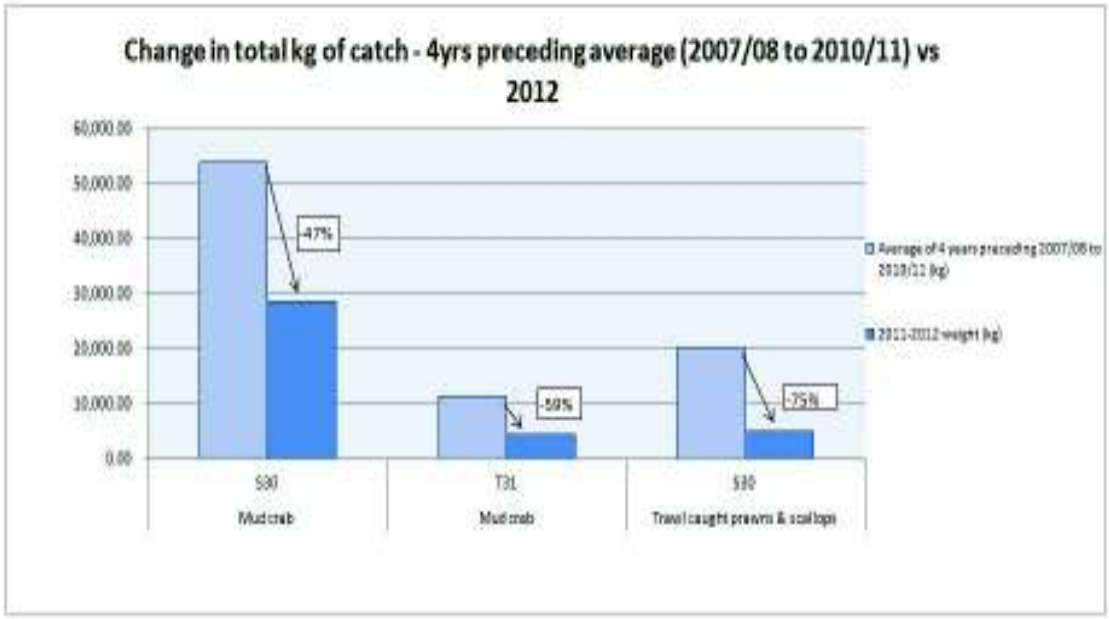


Figure 27: Changes to Mud crab, prawns and scallop catches around Gladstone: pre and post Harbour development (graph provided by Gladstone Fish Market, based on data supplied by Fisheries Queensland)

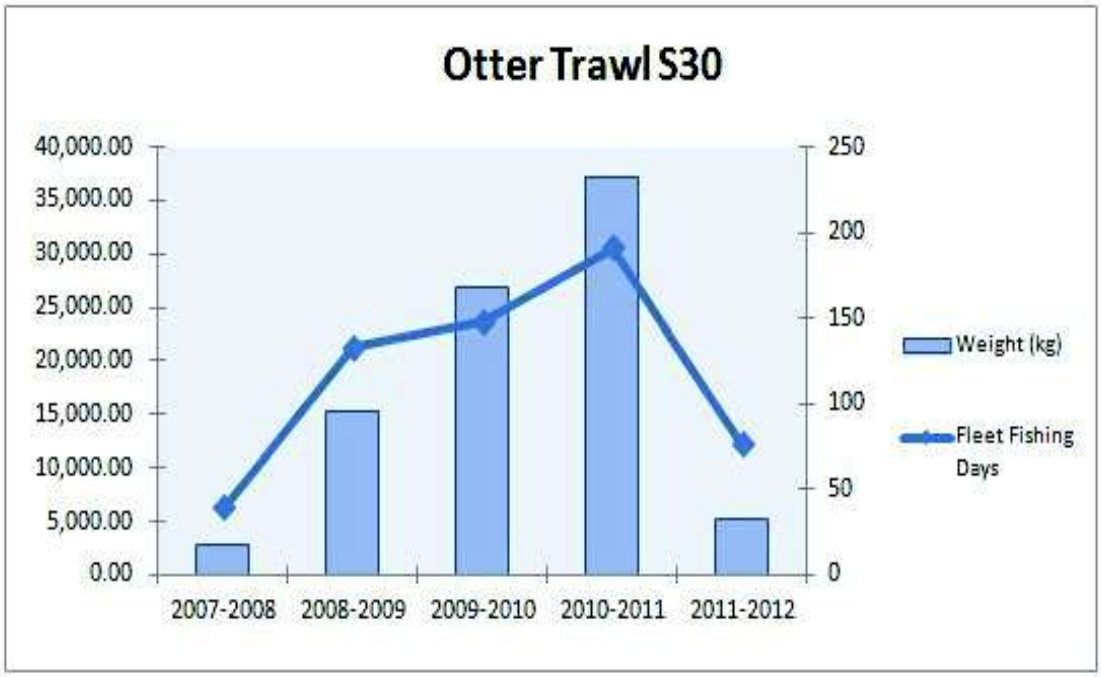


Figure 28: Changes in trawl catch 2007–July 2012 (graph provided by Gladstone Fish Market, based on data supplied by Fisheries Queensland)

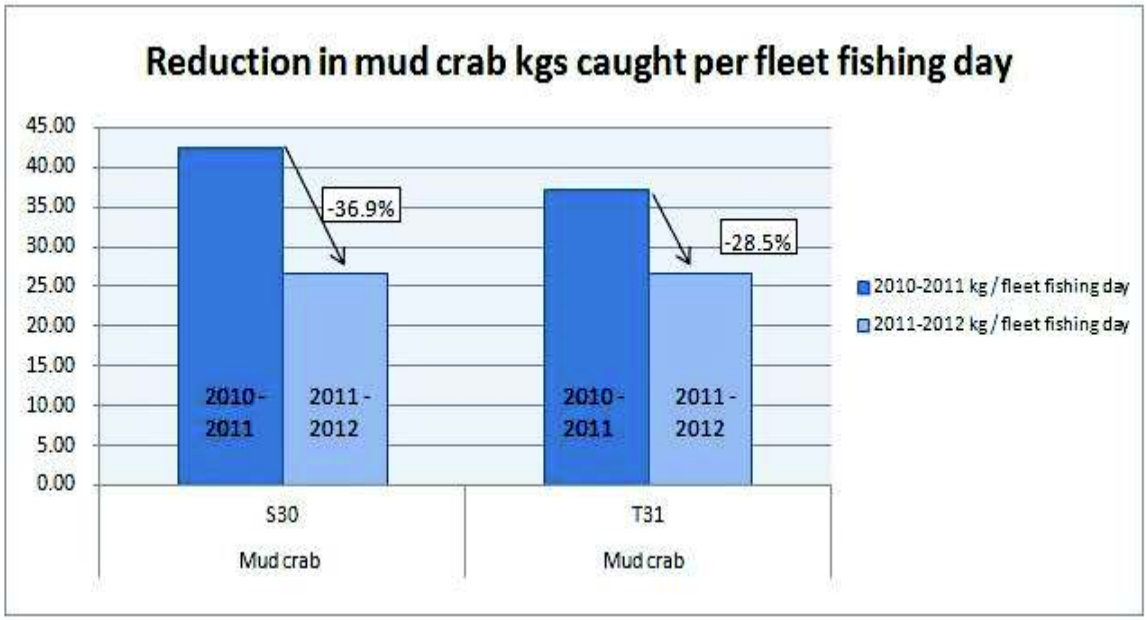


Figure 29: Changes to mud crab catch per fleet fishing day 2010–11 to 2011–12 (graph provided by Gladstone Fish Market, based on data supplied by Fisheries Queensland)

The freshwater inflow Dec 2010–Jan 2011 did not correlate temporally with the outbreak of fish disease which occurred in estuarine and marine areas of the harbour post May 2011 and is ongoing, as at 5 September 2012. The Harbour had returned to near full strength seawater salinity by the time the fish/dugong/turtle/dolphin/shark disease(s) were being widely observed and reported in July 2012.

Awoonga dam spillway was at 40 m as of July 2011. The graph below illustrates the time period where overtopping was occurring, noting that it stopped well before the mid-July outbreak of disease in fish in the Boyne River and elsewhere. Once the freshwater outflow slowed, the saline waters from the Harbour (and its high turbidity load) quickly penetrated up the Boyne River with tidal and wind forcing.

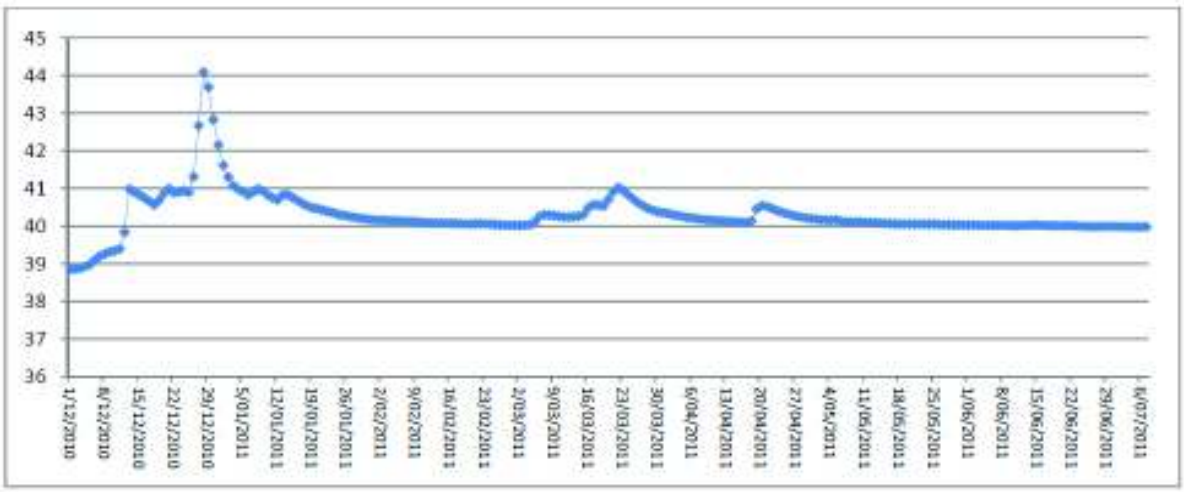


Figure 30: Spillway levels at Lake Awoonga from December 2010 to July 2011 (40 m AHD) Source: GAWB

Barramundi are normally found in fresh and salt water. Neither environment is stressful to them, as evidenced by aquaculture industries growing fish across the full salinity range. Disease is not

experienced when changing barramundi over from one salinity level to another. Such methods of salinity reversal are used routinely in aquaculture to remove parasites off these fish, and do not precipitate skin disease, or other diseases.

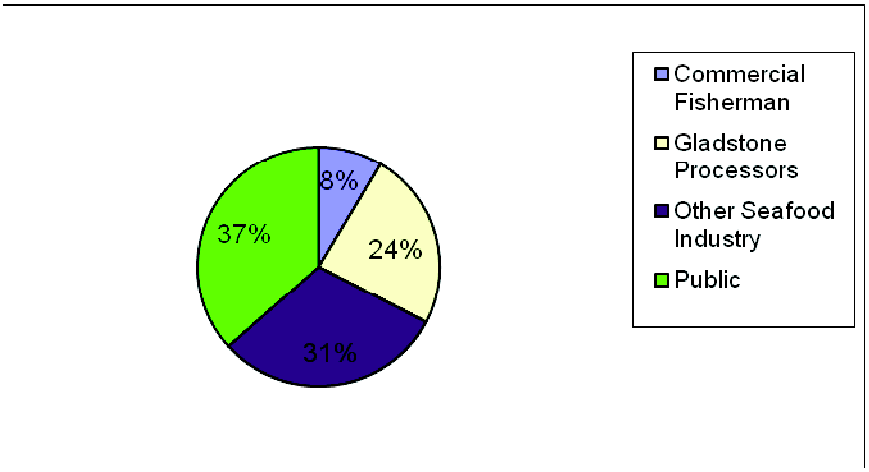
Of the estimated 20 000+ barramundi that went over the dam wall only around 1200 perished, due to injuries to the head and body from coming over the spillway. Observers from the Gladstone Area Water Board (GAWB) observers noted that deaths from initial trauma colliding with concrete/rocks once falling over the spillway, ceased within 4 weeks after the major spill event in Dec 10-Jan 11 (Gladstone Area Water Board, 2011). Examination of barramundi in the freshwater reaches of the Boyne River in April 2011 found them to be feeding and in generally good health (Diggles 2011a). GAWB collected numerous groups of barramundi which had come over the dam wall. The fish were taken to the hatchery and introduced them into treated (settled and filtered) marine water to assess their health. It was observed the barramundi made a smooth transition to marine water with negligible mortalities, as expected. The majority of barramundi (~ 200 t) estimated to have exited the dam in the late Dec 2010-Jan 2011 overtopping, were captured and sold by commercial fishermen, prior to the onset of disease. Hence the population density at the time of disease outbreak in the Boyne River around July 2011, was no longer exceptionally high.

Large numbers of barramundi have exited other Queensland dams, as documented by Infofish/Sunfish tagging data in spill events in Queensland, without similar disease events transpiring. GAWB staff observed the second spill event in March, noting that no barramundi went over the wall. GAWB also sampled fish in the dam and found them to be grossly normal in February 2011. This observation provides evidence that the disease outbreak which subsequently occurred on barramundi, did not emanate from the freshwater Awoonga Dam.

The continuation of disease has extended to the present day in the Harbour stocks of fish and crabs. The Harbour has been back around full oceanic salinity for more than 12 months. Government Department's claim that a rainfall event from 21 months ago (as of September 2012), is the cause of all and current fish diseases. There is a lack of scientific evidence to support this contention. In July and August 2012 numerous bream and mullet have been captured with red skin lesions, in the absence of significant rainfall events. In September 2012 ulcerated estuary cod and barramundi have been captured and photographed.

FFVS concludes that the available evidence does not support the hypothesis that the freshwater influx, or the influx of barramundi, caused the widespread aquatic animal disease event in Gladstone Harbour and nearby waters. Both were contemporaneous, rather than causal.

17. Source of funding for FFVS and acknowledgements



Particular thanks are due to:

- all who donated money their money and time, to enable this project to be undertaken;
- all of the commercial fishers who facilitated the sampling across a wide range of sites;
- Specialist Veterinary Pathologist, Dr Michelle Dennis; Phycologist, Dr Gustaff Hallegraef; staff at James Cook University and Australian Institute of Marine Science for facilitating sample processing;
- members of the FFVS sampling team: Dr Ben Diggles; Dr Christine Huynh; Rangi Faulder; John Talbot and
- Contributing scientists who provided peer reviewed papers and offered insights to the problems.

The timelines presented are as accurate as possible, given the unavailability of some key data. FFVS reserves the opportunity to alter the timelines should more data become available.

References

1. Ahern, K., Ahern, C., and Udy, J. (2008). In situ field experiment shows Lyngbya majuscula (cyanobacterium) growth stimulated by added iron, phosphorus and nitrogen. *Harmful Algae , 7*, 389-404.

2. Ahern, K., Ahern, C., and Udy, J. (2007). Nutrient additions generate prolific growth of Lyngbya majuscula (cyanobacteria) in field and bioassay experiements. *Harmful Algae , 6*, 134-151.

3. Albert, S., O'Neil, J., Udy, J., Ahern, K., O'Sullivan, C., and Dennison, W. (2005). Blooms of the cyanobacterium Lyngbya majuscula in coastal Queensland, Australia: disparate sites, common factors. *Marine Pollution Bulletin , 51*, 428-437.

4. Andersen, L. (2003). *A study into the epidemiology of mud crab (Scyalla serrata) shell disease.* Gladstone Australia: Centre for Environmental Management, Faculty of Arts Health and Science, Central Queensland University.

5. Andersen, L. (2004). Imposex: A biological effect of TBT contamination in Port Curtis, Queensland. *Australiasian journal of ecotoxicology , 10*, 105-113.

6. Andersen, L., Melville, F., and Jolley, D. (2008). An assessment of an oil spill in Gladstone, Australia- Impacts on intertidal areas at one month post-spill. *Marine Pollution Bulletin , 57*, 607-615.

7. Andersen, L., Norton, J., and Levy, N. (2000). A new shell disease in the mud crab Scylla serrata from Port Curtis, Queensland (Australia). *Diseases of Aquatic Organisms , 43*, 233-239.

8. Andersen L, Storey A, Fox S (2002). Assessing the effects of harbour dredging using transplanted oysters as biomonitors. Centre for Environmental management, Central QLD University.

9. Andersen, L., Revill, A., Storey, A., Boundy, K., Davis, A., Campbell, J., et al. (2005). *Metal Bioaccumulation through Food web Pathoways in Port Curtis.* Gladstone, Queensland: CRC for Coastal Zone Estuary and Waterway Management, Centre for Environmental Management Central Queensland University.

10. Angel, B., Jarolimek, C., King, J., Hales, L., Simpson, S., Jung, R., et al. (2012). *Metal concentrations in the waters and sediments of Port Curtis, Queensland.* CSIRO Wealth from Oceans Flagship Technical Report.

11. Apte, S., Duivenvoorden, L., Johnson, R., Jones, M., Revill, A., Simpson, S., et al. (2005). *Contaminants in Port Curtis: screening level risk assessment: Technical Report No. 25.* Indooroopilly, Queensland: Cooperative Research Centre for Coastal Zone, Estuary and Waterway Management.

12. Arthur, K., Limpus, C., and Whittier, J. (2008). Baseline blood biochemistry of Australian green turtles (Chelonia mydas) and effects of exposure to the toxic cyanobacterium Lyngbya majuscula. *Australian Journal of Zoology , 56*, 23-32.

13. Arthur, K., Limpus, C., Balazs, G., Capper, A., Udy, J., Shaw, G., et al. (2008). The exposure of green turtles (Chelonia mydas) to tumor promoting compounds produced by the cyanobacterium Lyngbya majuscula and their potential role in the aetiology of fibropapillomatosis. *Harmful algae , 7*, 114-125.

14. Au, D., Pollino, C., Wu, R., Shin, P., Lau, S., and Tang, J. (2004). Chronic effects of suspended solids on gill structure, osmoregulation, growth, and triiodthyronine in juvenile green grouper Epinephelus coioides. *Marine Ecology Progress Series* , 255-264.

15. Australian Government, Department of the Environment, Water, Heritage and the Arts. (2008). *EPBC Act Policy Statement 2.1 - Interaction between offshore seismic exploration and whales.* Australian Government.

16. Baker, R., Knittel, M., and Fryer, J. (1983). Susceptibility of chinook salmon, Oncorhynchus tshawytcha (Walbaum), and rainbow trout, Salmo gairdneri RIchardson, to infection with Vibrio anguillarum following sublethal copper exposure. *Journal of FIsh Diseases , 6*, 267-275.

17. Baldwin, D., Tatara, C., and Scholz, N. (2011). Copper-induced olfactory toxicity in salmon and steelhead: Extrapolation across species and rearing environments. *Aquatic Toxicology , 101*, 295-297.

18. Barton, B. (2002). Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology* (42), 517-525.

19. Batley, G. (2009). Development of guidelines for ammonia in estuarine and marine water systems. *Marine Pollution Bulletin* (58), 1472-1476.

20. Benedetti, M., Ciaprini, F., Piva, F., Onorati, F., Fattorini, D., Notti, A., et al. (2012). A multidisciplinary weight of evidence approch for classifying polluted sediments: Integrating sediment chemistry, bioavailability, biomarker responses and bioassys. *Environmental International , 38*, 17-28.

21. Berg, K., Lyra, C., Sivonen, K., Paulin, L., Suomalainen, S., Tuomi, P., et al. (2009). High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. *International Society for Microbial Ecology Journal , 3*, 314-325.

22. Berntssen, M., Kroglund, F., Rosseland, B., and Wendelaar Bonga, S. (1997). Responses of skin mucous cells to aluminium exposure at low pH in Atlantic salmon (Salmo salar) smolts. *Canadian Journal of Fisheries Aquatic Sciences , 54*, 1039-1045.

23. Berry, W., and Hill, B. (2003). *The Biological Effects of Suspended and Bedded Sediment (SABS) in Aquatic Systems: A review.* United States Environmental Protection Agency.

24. Bhattacharya, S., Bhattacharya, A., and Roy, S. (2007). Arsenic-induced responses in freshwater teleosts. *Fish Physiology and Biochemistry , 33*, 463-473.

25. Biosecurity Queensland (2012a). *Fish Health Sampling Report: Gladstone Harbour As at 27 February 2012.* Brisbane, Queensland: Department of Employment, Economic Development and Innovation.

26. Biosecurity Queensland (2012b). *Fish Health Sampling Report: Gladstone Harbour As at 27 February 2012.* Brisbane, Queensland: Department of Employment, Economic Development and Innovation.

27. Biosecurity Queensland (2012c). *Fish Health Sampling Reports as at 8 December 2011.* Brisbane: Department of Employment, Economic Deveopment and Innovation- Biosecurity Queensland.

28. Biosecurity Queensland (2012d). *Fish kill testing summary: Catfish, Boyne River, Gladstone, as at 28 September 2012.* Brisbane: Department of Agriculture, Fisheries and Forestry.

29. Bjerknes, V., Fyllingen, I., Holtet, L., Teien, H., Rosseland, B., and Kroglund, F. (2003). Aluminium in acidic river water causes mortality of farmed Atlantic Salmon (Salmo salar L.) in Norweigian fjords. *Marine Chemistry , 83*, 169-174.

30. Blatchford, E. (2011). Dead sting-ray found at Barney Point. Gladstone Observer, August 1, 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/08/01/dead-sting-ray-found-barney-pt/

31. Bolam, S., Barry, J., Bolam, T., Mason, C., Rumney, H., Thain, J., et al. (2011). Impacts of maintenance dredged material disposal on macrobenthic structure and secondary productivity. *Marine Pollution Bulletin , 62*, 2230-2245.

32. Bols, N. B. (2001). Ecotoxicology and innate immunity in fish. *Developmental and Comparative Immunology , 25*, 853-873.

33. Bonnet, C. B. (2000). Assessing the potential toxicity of resuspended sediment. . *Environmental Toxicology and Chemistry , 19*, 1290-1296.

34. Brinkmann, M., Hudjetz, S., Cofalla, C., Roger, S., Kammann, U., Giesy, J., et al. (2010). A combined hydraulic and toxicological approach to assess resuspended sediments during simulated flood events. Part-I multiple biomarkers in rainbow trout. *Journal of Soils and Sediments , 10*, 1347-1361.

35. Brodie, J., and Waterhouse, J. (2012). A critical review of environmental management of the 'no so Great' Barrier Reef. *Estuarine, Coastal and Shelf Science , 104-105*, 1-22.

36. Brodie, J., Kroon, F., Schaffelke, B., Wolanksi, E., Lewis, S., Devlin, M., et al. (2012). Terrestrial pollutant runoff to the Great Barrier Reef: An update of issues prorities and management responses. *Marine Pollution Bulletin* , in press.

37. Burton, G. (2010). Assessing contaminated sediments in the context of multiple stressors. . *Environmental Toxicology and Chemistry , 29*, 2625-2643.

38. Buruaem, L., Hortellani, M., Sarkis, J., Costa-Lotufo, L., and Abessa, D. (2012). Contamination of port zone sediments by metals from Large Marine Ecosystems of Brazil. *Marine Pollution Bulletin* , in press.

39. Buscaino G. F. F. (2010). Impact of an acoustic stimulus on the motility and blood parameters of European sea bass (Dicentrarchus labrax L.) and gilthead sea bream (Sparus aurata L.). *Marine Environmental Research , 69* (3), 136-142.

40. Caglan, A., Benli, K., and Koksal, G. (2005). The acute toxicity of ammonia on tilapia (Oreochromis nioticus L) larvae and fingerlings. *Turkish Journal of Veterinary and Animal Sciences , 29* (2), 339-344.

41. Chapman, P., Wang, F., Janssen, C., Persoone, G., and Allen, H. (1998). Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. *Canadian Journal of Fisheries Aquatic Science , 55*, 2221-2243.

42. Chen, T.-H., Lin, C.-Y., and Tseng, M.-C. (2011). Behavioural effects of titanium dioxide nanoparticles on larval zebrafish (Danio rerio). *Marine Pollution Bulletin , 63*, 303-308.

43. Cheung, R. N. (1982). Treatment of skin lesions in captive lemon sharks, Negaprion brevirostris (Poey), caused by monogeneans (Dermophthirius sp.). *Journal of Fish Diseases , 5*, 167-170.

44. Clark, W. (2004). Development of acid sulfate soil in sub-aerially disposed dredge spoil at Fisherman Islands, Brisbane, Australia. *Australian Journal of Soil Research , 42*, 553-567.

45. Cofalla, C., Hudjetz, S., Roger, S., Brinkmann, M., Frings, R., Wolz, J., et al. (2012). A combined hydraulic and toxicological approach to assess resuspended sediments during simulated flood events- part II: an interdisciplinary experimental methodology. *Journal of Soils and Sediments , 12*, 429-442.

46. Connolly, R., Currie, D., Danaher, K., Dunning, M., Melzer, A., Platten, J., et al. (2006). *Intertidal wetlands of Port Curtis: ecological patterns and processes, and their implications.Technical Report No. 43.* Brisbane: CRC for Coastal Zone, Estuary and Waterway Management.

47. Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., et al. (1997). The value of the world's ecosystem services and natural capital. *Nature , 387*, 253-260.

48. CQU Consulting. (2011) Port of Gladstone Western Basin Strategic Dredging and Disposal Project, QLD (EPBC 2009/4904), Annual Environmental Performance Report. Gladstone Ports Corporation. November 2011.

49. Cruz-Lacierda, E., Pagador, G., Yamamoto, A., and Nagasawa, K. (2011). Parasitic caligid copepods of farmed marine fishes in the Philippines. In M. Bondad-Reantaso, J. Jones, F. Corsin, and T. Aoki, *Diseases in Asian Aquaculture VII, Fish Health Section* (pp. 53-62). Selangor, Malaysia: Asian Fisheries Society.

50. Department of Agriculture, Fisheries and Forestry, Biosecurity Queensland. (2012). *Scallop Sampling: BUndaberg and Gladstone Waters, February 2012.* DAFF, Biosecurity Queensland.

51. Department of Environment and Heritage. (1998). *Fish Kill Reporting and Investigation Manual.* Queensland Government-Department of Environment and Heritage.

52. Department of Environment and Heritage Protection (2012b). *Sixth Update on the Water Quality of port Curtis and Tributaries Including data collected in the week of 5 March 2012.* Brisbane: Queensland Government.

53. Department of Environment and Heritage Protection (2012c). *Seventh update on the water quality of Port Curtis ad Tributaries including data collected in the week of 2 April 2012.* Brisbane: Queensland Government.

54. Department of Environment and Heritage Protection c. (2012). *Update on the Quality of Sediment from Port Curtis and Tributaries.* Brisbane: Queensland Government.

55. Department of Environment and Heritage Protection. (2012, July). *Department of Environment and Heritage Protection*. Retrieved August 6, 2012, from Gladstone Source: http://www.vision6.com.au/em/message/email/view.php?id=930162andu=50147

56. Department of Environment and Resource Management. (2012a). *Third Update on the Water Quality of Port Curtis and Tributaries including data collected in the weeks 21 November an 12 December 2011.* Brisbane: Queensland Government.

57. Department of Environment and Resource Management. (2012). *Fourth Update on the Water Quality of Port Curtis and Tributaries Including Data Collected in the Week of 9 January 2012.* Brisbane: Queensland Government.

58. Department of Environment and Resource Management. (2011, July). *Identifying acid sulfate soils.* Retrieved August 9, 2012, from DERM Factsheets I61: http://www.derm.qld.gov.au/factsheets/pdf/land/l61.pdf

59. Department of Environment and Resource Management. (2011). *Port Curtis and Tributaries: Comparison of Current and Historical Water Quality: October 2011.* Brisbane: The State of Queensland, Department of Environment and Resource Management.

60. Deveney, M., Chisholm, L., and Whittington, I. (2001). First published record of the pathogenic monogenean parasite Neobenedenia melleni (Capsalidae) from Australia. *Diseases of Aquatic Organisms , 46*, 79-82.

61. Di Nanno, M. C. (2007). Anaerobic Sediment Potential Acidification and Metal Release Risk Assessment by Chemical Characterization and Batch Resuspension Experiments. *Journal of Soil Sediments , 7* (3), 187-194.

62. Diamant, A. B. (1999). The use of fish metabolic, pathological and parasitological indices in pollution monitoring. II. The Red Sea and Mediterranean. *Helgoland Marine Research , 53*, 195-208.

63. Dietrich, D., Fischer, A., Michel, C., and Hoeger, S. (2007). Chapter 39: Toxin mixture in cyanobacterial blooms- a critical comparison of reality with current procedures employed in human health risk assessment. In *Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms* (p. 28).

64. Diggles, B. (2011a). Barramundi health check, Boyne River. DigsFish Services Pathology Report, DF11/25, 9 April 2011.

65. Diggles, B. (2011b). *Water Quality testing: Gladstone Harbour October-November 2011.* Bribie Island, Queensland: Gladstone Fish Market.

66. Dowling, R. (2008). *Investigation of Mangrove Dieback: Fitzroy River, Rockhampton.* Queensland Government, Environmental Protection Agency.

67. Downey, K. (2011). Boyne River Turtle Deaths Rise. Gladstone Observer, 19 April 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/04/19/boyne-river-turtle-deaths-rise/

68. Drabble, R. (2012a). Monitoring of East Channel dredge areas benthic fish population and its implications. *Marine Pollution Bulletin , 64*, 363-372.

69. Drabble, R. (2012b). Projected entrainment of fish resulting from aggregate dredging. *Marine Pollution Bulletin , 64*, 373-381.

70. Duke, N. (2008). Corrections and updates to the article by Duke et al. (2005) reporting on the unusual occurence and cause of dieback of the common mangrove species, Avicennia marina, in NE Australia. *Marine Pollution Bulletin , 56*, 1668-1670.

71. Duke, N., Bell, A., Pederseon, D., Roelfsema, C., and Bengston-Nash, S. (2005). Herbicides implicated as the cause of severe mangrove dieback in the Mackay region, NE Australia:

consequences for marine plant habitats of the GBR World Heritage Area. *Marine Pollution Bulletin* , 308-324.

72. Economic Associates. (2009). *Port of Gladstone Western Basin Dredging and Disposal Project: Economic Assessment: Final Report EIS Appendix Y.* Spring Hill, Queensland: Economic Associates.

73. Eggleton, J., and Thomas, K. (2004). A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environment International , 30*, 973-980.

74. Ellis, A. (1981). Stress and the modulation of defence mechanisms in fish. In A. Pickering, *Stress in Fish* (pp. 147-169). London: Academic Press.

75. Ellison, J. (1998). Impacts of Sediment Burial on Mangroves. *Marine Pollution Bulletin , 37* (8-12), 420-426.

76. Environmetrics Australia. (2011). *Towards a light-based monitoring program: A progress report to Gladstone Ports Corporation.* Parkville, Victoria: Environmetrics Australia.

77. Ernst and Young and the Centre for Agricultural and Regional Economics. (2004). *Economic Impact of the NSW Striped Marlin Fishery.* Ernst and Young.

78. Esslemont, G., Russell, R., and Maher, W. (2004). Coral record of harbour dredging: Townsville, Australia. *Journal of Marine Systems , 52*, 51-64.

79. Fabricius, K. (2012, May 18). *Australian Government: Australian Institute of Marine Science: Latest Releases.* Retrieved August 8, 2012, from Pollution Pluis Sedimentation Equals Rapid Coral Death: New Study: http://www.aims.gov.au/docs/media/latest-releases/-/asset_publisher/8Kfw/content/id/98479

80. Fisheries Queensland. (2012). *Fish Health Survey: Gladstone Harbour, as at 1 March 2012.* Brisbane, Queensland: Department of Employment, Economic Development and Innovation.

81. Forstner, U., Ahlf, W., and Calmano, W. (1989). Studies on the transfer of heavy metals between sedimentary phases with a multi-chamber device: combined effects of salinity and redox variation. *Marine Chemistry , 28*, 145-158.

82. Garcia, R., and Johnstone, R. (2006). Effects of Lyngbya majuscula (Cyanophycea) blooms on sediment nutrients and meiofaunal assemblages in seagrass beds in Morteon Bay, Australia. *Marine and Freshwater Research , 57*, 155-165.

83. Genemer, R., and Playle, R. (1999). The Bioavailability and Toxicity of Aluminium in Aquatic Environments. *Critical Reviews in Environmental Science and Technology* , 315-450.

84. Gerbersdorf, S., Hollert, H., Brinkmann, M., Wieprecht, S., Schuttrumpf, H., and Manz, W. (2011). Anthropogenic pollutants affect ecosystem services of freshwater sediments: the need for a "triad plus x" approach. *Journal of Soils and Sediments* , 1-15.

85. GHD. (2009a). *Gladstone Ports Corporation: Report for Western Basin Dredging and Disposal Project: Marine Ecology Assessment.* GHD.

86. GHD. (2009b). *Gladstone Ports Corporation: Report for Western Basin Dredging and Disposal Project Sediment Quality Assessment.* GHD.

87. GHD. (2009c). *Gladstone Ports Corporation: Report for Western Dredging and Disposal Project: Acid Suphate Soils Assessment September 2009.* GHD.

88. GHD. (2009d). *Executive Summary: Western Basin Dredging and Disposal Project: Environmental Impact Statement.* Retrieved August 10, 2012, from Department of Employment, Economic Development and Innovation, Queensland Government: www.deedi.qld.gov.au/cg/resources/project/gladstone-western-basin/executive-summary.pdf

89. GHD (2009e). Report for Western Basin Dredging and Disposal Project. Water Quality report . October 2009

90. GHD. (2011). *Gladstone Ports Corporation: Report for Marine Megafacuna and Acoustic Survey; Autumn Survey November 2011.* GHD.

91. Gladstone Area Water Board. (2011a). *Forward scientific panel at DEEDI water quality data at Hatchery 16_11_11 EDOCS_n159467_v_1.* Gladstone: Queensland Government.

92. Gladstone Area Water Board. (2011b). *Forward Water quality to Scientific Panel- John Robertson: EDOCS_n159452v1.* Gladstone: Queensland Government.

93. Gladstone Area Water Board. (2011). *GAWB response to request for information from scientific panel: EDOCS_n170218_v1_GAWB.* Gladstone: Queensland Government, Gladstone Area Water Board.

94. Gladstone Fish Health Scientific Advisory Panel. (2012). *Final Report 5 January 2012.* Brisbane: Queensland Government.

95. Gladstone Observer. (2011a). More dead turtles. Gladstone Observer, 31 August 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/08/31/more-dead-turtles-marine-deaths-facing-island/.

96. Gladstone Observer. (2011b). Further reports of dead turtles. Gladstone Observer, 1 November. http://www.gladstoneobserver.com.au/story/2011/11/02/further-reports-of-dead-turtles-curtis-island/.

97. Gladstone Observer. (2011c). Net ban call after marine death. Gladstone Observer, 16 June 2011. http://www.gladstoneobserver.com.au/story/2011/06/16/likely-extension-of-net-ban-marine-deaths/

98. Gladstone Observer. (2011d). Sick fish spotted in the Boyne. Gladstone Observer, 19 September 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/09/19/sick-fish-spotted-in-boyne-river-gladstone-harbour/

99. Gladstone Observer. (2011e).Sick crabs raise more issues. Gladstone Observer, 13 December 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/12/13/sick-crabs-raise-more-issues-gladstone-harbour/

100. Gladstone Observer. (2011f). Gladstone fish kill unknown. Gladstone Observer, 15 February 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/02/15/gladsdtone-fish-kill-unknown/

101. Gladstone Ports Corporation. (2012, August 10). *Origin and Destination of Cargoes*. Retrieved August 10, 2012, from http://www.cqpa.com.au/viewcontent/SHippingStatistics/CargoOriginDestination.aspx

102. Gladstone Ports Corporation. (2012b). Transitional Environmental Program: Western Basin Bund Sealing: Gladstone Ports Corporation: DA SPDE01611411, 18 June 2012.

103. Gotz, T., Hastie, G., Hatch, L., Raustein, O., Southall, B., Tasker, M., et al. (2009). *Overview of the impacts of anthropogenic underwater sound in the marine environment.* OPSAR commision.

104. Graneli, E., Edvarsen, B., Roelke, D., and Hgstrom, J. (2012). The ecophysiology and bloom dynamics of Prymnesium spp. *Harmful algae , 14*, 260-270.

105. Gross, E., and Martin, D. (1996). Iron Dependence of Lyngbya majuscula. *Journal of Aquatic Plant Mangement , 34*, 17-20.

106. Grossart, H.-P., Levold, F., Allgaier, M., Simon, M., and Brinkhoff, T. (2005). Marine diatom species harbour distinct bacterial communities. *Environmental Microbiology , 7* (6), 860-873.

107. Hart, B. (2011). *Port Curtis and Tributaries: Comparison of current and historical water quality.* Water Science Pty Ltd.

108. Hedge, L., Knott, N., and Johnston, E. (2009). Dredging and metal bioaccumulation in oysters. *Marine Pollution Bulletin , 58*, 832-840.

109. Heisler, J., Gilbert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., et al. (2008). Eutrophication and harmful algal blooms: A scientific consensus. *Harmful algae , 8*, 3-13.

110. Hendrix, S. (1985). Monogenetic trematodes from fishes of the New York Bight. *Annual Midwest Conference of Parasitology Presentations.* Ohio State University.

111. Herzfeld, M., Parslow, J., Andrewartha, J., Sakov, P., and Webster, I. (2004). *Hydrodynamic Modelling of the Port Curtis Region: Technical report 7.* Indooroopilly, Queensland: CRC for Coastal Zone, Estuary and Waterway Management.

112. Heterick, F., Knittel, M., and Fryer, J. (1979). Increased susceptibility of Rainbow Trout to Infectious Haematopoietic Necrosis Virus after exposure to copper. *Applied and Environmental Microbiology* , 198-201.

113. Hossain, MK., Khan, MMH., Alam, MA., Chowdhury, HK., Delwar Hossain, M., Feroze Ahmed, M., Kobayashi, K., Sakauchi, F., Mori, M. (2005) *Manifestations of arsenicosis patients and factors determining the duration of arsenic symptoms in Bangladesh.*Toxicology and Applied Pharmacology. 208. pp78-86

114. Infofish. (2012). *Recaptures of barramundi in the central Queensland Area from Corio Bay to Rodds Bay, Barramundi Season 2012.* Suntag CapReef infofish.

115. Irving. (2012). Sick fish flood NRG. Gladstone Observer. 24 May 2012. Accessed May 2012. http://www.gladstoneobserver.com.au/story/2012/05/24/sick-fish-flood-nrg/.

116. Jones, M.-A., Stauber, J., Apte, S., Simpson, S., Vincente-Beckett, V., Johnson, R., et al. (2005). A risk assessment approach to contaminants in Port Curtis, Queensland, Australia. *Marine Pollution Bulletin , 51*, 448-458.

117. Kalogianni, E., Alexis, M., Tsangaris, C., Abraham, M., Wendelaar Bonga, S., Iger, Y., et al. (2011). Cellular responses in the skin of the gilthead sea bream Sparus aurata L. and the sea bass Dicentrachus labrax (L.) exposed to high ammonia. *Journal of Fish Biology , 78*, 1152-1169.

118. Khan, R. (1991). Influnce of pollution on parasites of aquatic animals. *Advances in Parasitology , 30*, 201-238.

119. Khan, R. (1990). Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez spill. *Bulletin of Environmental Contamination and Toxicology , 44*, 759-763.

120. Khan, R. (2011). Chronic exposure and decontamination of a marine sculpin (Myoxocephalus scoprius) to polychlorinated biphenyls using selected body indices, blood values, histopathology and parasites as bioindicators. *Archive of Environmental Contaminants and Toxicology , 60*, 479-495.

121. Klaprat, D., Evans, R., and Hara, T. (1992). Environmental contaminants and chemoreception in fishes. In T. Hara, *Fish Chemoreception* (pp. 321-341). New York: Chapman and Hall.

122. Knittel, M. (1981). Susceptibility of steelhead trout Salmo Gairdneri Richardson to redmouth infection Yersinia ruckeri following exposure to copper. *Journal of Fish Disease , 4*, 33-40.

123. Knott, N. A. (2009). Contemporary ecological threats from historical pollution sources: impacts of large-scale resuspension of contaminated sediments on sessile invertebrate recruitment. *Journal of Applied Ecology , 46*, 770-781.

124. Law, M. (2001). Differential diagnosis of ulcerative lesions in fish. *Environmental Health Perspectives , 109* (Supplement 5), 681-686.

125. Leibovitz, L., and Lebouitz, S. (1985). A coccolithophorid algal dermatitis of the spiny dogfish, Squalus acanthias L. *Journal of Fish Diseases , 8*, 351-358.

126. Lim, C. K. (2001). Chapter 9: Dietary Iron and Fish Health. In C. a. Lim, *Nutrition and Fish Health* (pp. 189-200). Food Products Press.

127. LNG Port Dredging and Gladstone Ports Corporation. (2012). *Transitional Environmental Program: Western Basin Bund Sealing: Gladstone Ports Corporation DA SPDE01611411.* Gladstone, Queensland: Gladstone Ports Corporation.

128. Mackenzie, K. (1999). Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Marine Pollution Bulletin , 38* (11), 955-959.

129. Mahmood, T., Kovacs, T., Gibbons, S., and Paradis, J.-C. (2010). Implications of aerated stabilisation basin dredging on potential effluent toxicity to fish. *Water Environment Research , 82* (5), 440-447.

130. Marcogliese, D. (2005). Parasites of the superorganism: Are they indicators of ecosystem health. *International Journal for Parasitology , 35*, 705-716.

131. McCormack, C., and Rasheed, M. (2012a). *Gladstone Permanent Transect Seagrass Monitoring – February 2012 Interim Update Report.* Cairns: DEEDI Publication, Fisheries Queensland.

132. McCormack, C., and Rasheed, M. (2012b). *Gladstone Permanent Transect Seagrass Monitoring – May 2012 Interim Update Report.* Cairns: DAFF Publication, Fisheries Queensland.

133. McCormack, CV., Sankey, TL., Davies, JN., Carter, AB., Rasheed, MA. (2012). Gladstone Permanent Transect Seagrass Monitoring: August 2012 Update Report. Gladstone Ports Corporation, Queensland Government.

134. Mikkelsen, J. (2012). *Stirring up past industrial sins.* Queensland Telegraph. www.greatbarrierreef.org.au/464/. accessed September 15, 2012.

135. Millward, R. C. (2004). Mixtures of metals and hydrocarbons elicit complex responses by a benthic invertebrate community. *Journal of Experimental Marine Biology and Ecology* , 115-130.

136. Mirza, R., Green, W., Connor, S., Weeks, A., Wood, C., and Pyle, G. (2009). Do you smell what I smell? Olfactory impairment in wild yellow perch from metal-contaminated waters. *Ecotoxicology and Environmental Safety , 72*, 677-683.

137. Nakai, T. K. (1987). The effects of iron compounds on the virulence of Vibrio anguillarum in Japanese eels and ayu. *Fish Pathology* (22), 185-189.

138. Nayar, S. G. (2004). Environmental impact of metals from dredged and resuspended sediments on phytoplankton and bacteria assessed in in-situ mesocosms. *Ecotoxicology and Environmental Safety , 59*, 349-369.

139. Negri, A., Bunter, O., Jones, B., and Llewellyn, L. (2004). Effects of the blom-forming alga Trichodesmium erythraeum on the pearl oyster Pinctada maxima. *Aquaculture , 232*, 91-102.

140. Noga, E. (2000). *Fish Disease: Diagnosis and Treatment.* Iowa: Blackwell Publishing Professional.

141. Norrgren, L., Wicklund Glynn, A., and Malmborg, O. (1991). Accumulation and effects of aluminium in the minnow (Phoxinus phoxinus L.)at different pH levels. *Journal of Fish Biology , 39*, 833-847.

142. Nowacek, D. T. (2007). Responses of cetaceans to anthropogenic noise. *Mammal Review , 37* (2), 81-115.

143. Nowak, B., Crane, M., and Jones, B. (2005). *Aquatic Animal Health Subprogram: Development of national investigation and reporting protocols for fish kills in recreational and capture fisheries.* Australian Government, Fisheries Research and Development Corporation.

144. Ocean Maritime Consultants Pty Ltd. (2011). *Mapping and Assessment of Seagrass and Coral Reefs for Gladstone Ports Corporation Limited.* Magnetic Island, Queensland: Ocean Maritime Consultants.

145. Osborne, N., Seawright, A., and Shaw, G. (2008). Dermal toxicology of Lyngbya majuscula, from Morteon Bay, Queensland, Australia. *Harmful Algae , 7*, 584-589.

146. Osborne, N., Shaw, G., and Webb, P. (2007). Health effects of recreational exposure to Morteon Bay, Australia waters during a Lyngbya majuscula bloom. *Environment International , 33*, 309-314.

147. Osborne, N., Webb, P., and Shaw, G. (2001). The toxins of Lyngbya majuscula and their human and ecological health effects. *Environment International , 27*, 381-392.

148. Osbourne, N. (2012). Occupational dermatitis caused by Lyngbya majuscula in Australia. *International Journal of Dermatology , 51*, 122-123.

149. Overstreet, R. (1993). Parasitic diseases of fishes and their relationship with toxicants and other environmental factors. In J. Couch, *Pathobiology of Marine and Estuarine Organisms* (pp. 111-156). CRC press.

150. Palm, H. (2011). Fish parasites as biological indicators in a changing world: can we monitor environmental impact and climate change. In H. Mehlhom, *Progress in parasitology, parasitology research monographs 2* (pp. 223-250). Springer-Verlag.

151. Palm, H., and Ruckert, S. (2009). A new approach to visualise ecosystem health by using parasites. *Parasitology reserve , 105*, 539-553.

152. Partridge, G., and Michael, R. (2010). Direct and indirect effects of simulated calcarious dredge material on eggs and larvae of pink snapper Pagrus auratus. *Journal of Fish Biology , 77*, 227-240.

153. Patterson, R., Noga, E., and Germolec, D. (2007). Lack of evidence for contact sensitisation of Pfiesteria Extract. *Environmental Health Perspectives , 115*, 1023-1028.

154. Peng, S.-H., Hung, J.-J., and Hwang, J.-S. (2011). Bioaccumulation of trace metals in the submarine hydrothermal vent crab Xenograpsus testudinatus off Kueishan Island, Taiwan. *Marine Pollution Bulletin , 63*, 396-401.

155. Petus, C., and Devlin, M. (2012). *Using satellite maps to document the extent of sediment plumes associated with dredging activity in Gladstone Port's western basin, Queensland.* Townsville: Catchment to Reef Research Group, ACTFR,TROPWATER James Cook University .

156. Popper, A. a. (2009). The effects of human-generated sound on fish. *Integrative Zoology , 4*, 43-52.

157. Poulsen, A., and Escher, B. (2012). *Chemically induced immunosuppression and disease susceptibiltiy in marine wildlife: A literature review.* Brisbane, Queensland: ENTOX: National Research Centre for Environmental Toxicology.

158. Prashanth, M. (2012). Acute toxicity, behavioural and nitrogen metabolism changes of sodium cyanide affected tissues of Tilapia mossambica (Perters). *Drug and Chemical Toxicity , 35* (2), 178-183.

159. Queensland Coordinator-General. (2010). *Western Basin Dredging and Disposal Project: Coordinator-General's report for an environmental impact statement.* Queensland Government.

160. Queensland Government. (2011). *Maritime Safety Queensland: Queensland Notices to Mariners: 1074 (Temporary) fo 2010.* Gladstone: Queensland Government.

161. Rasheed, M., Thomas, R., Roelofs, A., Neil, K., and Kerville, S. (2003). *Port Curtis and Rodds Bay seagrass and benthic macro-invertebrate community baseline survey.* Cairns: Queensland Government, DPI Information Series QI03058.

162. Redding, J., Schreck, C., and Everest, F. (1987). Physiological effects on Coho salmon and Steelhead of exposure to suspended sediments. *Transactions of the American Fisheries Society , 116*, 737-744.

163. Reid, S., Isaac, G., Metikosh, S., and Evans, J. (2003). Physiological response of rainbow trout to sediment released during open-cut pipeline water crossing construction. *Water Qual. Res. Journal Canada , 38* (3), 473-481.

164. Rice, C. a. (1987). PCB availability assessment of river dredging using caged clams and fish. *Environmental Toxicology and Chemistry , 6*, 259-276.

165. Richardson, W., Greene, C., Malme, C., and and Thomson, D. (1995). *Marine Mammals and Noise.* NY: Academic Press.

166. Rodsaether, M., Olafsen, J., Rea, J., Myhre, K., and Steen, J. (1977). Copper as an initiating factor of vibiosis (Vibrio anguillarum) in eel (Anguilla anguilla). *Journal of Fish Biology , 10*, 17-21.

167. Ronnback, P. (1999). The ecological basis for economic value of seafood production supported by mangrove ecosystems. *Ecological Economics , 29*, 235-252.

168. Rzymski, P., and Poniedzialek, B. (2012). Dermatotoxins synthesized by blue-green algae (Cyanobacteria). *Postepy Dermatologii i Alergologii* , 47-50.

169. Schlenk, D., Wolford, L., Chelius, M., Steevens, J., Chan, KM. (1997). Effect of arsenite, arsenate, and the herbicide monosodium methyl arsonate (MSMA) on hepatic metallothionein expression and lipid peroxidation in channel catfish. *Comparative Biochemistry and Physiology. Part C: Pharmacology, Toxicology and Endocrinology*. 118. 2. 177-183

170. Schmitt, C., and Dethloff, G. (2000). *Biomonitoring of Environmental Status and Trends (BEST) Program: Selected Methods for Monitoring Chemical Contaminants and their Effects in Aquatic Ecosystems: Information and Technology Report USGS/BRD-2000-0005.* Columbia: US Geological Survey, Biological Resources Division.

171. Schwedler, T., Tucker, C., and Beleau, M. (1985). *Channel catfish culture.* Amsterdam: Elsevier.

172. Scott, G., and Sloman, K. (2004). The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquatic Toxicology , 68*, 369-392.

173. Simpson, S., Radford, A., Tickle, E., Meekan, M., and and Jeffs, A. (2011). Adaptive Avoidance of Reef Noise. (U. o. A. Peter Klimley, Ed.) *PLoS ONE* , e16625.

174. Sindermann, CJ. (2006). Coastal Pollution: Effects on Living Resources and Humans. CRC Press.

175. Southall, B., Bowles, A., Ellison, W., Finneran, J., Gentry, R., Greene Jnr, E., et al. (2007). Marine mammal noise exposure criteria: Initial scientific recommendations. *Aquatic mammals* (33), 411-521.

176. Sparkes, D. (2011a). Vet calls for facts of deaths. Gladstone Observer, 27 July 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/07/27/vet-calls-for-facts-of-marine-deaths-harbour/

177. Sparkes, D. (2011b). Dying sea animals concern experts. Gladstone Observer, 18 July 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/07/18/more-protection-needed-marine-animals/

178. Sparkes, D. (2011c). Blind barramundi raises questions. Gladstone Observer, 22 August 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/08/22/blind-barramundi-raise-questions-gladstone-harbour/

179. Sparkes, D. (2011d). Dredging halted in the Harbour. Gladstone Observer, 30 September 2011. Accessed October 2012. http://www.gladstoneobserver.com.au/story/2011/09/30/dredging-halted-demand-for-probe/

180. Sparkes, D. (2012). Dead catfish at Boyne River Mouth. Gladstone Observer, April 4, 2012. Accessed April 2012. http://www.gladstoneobserver.com.au/story/2012/04/04/dead-catfish-boyne-river-mouth/

181. Stitt, S. (2012, March 2). *Cyanide leak from Orica's Gladstone facility*. Retrieved September 6, 2012, from EJS: Environmental Justice Society: www.environmentaljustice.com.au/2012/03/cyanide-leak-from-oricas-gladstone-facility/

182. Sturve, J. B. (2005). Effects of dredging in Göteborg Harbor, Sweden, assessed by biomarkers in eelpout (Zoarces viviparus). . *Environmental Toxicology and Chemistry , 24*, 1951-1961.

183. Sun, F. W. (2012). Effects of three different PAHs on nitrogen-fixing bacterial diversity in mangrove sediment. *Ecotoxicology , 21*, 1651-1660.

184. Thoney, D. (1991). Monogenea (Platyhelminthes) as hazards for fish in confinement. *Annual Review of Fish Diseases* , 133-153.

185. Tierney, K., Baldwin, D., Hara, T., Ross, P., Scholz, N., and Kennedy, C. (2010). Olfactory toxicity in fishes. *Aquatic Toxicology , 96*, 2-26.

186. Torud, B., and Hastein, T. (2008). Skin lesions in fish: causes and solutions. *Acta Veterinaria Scandinavica , 50*.

187. Udomkusonsri, P., and Noga, E. (2005). The acute ulceration response (AUR): A potentially widespread and serious cause of skin infection in fish. *Aquaculture* , 63-77.

188. United States Environmental Protection Agency. (2012, July 30). *National Recommended Water Quality Criteria*. Retrieved August 10, 2012, from Water Quality Criteria: http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm

189. Vasas, G., M-Hamvas, M., Borics, G., Gonda, S., Mathe, C., Jambrik, K., et al. (2012). Occurence of toxic Prynesium parvum blooms with high protease activity is related to fish mortality in Hungarian ponds. *Harmful algae , 17*, 102-110.

190. Vincente-Beckett, V., Shearer, D., Munksgaard, N., Hancock, G., and Morrison, H. (2006). *Metal and polycyclic aromatic hydrocarbon contaminants in benthic sediments of Port Curtis.* Indooroopilly, Queensland: Cooperative Research Centre for Coastal Zone, Estuary and Waterway Management.

191. Vision Environment Queensland. (2011). *Port curtis Ecosystem Health Report 2008-2010.* Gladstone: Port Curtis Integrated Monitoring Program (PCIMP).

192. Vision Environment. (2012). *WBDEM Water Quality Data Summary.* Retrieved August 10, 2012, from www.westernbasinportdevelopment.com.au/media/pdf/metals water quality monitoring graphs.pdf

193. Weber, M., de Beer, D., Lott, C., Polerecky, L., Kohls, K., Abed, R., et al. (2012). Mechanisms of damage to corals exposed to sedimentation. *Proceedings of the National Academy of Science* , 1-10.

194. Weinstein, JE., West, TL., Bray, JT. (1992). Shell disease and metal content of blue crabs, *Callinectes sapidus*, from the Albemarle-Pamlico Estuarine System, North Carolina. Archives of Environmental Contamination and Toxicology. 23. 355-362.

195. Wilber, D., and Clarke, D. (2001). Biological effects of suspended sediments: a review of suspended sediment impacs on fish and shellfish with relation to dredging activities in estuaries. *North American Journal of Fisheries Management , 21*, 855-875.

196. Wilkens, S. S. (2012). Induction of settlement in mussel (Perna canaliculus) larvae by vessel noise. *Biofouling: The Journal of Bioadhesion and Biofilm Research , 28* (1), 65-72.

197. Wilkinson, M., and Hichens, C. (2011, November 8). *Great Barrier Grief*. Retrieved September 6, 2012, from Four Corners: www.abc.net.au/4corners/stories/2011/11/03/3355047.htm

198. Williams, B. (2011, November 28). *Possible algae link to fish deaths.* Retrieved August 9, 2012, from Courier Mail: http://www.couriermail.com.au/ipad/possible-algae-link-to-fish-deaths/story-fn6ck51p-1226207534864

199. Wysocki, L. D. (2006). Ship noise and cortisol secretion in European freshwater fishes. *Biological Conservation* (128), 501-508.

200. Yanong, R., Francis-Floyd, R., Curtis, E., Klinger, R., Cichra, M., and Berzins, I. (2002). Algal dermatitis in cichlids. *Journal of American veterinary Medicine Association , 220* (9), 1353-1358.

201. Zeilokoff, J. (1993). Metal pollution-induced immunomodulation in fish. *Annual Review of Fish Diseases* , 305-325.

APPENDIX 1: FFVS Interim Report 001

Future Fisheries
VETERINARY SERVICE Pty Ltd
ABN: 520 830 961 17
PO Box 364 Lennox Head NSW 2478
Phone 0437 492 863
matty.landos@gmail.com

Project Update 001:

QSIA managed funding which was provided through the charity, Gladstone Fishing Research Fund, to commission FFVS to commence an investigation of the presence and cause of reported aquatic animal health problems in the Gladstone harbour and nearshore waters. Dr Matt Landos and Dr Ben Diggles travelled to Gladstone on 18-25 January, 2012 and undertook intensive sampling of a range of aquatic animals through being onboard with commercial fishers.

Sampling trips were undertaken at:

-Upper Boyne River- sampling barramundi, bull shark, mullet and catfish

-Lower Boyne River- sampling barramundi (not listed on map yet)

-Nearshore Hummocky- sampling barramundi, a turtle, shark

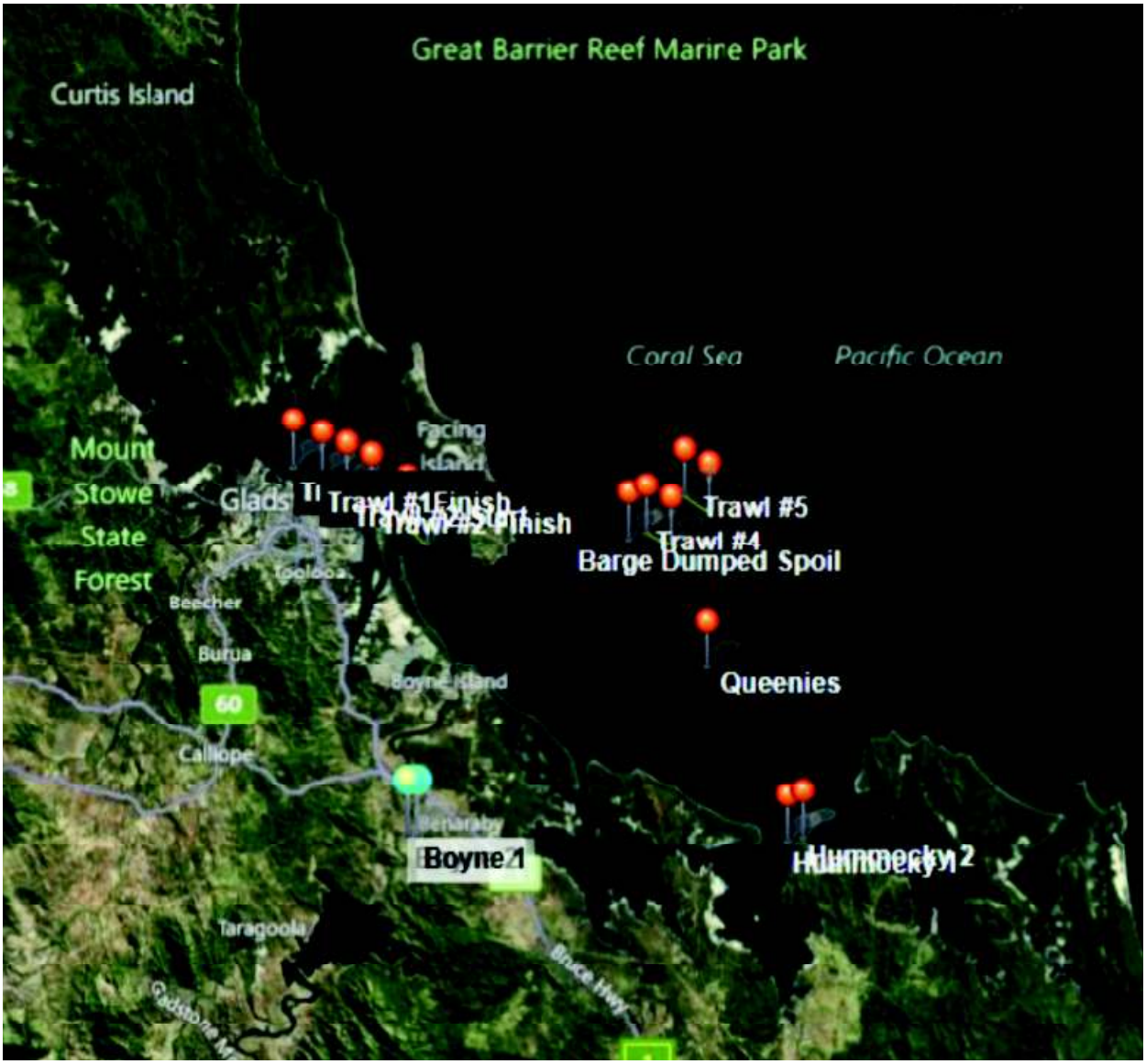
-Offshore near spoil dump site- sampling queenfish and shark

-Friends Point and Grahams creek- sampling mudcrab (not listed on map yet)

-Colosseum- sampling mudcrab and mangrove dieback (not listed on map yet)

-7 Mile/Turkey beach- sampling whiting, mullet, mudcrab (not listed on map yet)

-3 harbour trawl shots and 2 spoil ground shots sampling prawn and bycatch



Captured animals were examined for the presence or absence of external lesions such as ulcers on their skin, a tucked up abdomen and damaged gills. Where animals were observed with lesions, 5 typically affected animals were sampled for histological examination, and a range of bacterial culture plates were set up to examine for the presence of bacterial infections.

A second trip is planned to collect control samples from a site remote to the dredging in 3 weeks time. In Gladstone further sampling will focus on scallops and collecting more water quality and algal samples.

I estimate that it will take me at least 1 to 2 months to conduct the necessary scientific testing of samples taken and then prepare my report and conclusions. However, I am able to report some preliminary observations of fish health issues, and also the results of some of the gross pathology examinations I conducted.

Boyne River, Highway Bridge

I observed a very high prevalence of sick barramundi in the Boyne River, with the vast majority having tucked up abdomen's indicating an absence of feed in the gut. This was corroborated by necropsy findings which did not identify ingesta in any of the barramundi sampled. I observed abundant baitfish (mullet) in the river immediately adjacent the locations where barramundi were caught. The mullet were sampled by cast net, and did not exhibit any gross external signs of disease. The barramundi were lethargic, and a high proportion developed a reddening of the scales along the ventral midline which was apparent when fish were removed from the water. Some fish had ulcerative skin and eye lesions, some of which appeared to be healing.

At necropsy, a significant proportion of the sick stock had large volumes of peritoneal effusion, a small dark liver with small spots of pigment on the surface, gross changes to gill with multifocal pale proliferative areas of lamellae, and in many cases an enlarged spleen. Gill tissues from 2 fish were examined under the microscope which identified low numbers of monogenean gill flukes, a suspect myxosporidial cyst and suspect sanguillicolid eggs within lamellae. The cause of the pale colour was not discerned. No ectoparasites were identified either by eye, or under light microscope on the skin of these fish. Samples were collected for histology and microbiology and lesions documented with digital photography.

In simple terms, all the barramundi captured were quite sick, regardless of the presence or extent of skin lesions and redness. The vast majority of the captured barramundi, even those with no apparent external skin abnormalities, displayed tucked up abdomens and all were lethargic when handled. I would not recommend human consumption of any of the barramundi which I sampled for histopathology.

Reddening along ventral midline of barramundi

Enlarged spleen barramundi

Abnormal liver barramundi

Peritoneal effusion barramundi

Tucked up abdomen

Healing eye lesion

Healing skin lesion

Active pale white lesion on gill

Fluid within serosa of liver

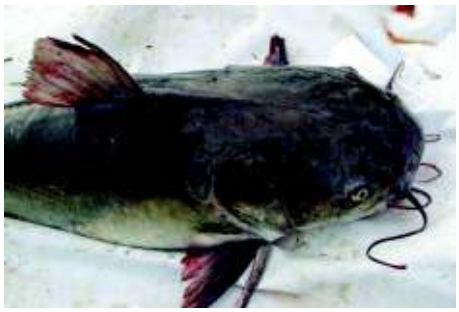
Several bull sharks were captured in the Boyne River also, all of which had skin lesions associated with parasites (suspect skin fluke). The ventral skin of many of the sharks exhibited erythema (redness) soon after they were removed from the water. The areas affected included areas which were remote from the location of contact with the net in which they were captured.

Ulcerated skin lesion associated with parasites bull shark

Skin lesion adjacent gill and red abdomen bull shark

A large fork tailed catfish exhibited erythema in fins soon after removal from the water. Another smaller fork tail catfish was observed to be emaciated. No internal lesions were identified. Samples were

collected for histology and microbiology. The emaciated fish had ingesta present in its gastrointestinal tract, suggesting the fish was sick rather than starving.



Reddened fins, sclera of the eye & rostrum of catfish

The salinity at the Boyne boat ramp close to the river mouth was 31 ppt on 22 January 2012. We then travelled up the river around 14 kilometres where the nets were set adjacent to the highway bridge. The salinity at that location was 31 ppt, indicating near sea level concentrations of salt at the surface with higher salt concentrations registered at greater depths. A further test on 21 January 2012 in the upper Boyne, around 2 kilometres upstream of the Boyne railway bridge was 30 ppt, indicating slightly brackish water.

Hummocky, near Turkey Beach

Samples were captured using gill mesh netting at this location to the south of Gladstone harbour. Two black tip sharks had skin erosions between their dorsal fins associated with suspected skin fluke parasites. Two barramundi were examined which appeared normal from a visual inspection. Eight other sharks were examined and appeared to be externally normal. A 35cm Hawksbill turtle was examined which had a peritoneal effusions and pericardial effusion. The turtle was emaciated but had ingesta in its intestines, indicating that it had been eating. Tissues were collected for pathology.

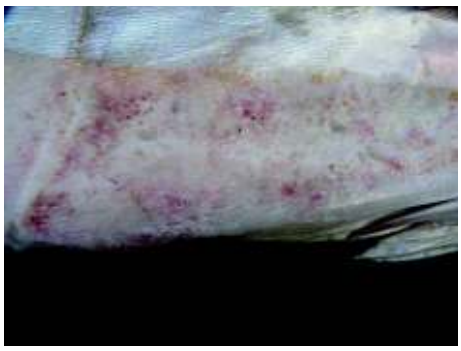
The salinity at Hummocky on 20 January 2012 was 36 ppt, a full marine sea water concentration and indicative of an absence of fresh water influence.

Emaciated Turtle with food still in its intestine

Dredge Spoil Dump Site: east of Gladstone harbour entrance

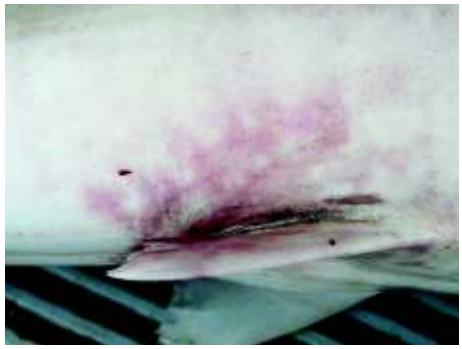
Pelagic fish and sharks were sampled near to the dredge spoil dumping ground. Dredge spoil dumping from a barge was observed nearby during the fish sampling process. A population of 27 queenfish were sampled. All had infestations of a caligad-like organism on the skin around the pectoral/pelvic area. High numbers, 18 in total, also displayed areas of erythema (redness).

Queenfish skin lesions caught on shot near spoil grounds



Queenfish red skin lesion with parasites visible

A weasel shark was recorded with red lesions on its skin soon after removal from the water. Some suspected copepods were attached to the skin of other sampled sharks. Fish were sampled for histopathology and digital images collected of lesions.



Copepods attached to Shark skin adjacent fin - Shark skin lesion present in addition

to skin flukes

Shark skin lesion

Shark copepods

The salinity at this location on 20 January 2012 was 36 ppt, a full marine sea water concentration and indicative of an absence of fresh water influence.

Friends Point, Gladstone inner harbour

At this location mud crabs taken by pot were examined. The mud crabs sampled demonstrated a high incidence of shell disease. 17 of 76 crabs sampled at Friend's Point exhibited mild to severe changes in the shell, ranging from 1-2mm orange coloured spots on the carapace to severe full thickness shell erosions.

Large numbers of suspected barnacles were adherent to the gills of the crabs examined at necropsy. Samples were collected for histological analysis, and digital photographs of all crab lesions recorded.

Only one small cod was captured in the pots which were examined. The absence of small fish in crab pots is unusual.

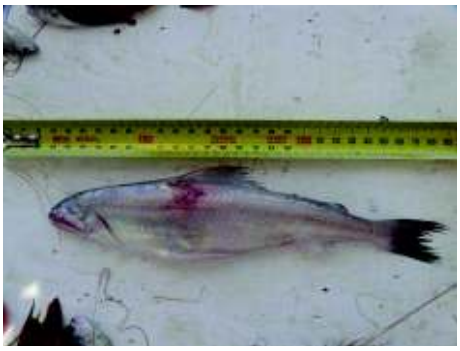
The salinity at this location on 23 January 2012 was 36 ppt, indicating near full strength sea water with no fresh water influence.

Carapace and exoskeleton lesions of mudcrabs

Shipping Channel, Gladstone inner and outer harbour

3 trawl net shots were undertaken in the harbour. A small amount of prawn and bycatch fish only was captured, and was then examined. The rate of lesions on the examined prawn and small fish was less than 1%.

Pleopods eroded from Banana Prawn taken from Harbour trawl.



Ghost grinner skin lesion

(from trawl)

Sole reddening of anterior end

of fish

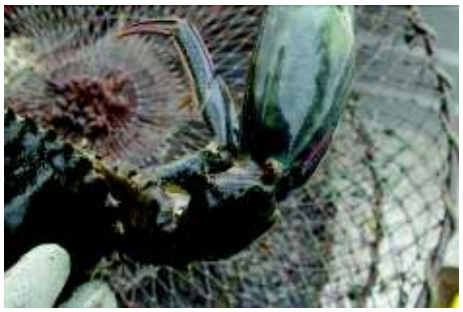
Two trawl shots were then undertaken adjacent the dredge dump site. No scallop shell was captured. Despite the trawl nets being down for approximately 45 mintues, less than 1kg of total catch was able to be achieved, mostly squid. No benthic fish were able to be captured. I observed that there was very little aquatic biota in the dredge spoil dump site area.

The salinity at this location on 20 January 2012 was 37 ppt on all 5 trawl shots, indicating full strength sea water with no fresh water influence.

Colosseum Inlet, south of Gladstone harbour

Crab catch was examined from 13 pots in the Colosseum. A high incidence of shell disease was observed. 12 of 33 crabs showed signs of lesions on the carapace which ranged in severity from 1-2mm orange discolourations of shell, to full thickness carapace erosions. Digital images of lesions were collected and tissue samples preserved for pathology.

White mangrove die back



Orange rust coloured lesions on mudcrab

Ulcer on claw of mudcrab

Ulcer on claw of mudcrab

Carapace erosion mudcrab

Carapace erosion mudcrab

Carapace erosion mudcrab

Carapace erosion mudcrab

Mangrove die back

I observed that a low quantity of juvenile crabs were present. Only 2 of the 33 were under 14cm carapace width. I also observed that there were few fish found in the pots. Only 2 fish (1 bream and 1 toadfish) were observed in the 13 pots which were pulled.

There are clear signs of mangrove dieback, most obvious in white mangroves, with some dieback also in green mangrove trees in the Colosseum Inlet area.

The salinity at this location on 24 January 2012 was 35.7 ppt, indicating near full strength sea water with very little fresh water influence.

7 Mile, Rodd's Bay /Turkey Beach area, south of Gladstone harbour

Fish species were sampled in this area using net and crab pot apparatus.

Crabs were sampled from 17 pots in the Turkey Beach area of Rodds Bay. 3 of 45 crabs were observed with 1 to 3 mm orange rust spots or ulcer through the carapace.

45 crabs were captured in total, but only 4 were juvenile crabs less than 14 cm in width. 5 small bream and 1 estuary cod were found in total in the 17 pots.

One net shot was undertaken in the Moondoolan area using 2 inch mesh. 1 of 45 sand whiting was observed to have a skin lesion. 5 of 150 mullet were observed to have skin lesions. 2 juvenile queen fish were taken and appeared externally normal. 1 large white shovel nose shark was taken with 20 external parasites, suspect caligad, sampled. Photographs were taken and samples collected for pathology.

The salinity at this location on 25 January 2012 was 28.2 ppt, indicating minor fresh water influence, probably as a result of heavy overnight rain.

Shell ulcer on mudcrab

Red lesion on mullet

Red lesion around vent of mullet

Red lesion on shoulder of mullet

Red lesion around the vent of mullet

Red lesion on flank of whiting

Preliminary Observations

1. In my experience the presentation of this collection of symptoms in wild fish, coincidentally in multiple sites, is unusual. Wild fish generally do not suffer from disease outbreaks in the absence of an environmental trigger, such as a pollution event, or the introduction of an exotic pathogen.

2. The presence of ongoing disease in multiple species suggests the causative factor(s) are still active. The duration of time which has elapsed between the observation of lesions on a range of aquatic biota between 20/01/12 – 25/01/12 by myself and the flood event in late 2010 suggests that other factors are contributing to disease expression today, rather than the flood. The salinity of sampling locations also suggests the freshwater from the flood in late 2010 is no longer present, and is unlikely to be continuing to contribute to the ongoing expression of disease in the aquatic animals.

3. My findings of lesions on a very high percentage of Queenfish out in an ocean area adjacent to the Gladstone dredge spoil dump site would also suggest that the 2010 flood is unlikely to be involved in their causation.

4. Further laboratory sampling will assist in elucidating the role of infectious and environmental stress factors in causation of the abnormalities of fish, crab and prawn tissue in Gladstone waterways.

APPENDIX 2: FFVS Interim report 002

Future Fisheries
VETERINARY SERVICE Pty Ltd
ABN: 520 830 951 17
PO Box 7142 East Ballina NSW 2478
Phone 0437 492 863
matty.landos@gmail.com

Project Update 002: **Date: 16 April 2012**

Dr Matt Landos BVSc(HonsI)MANZCVS(Aquatic An Health)

Supported by Gladstone Fishing Research Fund

www.gladstonefishingresearchfund.org.au

Contents

Background

Gladstone Fishing Research Fund is a public charity which has funded FFVS to commence an investigation of the presence and cause of reported aquatic animal health problems in the Gladstone harbour and nearshore waters. Dr Matt Landos and Dr Ben Diggles travelled to Gladstone on 18-25 January, 2012 (findings in Update 001).

The findings of a second trip undertaking a prevalence study on shell lesions in mudcrabs was undertaken on 15-16 February, 2012 and a third trip undertaking sampling of fish, prawns and crabs around Gladstone Harbour on 22-29 February 2012.

FFVS also visited Gladstone Area Water Board Hatchery and Office and requested data from hatchery and office on water flows from Awoonga Dam and testing undertaken at the hatchery, in addition to materials supplied to the Panel. These documents have been supplied and will be considered in the final report as data contributing to the conclusions of the investigation. The data in these documents do not support the DERM/DEEDI/GPC hypothesis that freshwater was the cause of sickness in the barramundi, or all of the other aquatic animals in Gladstone Harbour, or on the oceanic side of Facing Island.

Sampling for the second trip was undertaken at:

-Oceanic side of Facing Island- queenfish, shovelnose shark, bronze whaler shark, blubberlip bream, blacktip shark, black jew, coral

-Sable Chief Rocks- sampling blacktip shark, weasel shark, scallop hammerhead, bronze whaler shark, slatey bream, batfish, school shark, coral and shark

-Upper Boyne River- sampling barramundi

-Harbour trawl- banana prawn, tannum tiger prawn, soapie jewfish, herring, grunter, stingray, sole

-Gladstone Harbour- Southern end Curtis Island- sampling barramundi

-7 Mile estuary- crab, oyster cracker, shovelnose shark, shark, barramundi

Dr Matt Landos met with the UNESCO team during their visit to Gladstone, and passed on the preliminary findings to the team. The presentation provided to UNESCO has been uploaded to the website www.gladstonefishingresearchfund.org.au

Methods

Captured animals were examined for the presence or absence of external lesions such as ulcers on their skin, a tucked up abdomen and damaged gills. Where animals were observed with lesions, typically affected animals were necropsied, and sampled for histological examination, with some bacterial culture plates set up to examine for the presence of systemic bacterial infections.

Observations of fishers during the outbreak were also recorded.

Further sampling

A further trip is planned to collect control samples from a site remote to the dredging in the next week. In Gladstone further sampling will focus on scallops. With the final project report expected to be complete around mid-late May depending on the return of samples from the laboratory.

Publication of results

The results of laboratory submissions received to date will be attached to this report as appendices and will be uploaded to the website: www.gladstonefishingresearchfund.org.au

Crab shell disease prevalence survey

Utilising the Grading scale for lesion severity, developed by Leonie Andersen in her PhD (2003) on mudcrab shell disease, FFVS undertook a wide screening of mudcrab catches in the Gladstone region.

Grade 1 lesion: Non-perforated <5mm diameter discolouration of shell (rust spot)

Grade 2 lesion: Non-perforated >5mm diameter discolouration of shell (rust spot)

Grade 3 lesion: Perforated partially or fully < 5mm diameter

Grade 4 lesion: Perforated partially or fully > 5mm < 20mm diameter

Grade 5 lesion: Perforated partially or fully > 20mm diameter

Sites sampled included:

-Narrows/ Deception/ Mosquito Creek/ Badgers Creek

-Colosseum

- East Harbour sites (Enfield Creek, Barge landing, Nutmeg Creek)

- Western Harbour (Graham's Creek, Flying fox river, adjacent dredges)

-Comparison site (Stanage Bay- collected by commercial fishers delivered to Gladstone Fish Market)- further sampling in coming weeks.

Some preliminary data mapped to show locations- further data yet to be loaded onto maps.

Summary of findings

A broad trend was observed: The further away from the Western Basin dredging activity, the lower the prevalence of observed shell lesions in mudcrabs.

All sites around Gladstone recorded a higher level than a comparison site, which is remote to Gladstone and the dredging activity, but was in the area affected by substantial rainfall from Cyclone Yasi.

The data suggests that freshwater inflows do not appear to be a causal factor in mudcrab shell disease. Leonie Andersen associated shell disease with elevated exposures to metals including copper and zinc. The type of lesions being observed are morphologically consistent with those described in her PhD. As such, it is likely that a similar cause is likely to be behind the current elevation in shell disease rates.

Dr Andersen found 14.3% of Gladstone crabs sampled had shell disease. From the results detailed below, the rates of shell disease are now substantially higher at 43.8% across 623 crabs sampled.

A suite of samples for histopathology are still pending at the laboratory, where more detail on the health of other organs will also be determined. Increased rates of gill parasitism were observed from initial sampling in July (Interim Report 001).

DERM monitoring has identified elevated levels of some metals in harbour water including aluminium and copper. Sampling undertaken by Gladstone Fish Market has also identified elevations of arsenic in some water samples collected since the commencement of dredging.

Published literature has identified that dredging can increase the bioavailability of metals in sediment, through resuspension, and activation through oxidation of acid-sulfate sediments.

The Gladstone Fishing Research Fund is seeking further funds to undertake metals testing on mudcrabs.

Narrows/ Deception/ Mosquito Creek/ Badgers Creek

Total number crabs	70
Male	47
Female	23
% no shell lesions	50%
Average lesion grade	2.1
Grade 1	27.1%
Grade 2	7.1%
Grade 3	4.3%
Grade 4	5.7%
Grade 5	5.7%

Colosseum

Total number crabs	139
Male	83
Female	56

Absence of shell lesions	66.2%
Average lesion grade	2.15
Grade 1	13.7%
Grade 2	12.2%
Grade 3	0.7%
Grade 4	3.6%
Grade 5	3.6%

East Harbour sites (Enfield Creek, Barge landing, Nutmeg Creek)

Total number crabs	122
Male	59
Female	63
Absence of shell lesions	39.3%
Average lesion grade	1.71
Grade 1	38.5%
Grade 2	10.7%
Grade 3	5.7%
Grade 4	3.3%
Grade 5	2.5%

Western Harbour (Graham's Creek, Flying fox river, adjacent dredges)

Total number crabs	87
Male	43
Female	44
Absence of shell lesions	56.32%
Average lesion grade	1.55
Grade 1	29.9%

Grade 2	6.9%
Grade 3	3.5%
Grade 4	3.5%
Grade 5	0.0%

*Comparison site- Stanage Bay**

Total number crabs	131
Male	131
Female	0 (commercial catch only allowed to keep male crabs) crabs assessed post-transport to Gladstone Fish Market
Absence of shell lesions	82.4%
Average lesion grade	2.87
Grade 1	3.1%
Grade 2	2.3%
Grade 3	6.9%
Grade 4	4.6%
Grade 5	0.8%
Crabs under 15cm	N/A as commercial harvest

* Further sampling planned to match other sites- data will be added once available.

7 Mile Estuary (sampled 29/2/12)

Total number crabs	135
Absence of shell lesions	67.4%
Average lesion grade	2.18
Grade 1	16.3%
Grade 2	3.7%
Grade 3	5.1%

Grade 4	5.1%
Grade 5	2.2%

Fish sampling from February 2012

Ocean side of Facing Island & Sable Chief Rocks- 22/2/12

This location is remote from the harbour and the dredges. It is however in direct line of sight of the dredge spoil dumping ground approximately 5 nautical miles away. It is a full marine oceanic site, with associated coral reef.

A shot of net was run through this area and retrieved at dawn on 22/2/12. A film crew was on board to record the event.

The coral in this location was surveyed by other research groups after the 2010-11 flood and was reported not to have been affected by the flood water. Water clarity was poor at the site.

Pieces of coral which were caught in the net appeared to be sick (images below). Samples have been sent to a laboratory for further analysis. The images were sent to a coral expert at AIMS who suggested it may be white syndrome or some kind of protozoal disease, however further investigations were required.

Two blubberlip bream were caught. One had severe changes to both eyes, which appeared cloudy. Both had redness in their skin in between the scales. Both had gross marked intestinal parasitism on necropsy. Samples were collected for histopathology.

One black jew was captured which had unusual redness around its eyes.

Many species of sharks were caught, most of which had skin reddening apparent. Some had full skin thickness skin ulcers.

Six queenfish were caught, none had external skin lesions, however one had gross changes to the gill indicative of gill disease and marked gross intestinal parasitism.

One shovelnose shark was captured which had significant reddening around its claspers (reproductive organs), the animal also had marked internal parasitism, with free fluid in its abdomen. It was sampled for histopathology.

The two shots were remarkable for the high proportion of grossly diseased fish which were captured. Given the location remote from the harbour, and the timing of sampling some 13.5 months after the flood, it is my professional opinion that the disease being observed in this fish population has nothing to do with the flood event at all. Further, the absence of barramundi at this location suggests that disease in this fish population has nothing to do with barramundi coming over the wall of Awoonga dam either.

The association with exposure to dumped dredge spoil is under further investigation. The reported changes to water turbidity are consistent with the movement of dredge spoil to expose the animals at this site north of the dumping ground. The temporal association is consistent with exposure to dredge spoil. The level of parasitism across a range of species is suggestive of broad immunosuppression, which can be caused by exposure to sub-optimal water quality. The level of disease observed in some animals would be expected to inhibit feeding and reproductive behaviour.

All images were sent to DEEDI/DERM. Recommendations were provided to DERM to investigate the apparent coral sickness/mortality event at the site.

Sick Coral

Diseased gills blubberlip bream

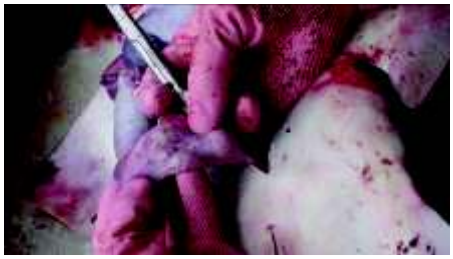
Reddened lips blubberlip bream

Diseased eye blubberlip bream

Reddening around eye Black jew

Red skin hammerhead

Full thickness skin ulcer bronze whaler



Granulomas in intestine of shovelnose shark

Red skin shark

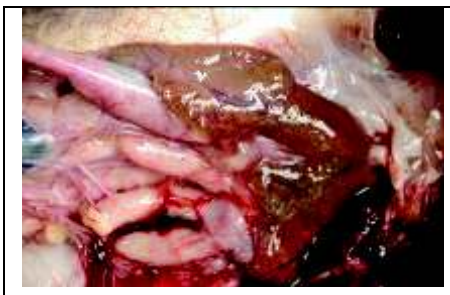
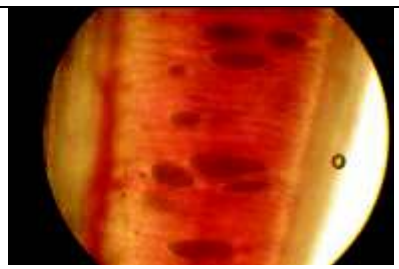
Upper Boyne River- 24/2/12

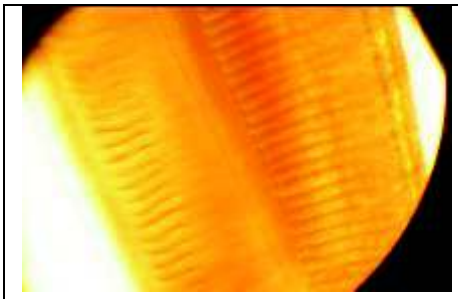
Several shots were made in the Boyne river, upstream from the Benaraby bridge. One 45-50cm barramundi was captured which appeared grossly externally normal. The gills were examined microscopically and found to be normal. A larger 90cm fish was also captured. It had a large ulcer on its side (see photo below). The lesion appeared superficial. There were no deeper muscle lesions to suggest any kind of trauma. A film crew was on board to record the event.

The fish had severe gill disease observed under light microscopy associated with parasitic cysts (likely microsporidial/myxosporidial) and suspect sanguilicolid eggs (see photo below). Similar to the diseased fish captured in January this fish had free fluid in its abdomen, and oedema on its liver, demonstrating that the disease conditions have not resolved in the fish populations.

The fish was captured in full freshwater, as recent rain had seen some over-topping of the Awoonga spillway. No external parasites were evident on the skin of the fish.

The small barramundi had a tag in it, the tag information is being retrieved to document where it was caught and tagged.

		
Oedema on barramundi liver	Parasitic cysts in gill under microscope 100x magnification	Suspect sanguilicolid eggs in barramundi

		
Normal gill in small barramundi	Severe skin ulcer barramundi	Close-up skin ulcer barramundi

Bacterial cultures of head kidney and liver had no significant growth after 96 hours of incubation.

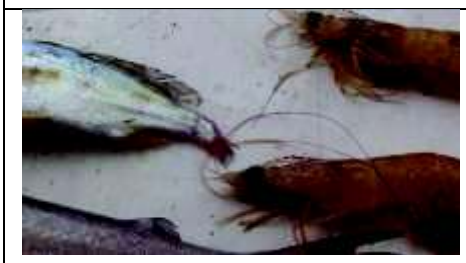
Harbour trawl- 27/2/12

Several harbour trawl shots were undertaken. The volume of catch was well below that reported at a similar time in the previous season. With over 2 hours of trawling for less than 10kg of banana prawns and just over 10kg of tannum tiger prawns. The prevalence of fish or prawns exhibiting external signs of disease was low (<1%), similar to the previous trip. Fish and prawns with signs of disease were necropsied and preserved for histopathology examinations.

A small sole ~ 17cm was caught, which had red spots over the surface of the underside of its body and throughout its fins. It was sampled for histopathology.

Lesions on other biota are documented in photographs below.

Turbidity had improved slightly from the previous trip to this area in January. It was noted that improvements in turbidity were associated with the shut-down and maintenance of the large cutter suction dredges. Plumes of sediment were however clearly visible emanating from the backhoe dredge.

		
Eroded red tail fin of grinner	Shell disease on banana prawns	Reddened stingray vent
		
Eroded scaienid tail fin	Reddened ray eye	Internal eye lesions

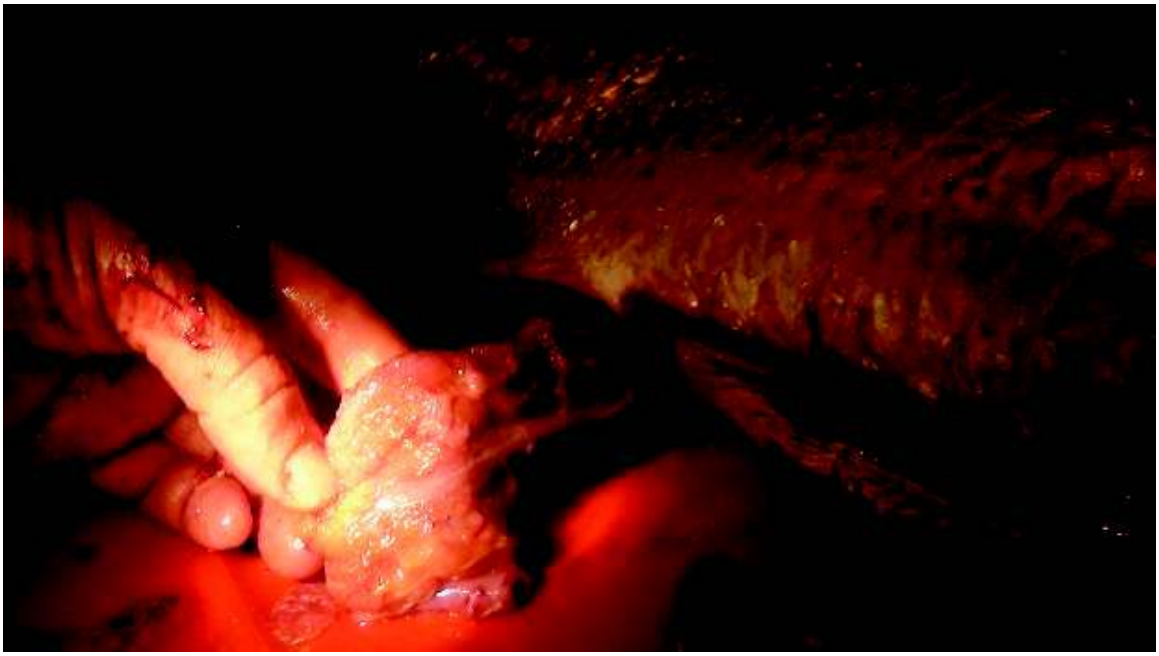
Elevated turbidity evident adjacent “big Boss” dredging operations.

Pile driving impulses could be felt through the deck of the prawn trawler when two nautical miles from the pile driving on Curtis Island. It is likely that noise pollution from increased shipping, pile driving and dredging is impacting on aquatic animals in a negative fashion.

Gladstone harbour- southern end of Curtis Island- 28/2/12

Two ring shots were done to capture 3 barramundi at the southern end of Curtis Island and on an adjacent island. Two of the three fish had ingesta in their stomach.

One had abnormal proliferative red intestinal lining. Another had white patches on the gills similar to the diseased fish captured in the Boyne river. The fish with white gills had a tucked up abdomen. Large numbers of recently dead oysters have been observed around the Calliope boat ramp also.



Proliferative intestinal mucosa of barramundi

7 mile estuary-crabbing 28/2/12

		
Rust spots on shell	Rust spots and shell ulceration	Severe shell ulceration/deformity

		
Marked rust spots		

135 crabs were examined from pots across the 7 Mile estuary.

The scoring system from Leonie Andersen's PhD was used to grade the severity of lesions in the examined crabs.

67.4% were normal, without any lesions at all.

16.3% had grade 1 lesions: Non-perforated <5mm diameter

3.7% had grade 2 lesions: Non-perforated >5mm diameter

5.1% had grade 3 lesions: Perforated partially or fully < 5mm diameter

5.1% had grade 4 lesions: Perforated partially or fully > 5mm < 20mm diameter

2.2% had grade 5 lesions: Perforated partially or fully > 20mm diameter

No material was collected for pathology or toxicology from this sampling exercise.

Only one small cod was captured in the pots which were examined. The absence of small fish in crab pots is unusual.

Only 5 crabs under 15cm were observed from the pulling of all pots on the day. This is reported to be highly unusual, as in a normal season small crabs are frequently found on the crab ropes and outside of the pots.

7 mile- fishing gill mesh- 29/2/12

Two net shots were undertaken in the estuary with the fishers sleeping on the boat adjacent the nets.

Multiple diseased fish were captured. Prominently three of three oyster crackers had bilateral pectoral fin erosions. Two of three fish had 2-3 caligid parasites visible on the skin. One had prominent intestinal nematode infestation. Each fish was necropsied and samples were preserved for pathology. Three ~75cm catfish had up to 8 visible caligid parasites on their skin. One shark had

ulceration between the dorsal fins, associated with some fluke-like parasites. One barramundi was captured which had white patches on its gill. It had some ingesta in its stomach. This fish was sampled for pathology. Two other barramundi captured had no gross external lesions. Two large whitespot shovelnose sharks were captured, and had no gross external signs of disease. One eagle ray was captured, it had no visible external lesions. Two threadfin salmon had no visible external lesions.

The lesions on the oyster crackers are not a normal finding on wild fish. They are inconsistent with the hypothesis that the problems in the fishery are due to the Dec10-Jan 11 flood, and inconsistent with the causation proposed by DERM/DEEDI that the large number of barramundi which came over the wall were the cause. The 7 mile estuary is more than 23.5km from the mouth of the Boyne River. The salinity was 34.5ppt which is close to full strength marine seawater.

Fin lesions are common on fish which are suffering from a stressor. The intermittent exposures to fluxes of dredging spoil from the dumping ground moving with the tide and winds into this estuary are a plausible stressor. No other stress factor was identified in this estuary.

The multi-species, multi-parasite load pattern observed in these samples is consistent with that observed in all other sampling around the Gladstone area. It confirms that diseases are ongoing in the fish population. It is common for fish which are suffering from sub-lethal diseases to suffer from impaired reproductive performance. The potential impact on recruitment to the local and regional fish population is expected to be significant. Gladstone harbour had been recognised for its extensive nursery ground areas.

		
Reddening and erosion of pectoral fin- oyster cracker 1	Reddening and swelling of pectoral fin- oyster cracker 2	Reddening and severe erosion of pectoral fin- oyster cracker 3
		
White patches on barramundi gills	Skin reddening shark	Skin ulceration shark with intra-lesion parasites

Some key histological findings

It is clear from the range of histological reports attached as appendices that the examination of fish externally by eye, is insufficient to classify them as healthy- which is the approach used in the DERM report.

There are numerous internal disease processes going on, many associated with parasites. Whilst parasites can be normally found in wild fish, the intensity of infections observed, the multi-species spectrum observed, and the accompanying tissue damage are abnormal in wild fish in my opinion, and are likely to result in decreased health/reproductive outcomes for the affected fish/aquatic animals.

Hawksbill turtle

The gross observations at necropsy of fluid in the pericardium and ascites in the abdominal cavity were consistent with the histological findings of heart disease. The turtle was likely to have high loads of parasites(flukes) in its heart, associated with inflammation to the heart muscle and other body tissues.

Parasite loads can be elevated for a range of reasons- such as increased population density, or immune system compromise driven by increased levels of environmental stress such as poor water quality, inadequate nutrition or exposure to toxins, like heavy metals.

Barramundi

Barramundi have been sampled from the Boyne River, adjacent Hummocky Island, 7 mile estuary and at the Southern end of Curtis Island. The fish have been suffering from a broad range of parasitic conditions. The intensity of parasitism, and spectrum of organisms are suggestive of an immunocompromised host.

Microsporidian, myxosporidian parasitic cysts have been documented in the gills. Blood fluke eggs (suspect Sanguilicolid) have been documented in the gills. Fluke parasites have been identified on the external surface of the gills.

Rates of gill disease in sampled fish were very high. The levels of disease would be expected in my opinion to impair gill function and potentially physiologically stress the fish. This may result in inappetence, failure to grow, reduced or nil reproductive performance.

Further compounding the issues observed in the gills were moderate heart disease (inflammation), liver disease (hepatitis) with a erosive skin condition (dermatitis).

The cause of the dermatitis was not apparent in the barramundi examined.

Dermatitis is a very common lesion across all the biota examined. This epidemiological pattern is suggestive of a water-borne irritant. As no infectious causes have been identified for this lesion- non-infectious causes need to be considered, such as the impact of exposure to dredge spoil and toxicants. Freshwater does not cause the observed effects on barramundi skin. The timing of the presentation of the lesions, that is Jan-Feb 2012) does not fit with any trauma from moving out of Awoonga dam down the spillway in Dec 2010-Jan 2011. Fish skin heals rapidly in good water quality from trauma (within weeks).

The gut of the barramundi was frequently inflamed with large numbers of parasitic larvae migrating through the gut wall. This finding was surprisingly common across all fish sampled. Whilst it is common for marine fish to have some intestinal parasite burdens, the extent and intensity across all examined fish is remarkable, and consistent with some form of common immunosuppression.

The timing of onset of reported sick barramundi in the Boyne River, coincides with the cessation of water coming over the Awoonga Dam spillway. Marine parasites (Neobenedenia) were observed on some sick fish at that time in mid 2011, demonstrating that saltwater had penetrated up the Boyne River from the Harbour. Dredging had commenced in the harbour at this time, and turbidity increased substantially, as documented by the Gladstone Area Water Board hatchery. Sickness coincided with exposure to this marine water and whatever was contained in it (algae, suspended sediment, bacteria, toxins, etc).

Barramundi normally live in both full salt and full freshwater, and do not routinely get sick when transferring from fresh to salt, or visa versa. Indeed in aquaculture, freshwater bathes are used to treat fish with marine parasites, and salt is used to treat fish which have contracted freshwater parasites.

Scallops

Three of four rough shelled scallops were found to be suffering bacterial infections in the mantle, in addition to moderate to severe parasitic infections in other tissues. Such infections are uncommon in healthy bivalve populations and are indicative of animals suffering from some form of stress. The water had been at full marine strength salt concentrations for many months prior to this sampling in January. Such infections are likely to compromise the health of the bivalves leading to increased

mortalities. The loss of benthic bivalve populations can have substantial negative effects on food webs.

Subsequent trawling in February failed to capture any more live scallop inside the harbour. Dredging has been published (Knott, Aulbury, Brown, & Johnston, 2009) to have negative impacts on benthic bivalves, due to exposure to increased suspended sediment, and sediment borne toxicants. Both are likely to have occurred with current activities in Gladstone harbour.

Queenfish

The high levels of caligid parasites observed on fish, leading to grossly visible skin lesions (reddening) are unusual on wild fish. The fish also had severe parasitic infections in the stomach and intestine, with migrating larvae causing a granulomatous inflammatory response and peritonitis. The fish also had a proliferative inflammatory gill response to the presence of a load of suspected sanguilicolid eggs. There was also a protozoal infection in the intestines causing cell death (necrosis) and inflammation.

The coincidence of lesions in queenfish, and other fish sampled, are suggestive of immunosuppression. All fish sampled have an ongoing risk factor in common, as at January-February 2012: exposure to dredge spoil resuspended sediment.

The freshwater influx has long passed and is not expected to be playing any role in the current range of conditions the fish are observed to be suffering from.

Prawns

The prawns were found to be suffering from microscopic parasitic infections of flukes on their gills, and flukes in their muscle. The grossly visible shell lesions were corroborated histologically, with ulcerations of shell described, and inflamed and swollen muscle around the lesions. Some had bacteria associated with the ulcerated areas. The pathologist noted sediment accumulations in the exoskeleton and on gills. One prawn had an inflamed intestine also.

Preliminary Observations

Gross visual observations of fish and crabs indicate there are significant, ongoing disease processes in fish populations in the Gladstone region.

The histological assessment indicates that using externally visual signs alone under-predict the extent and severity of disease in the sampled animals.

The timing of collections (Jan-Feb 2012) and type of pathological changes do not support the hypothesis that freshwater was the cause of the ongoing sickness in aquatic animals.

The diversity of species affected do not support the hypothesis that the entry of barramundi from Awoonga dam to Gladstone Harbour is a sufficient cause to explain the extent and types of disease recorded in all other biota examined.

The spatial location of collections of sick fish, on the oceanic side of facing island, and appearance of sick coral are inconsistent with the explanation that the events observed were caused by a freshwater influx in Dec 2010-Jan 2011.

All observed signs in fish, can be explained by immunosuppression secondary to a water based stress factor, which has exposed (and likely continues to expose) all animals sampled. Dredging resuspended sediment and dumped dredge spoil can plausibly have exposed all areas which have been sampled within the project sampling to date, from Turkey Beach in South, to Narrows in the North, and the ocean side of Facing Island to the East, depending on prevailing wind, tide and ocean currents. Noise from dredging, ships and pile driving is also likely to have exposed much of the harbour based biota.

Appendix 1: Turtle pathology report

PATHOLOGY REPORT

School of Veterinary and Biomedical Sciences
James Cook University
Post Office
James Cook University
TOWNSVILLE QLD 4811

Date Received:	10-2-12		Specimen No.:	12-175			
Submitted by:	Dr Ellen Ariel		Date Reported:				
Owner:	Matt Landos		Address:	School of Veterinary & Biomedical Sciences James Cook University Townsville QLD 4811			
Animal name:	Hawksbill DERM #?						
Species:	*Eretmochelys*	Breed: imbricata	Hawksbill Turtle	Sex:		Age:	Juv

Clinical history: ***"Weak turtle captured during fish health sampling at Hummocky Island, south of Gladstone.?"***

Specimen submitted: Lung, heart, intestine, adipose tissue, liver

Histopathological examination:
Liver: Diffuse congestion distends sinusoids. Within portal areas there are pigmented trematode eggs, sometimes present within adjacent hepatic parenchyma, or within small vessels or periportal connective tissue or in clusters, surrounded by fibrous connective tissue and chronic inflammation with multinucleated giant cells.
Adipose tissue: Irregular tissue section with serous atrophy, numerous melanomacrophages and oedema
Lung: Multifocally, often associated with terminal bronchioles, there are trematode eggs with associated chronic inflammation (multinucleated giant cells). The peripheral pleura displays serous atrophy.
Heart: Multiple heart portions with adult trematodes within the lumen, as well as egg granulomas associated with the tissue parenchyma. Several sections of valve, characterized by hyperplastic endocardial mesenchymal tissue with luminal surface inflammation, consisting of heterophils, lymphocytes and macrophages. The luminal surface inflammation results in an irregular, inflamed, lumenal outline.
Intestine: Multiple sections. All sections have multifocal trematode eggs, which may display one or more of the following: Subserosal granuloma with multinucleate giant cells and fibrous connective tissue surrounding a cluster of eggs; eggs within the lamina propria, or villi; eggs within vessels and eggs within the subserosa.

Diagnosis: Heart: Endocarditis, chronic-ongoing, with endocardial hyperplasia, focally extensive, severe
Heart: Trematodes, adults and eggs, within ventricular spaces as well as within the cardiac muscle, morphologically consistent with Spirorchids
Lung and intestine: Granulomatous inflammation, multifocal, with trematode eggs, marked

Comments: The number and density of trematode eggs within the submitted tissues suggests a very high parasite burden. The lesions noted within the heart likely contributed to an inability to sustain stress, or may have been the primary cause of death. Interestingly, some trematode eggs within small vessels were not observed to promote fibrin/coagulation, although in clusters. This may be as a result of the sectioning/temporal relationship, or may reflect an adaptation of the parasite to ensure the continued good health of the host.

Dr Linda Johnson DVM MS MPH Diplomate ACVP
Dr Jenni Scott BVMS (Hons) PhD MACVSc
Dr Liz Parker BVSc MACVSc MVPHMgt

[B2 - 6281 57162 0102]

Appendix 2: Pathology Report 1- 12-4021148

DR MATT LANDOS LANDOS, GLADSTONE PROJECT FISH
FUTURE FISHERIES VETERINARY SERVICE PO BOX 364 LENNOX HEAD NSW 2478
Collected:03/02/12 **Lab Ref:**12-4021148
Collect Time:10:15 **Your Ref:**
Printed:06/03/12
BRI 2012 08116

Specimen: MULTIPLE FISH SPECIMENS
ADDITIONAL REPORT - 21/02/12

Macroscopic: (DO/EM)

The specimen consists of thirteen fish specimens.

A (near spoil ground queen fish 1): The specimen consists of multiple pieces of internal organs and skin. RS taken through each of the tissues and RS are taken through each of the samples in blocks A to E.

B (near spoil ground queen fish 2): The specimen consists of multiple pieces of tissue. RS taken blocks F to J with block J containing a cross section of the eye.

C (near spoil ground hummocky 1 queen fish 3): The specimen consists of multiple pieces of tissue. RS taken blocks K to N. Block O contains a sample through fish eye.

D (near spoil ground hummocky 1 queen fish 4): The specimen consists of multiple pieces of tissue. RS taken blocks P to S with one section through the eye in block T.

E (near spoil ground hummocky 1 queen fish 5): The specimen consists of multiple pieces of tissue. RS taken blocks U to W.

F (hummocky 1 queen fish 6): The specimen consists of multiple pieces of tissue. RS taken blocks X to AA with one section through the eye in block AB.

G (hummocky 1 near spoil ground shark 1): The specimen consists of multiple pieces of tissue. RS taken blocks AC to AF.

H (hummocky 1 shark reece 2): The specimen consists of multiple pieces of tissue. RS taken blocks AG to AJ. A section through the eyeball in block AK.

T (bridge boyne barramundi 1): The specimen consists of multiple pieces of tissue. RS taken blocks AL to AQ.

U (bridge born barramundi 2): The specimen consists of multiple pieces of tissue. RS taken blocks AR to AW.

V (bridge boyne barramundi 3): The specimen consists of multiple pieces of tissue. RS taken blocks AX to BA.

W (bridge boyne barramundi 4): The specimen consists of multiple pieces of tissue. RS taken blocks BB to BF.

X (bridge boyne barramundi 5): The specimen consists of multiple pieces of tissue. RS taken blocks BG to BL. Two eyes are submitted and RS are taken from each in blocks BM and BN.

Microscopic:

A (near spoil ground queen fish 1): There is mild to moderate renal tubular proteinosis. No glomerular lesions are identified. Small aggregates of mononuclear leucocytes are scattered throughout the liver in a random distribution. A few inactive granulomas are scattered throughout the small intestinal submucosa and peritoneum. These are associated with marked eosinophilic granular cell infiltrate and few mononuclear leucocytes. One granuloma contains profiles of round structures suggestive of degenerate nematode larvae.

Low to moderate numbers of coccidia-like organisms are associated with the surface of enterocytes. Mild epithelial degeneration and moderate non-suppurative mucosal infiltrate is associated with their presence. Mature nematode larvae are encysted within the peritoneum of the pyloric cecae. These are 80 um diameter on average, have coelomyarian-polymyarian musculature, a pseudoceolum, well developed digestive tract, lateral
cors, and an ornate cuticle with longitudinal ridges. They are associated with variable mixed leucocyte infiltrate. Rare similar appearing adult nematodes are within the lumen of cecae. These have thin shelled morulated eggs. There is heavy submucosal infiltrate of primarily eosinophilic granular cells affecting the stomach. A few small granulomas are throughout but no infectious agents are seen.
There are segmental foci of gill lamellar fusion due to epithelial cell hyperplasia and mononuclear leucocyte infiltration. Some of these appear to be centred on profiles of what appear to be embryonated eggs, consistent with sanguinocolid infection. The section of skin and body wall has an area of epidermal erosion. The underlying subcutis has mild mononuclear leucocyte infiltrate. No significant pathology is identified within sections of skeletal muscle, heart, spleen or pancreas.

B (near spoil ground queen fish 2): Rare structures suggestive of sanguinocolid eggs are within the gill lamellae. Renal changes are similar to fish A. Epicardial vessels occasionally have perivascular aggregates of eosinophilic granular cells and mononuclear leucocytes. No epidermis is present within a section of body wall and skin. There are scattered necrotic stromal cells within the superficial dermis, suggestive of true ulceration. No infectious agents are seen. Nematodes similar to those described within the pyloric cecae are within the mesentery associated with intestine and spleen. Intestinal and pyloric cecae changes are similar to those described for fish A. No significant pathology is identified within sections of spleen, pancreas, skeletal muscle or eye.

C (near spoil ground hummocky 1 queen fish 3): The spleen is moderately congested. A discrete segment of epidermal ulceration is present. There is necrosis of underlying tissues, extending into the body wall muscle. No infectious agents are seen. Gastric, intestinal, kidney and mesenteric changes are similar to those described for other fish, however, mesenteric larvae and coccidia are in much greater numbers.
The degree of gastritis is severe and early stage nematode larvae are present within several granulomas.

D (near spoil ground hummocky 1 queen fish 4): The dermis is much more heavily pigmented than that of the previous three fish. Erosion/ulceration is not identified. A single myofibre of the body wall contains encysted cytoplasmic microspora. Clusters of structures consistent with sanguinocolid eggs are within the vessels of multiple filaments. There are a couple of sections of a multicellular organism, 2mm in diameter, which is not clearly associated with any tissue. The organisms is dorsoventrally flattened, has a hyaline tegument/exoskeleton, and multiple appendages with segmentation and striated muscle. It is potentially an arthropod/crustacean (derived from the external surface of the fish?). Kidney, intestinal and pyloric cecae changes are similar to those described for other fish. No significant pathology is identified within sections of spleen, pancreas, heart or eye.

E (near spoil ground hummocky 1 queen fish 5): A few larval cestodes are encysted within the liver. Skin changes are similar to those of fish D. Similar pathology affects the stomach, kidney, pyloric cecae and intestine. No significant pathology is identified within sections of gill, spleen, heart of pancreas.

F (hummocky 1 queen fish 6): A few nematodes are encysted within the spleen. These appear similar to those previously described. Similar pathology affects the pylori cecae, intestine, stomach, kidney and gill. No significant pathology is identified within sections of heart, skin, body wall or eye.

G (hummocky 1 near spoil ground shark 1): Low numbers of an oval to pyriform single cell organism are present within renal tubules. These are up to 6 microns in diameter and have central basophilic round 1 micron in diameter endospores or daughter cells. They are associated with little tissue response. The intestine has heavy overgrowth of mixed bacteria with little associated host response. Several monogenean flukes are associated with the gill surface. There is mild epithelial hyperplasia at the site of their attachment. No significant pathology is identified within sections of liver, stomach, lymphoid organ, skin, body wall or heart.

H (hummocky 1 shark reece 2): A few granulomas are within the submucosa of the spiral valve. There is mild lymphocytic gastritis which is associated with mild epithelial injury, loss, and regeneration. There are rarely associated coccidia-like organisms. In one section of intestine is a focus of severe mural acute inflammation. No significant pathology is identified within sections of lymphoid organ, rectal gland, kidney, heart, brain, or liver.

Deeper sections reveal a few myxozoan cysts within the gill. These are similar to those described for the barramundi.

Deeper sections of the eye reveal an area suggestive of a corneal erosion.

No infectious agents are seen within the mural enteritis with gram, PAS, or ZN stains.

T (bridge boyne barramundi 1): Monogenean flukes are associated with lamellar surfaces. There are many cysts containing Myxozoa at the base of the lamellae; these are 2 microns in length, are tear drop shaped, and have 2 small polar capsules. Hepatic melanomacrophage centres are increased. Migrating larva are within the stomach and intestine wall and there is mild mixed to eosinophilic inflammation. A few granulomas are within the mesentery also. The epidermis is segmentally eroded. In areas it is ulcerated and there is mixed inflammation within the exposed dermis. No infectious agents are seen. No significant pathology is identified within the heart.

No infectious agents are seen when mesenteric granulomas are stained with gram or AN stains.

U (bridge boyne barramundi 2): Melanomacrophage centres are markedly increased in the liver, spleen, and kidney. There is mild non-suppurative inflammation within portal areas. Larvae are insisted in the submucosa of the intestine. These are associated with moderate mixed inflammation. Marked mixed inflammation is within the gastric submucosa. No infectious agents are seen. Gills are similar to fish T. The epidermis is lacking in a section of skin and body wall. The dermis has mild nonsuppurative infiltrate. No infectious agents are seen. No significant pathology is identified in a section of heart.

No fungal agents are seen in the skin when stained with PAS.

V (bridge boyne barramundi 3): Embryonated eggs, consistent with Sanguinicolid eggs, are within the base of lamellae. These are associated with lamellar fusion and mixed inflammation. Liver, kidney and spleen have markedly increased melanomacrophage centres. A few blood fluke eggs are present within the liver and kidney surrounded by small granulomas. Encysted larvae are within the intestinal submucosa with mixed inflammation. The stomach is similar to other Barramundi. Blood fluke eggs are within the myocardium, associated with mixed inflammation.

W (bridge boyne barramundi 4): Monogenean flukes are associated with lamellar surfaces. Hepatic changes are similar to other fish. There is very little pancreatic tissue. Low to moderate numbers of Epi-eimeria are associated with intestinal mucosal surfaces. There is moderate nonsupporative enteritis. The spleen, kidney and liver have markedly increased melanomacrophage centres. There is mild to moderate mural mixed gastritis with occasional degenerate insisted larva. There is an adult nematode within the lumen of the stomach which has a thin cuticle with longitudinal ridges, a pseudo-coelum, and embryonated thick walled eggs. Scattered degenerate blood fluke eggs are throughout the myocardium. There are rare cysts containing Microsporidia within the myocardium. There is severe epidermal ulceration with mixed leucocyte infiltrate in the dermis. There is early fibroplasia and formation of lymphoid follicles and melanomacrophage centres in the dermis. No infectious agents are seen.

No fungal agents are seen in the skin when stained with PAS.

X (bridge boyne barramundi 5): The spleen, kidney and liver have markedly increased melanomacrophage centres. Scattered granulomas are within the mesentery, occasionally bordered by mixed inflammation. They are also in the intestinal submucosa. The stomach is similar to other fish. There is mild myocarditis associated with degenerate blood fluke eggs. Myxozoan cysts are within the gills. Similar severe ulcerative dermatitis is present. No significant pathology is identified within the eye.

No infectious agents are seen when mesenteric granulomas are stained with gram or ZN stain.

DIAGNOSIS:
QUEEN FISH 1-6:
MESENTERIC/PYLORIC CECAE NEMATODIASIS, MARKED, WITH MILD MIXED TO GRANULOMATOUS PERITONITIS (6 FISH).

MILD TO MODERATE NON-SUPPURATIVE AND NECROTISING ENTERITIS WITH INTERLESIONAL COCCIDIA-LIKE ORGANISMS (6 FISH).

MILD PROLIFERATIVE BRANCHITIS WITH INTRALESIONAL SANGUINOCOLID EGGS (4 FISH).

SEVERE EOSINOPHILIC MURAL GASTRITIS WITH INTRALESIONAL NEMATODE LARVAE (4 FISH).

MULTIFOCAL EROSIVE TO ULCERATIVE DERMATITIS, ACUTE (3 FISH).

SHARK 1-2:
MILD RENAL TUBULAR PROTOZOA (1 SHARK)

LAMELLAR DISTOMIASIS WITH MILD PROLIFERATIVE BRANCHITIS (1 SHARK)

MILD LYMPHOCYTIC GASTRITIS WITH COCCIDIA (1 SHARK)

SEVERE FOCAL ACUTE MURAL ENTERITIS, AETIOLOGY UNKNOWN (1 SHARK)

BARRAMUNDI 1-5:
MILD-MARKED MIXED GASTRITIS AND ENTERITIS WITH FOCAL GRAULOMAS AND INTRALESIONAL DEGENERATE LARVAE (5 FISH).

LAMELLAR DISTOMIASIS (3 FISH).

LAMELLAR ENCYSTED MYXOZOA (3 FISH).

EROSIVE TO ULCERATIVE DERMATITIS, AETIOLOGY UNKNOWN (3 FISH).

MILD NON-SUPPURATIVE PORTAL HEPATITIS (3 FISH).

MILD SNAGUINOCOLID BRANCHITIS (1 FISH) AND MODERATE MYOCARDITIS (3 FISH).

MODERATE COCCIDIA-ASSOCIATED ENTERITIS (1 FISH).

COMMENTS:
QUEEN FISH 1-6: These fish are affected with an assortment of parasite-induced lesions and ulcerative dermatitis. The parasite lesions would not seem of severe physiological consequence when considered independently, however, it seems remarkable to observe such a consistent assortment including a number of different types of parasites in a wild population. The cause of the dermatitis is not microscopically apparent. It may at least in part involve ectopic parasites that have been lost during processing (the samples of one fish included a free arthropod/crustacean like organism). Fungal or bacterial causes are unlikely. Other environmental factors which may damage the integument would need to be investigated with methods other than histology.

SHARK 1-2: These sharks did not have a consistent theme of lesions. However, both were affected with various forms of parasitism.

BARRAMUNDI 1-5: Similar to the queen fish, the barramundi have a diverse assortment of parasite associated disease which is somewhat consistent among the sampled fish. I would not expect any one lesion to be fatal

on its own, but in combination with other lesions, I would expect fish health to be poor. The cause of the dermatitis is not apparent.

Reported and checked by Dr Michelle Dennis, Specialist Veterinary Pathologist.
/V00000/D32500/L55100/M43000

03/02/12 LANDOS, GLADSTONE PROJEC FISH 12-4021148

Appendix 3: Pathology report 2: 12-2755432

DR MATT LANDOS LANDOS, GLADSTONE PROJECT FISH
FUTURE FISHERIES VETERINARY SERVICE PO BOX 364 LENNOX HEAD NSW 2478
Collected:21/01/12 **Lab Ref:**12-2755432
Collect Time:00:00 **Your Ref:**
Printed:09/03/12
BRI 2012 11038

Specimen: MULTIPLE FISH TISSUE

Macroscopic: (LC/JS)

1. A. Upper Boyne Barramundi 2: The specimen consists of multiple pieces of pale tan tissue including an eye ranging in size from 50 x 40 x 15mm down to 20 x 5 x 3mm. The eye measures 35 x 25 x 20mm. RSs taken across blocks B to H. Blocks A, I and J include the eye and the scaly part of the fish which will be cut by Dr Brett Stone. Block H submitted for decal prior to routine processing.
1RS of skin in block A. One additional further decalcified section block J. One sagittal section of eye in block I.

2. B. Upper Boyne Barramundi 3: The specimen consists of multiple pieces of tissue, the largest being a scaly light tan piece of tissue measuring 60 x 55 x 20mm down to 10 x 5 x 3mm. There is a dark tan piece of tissue measuring 12 x 8 x 3mm. RSs taken across blocks K to M. The portion of thin and scaly fish will be cut by Dr Brett Stone across Blocks O to Q.
2RS sections of gill in block O. 1RS of skin in block P. One additional section and further decalcified skin in block Q.

3. C. Upper Boyne Bull Shark 4: The specimen consists of multiple pieces of grey to light tan tissue, ranging in size from 50 x 21 x 3mm down to 15 x 10 x 5mm. RS of each piece across Blocks R to X.

4. D. Boyne Upper Bull Shark 5: The specimen consists of multiple pieces of grey to pale tan tissue, ranging in size from 55 x 30 x 3mm down to 10 x 10 x 3mm. RS of each piece across Blocks Y to AF.
NB - blocks Y to AA have been submitted for brief decal.

5. E. Boyne Upper Barramundi 6: The specimen consists of multiple pieces of grey to pale tan tissue, the larger covered with scale and measuring 50 x 40 x 15mm and the smaller measuring 8 x 8 x 3mm. The scaly piece of tissue will be cut by Dr Brett Stone across blocks AG to AH. RS of each piece blocked across Blocks AI to AM.
2RS sections in each block AG and AH.

6. F. Boyne Upper Bull Shark 7: The specimen consists of multiple pieces of grey to pale tan tissue, ranging in size from 150 x 25 x 2mm down to 13 x 10 x 3mm. RSs of each piece blocked across Blocks AO to AS.
NB - Block AR will be submitted for brief decal prior to routine processing.

7. G. Boyne Upper Bull Shark 8: The specimen consists of multiple pieces of grey to pale tan tissue, ranging in size from 50 x 25 x 5mm down to 10 x 7 x 3mm. RSs taken across Blocks AT to AZ.
NB - Blocks AX to AZ will be submitted for brief decal prior to routine processing.

8. H. Boyne Upper Catfish 9: The specimen consists of multiple pieces of grey to pale tan tissue including an eye, ranging in size from 55 x 20 x 20mm down to 15 x 15 x 8mm. The eyeball measures 29 x 20 x 20mm and will be cut by Dr Brett Stone in block BA. RS of each piece of remaining tissue taken across Blocks BB to BH.
NB - Blocks BF to BH will be submitted for brief decal prior to routine processing.
One sagittal section of eye in block BA.

9. I. Boyne Upper Catfish 10: The specimen consists of multiple pieces of grey to pale tan tissue measuring 32 x 15 x 8mm down to 8 x 5 x 3mm. Also included in the tissue is an eye measuring 13 x 10 x 7mm. This will be cut by Dr Brett Stone in block BI. RSs blocked across Blocks BJ to BL.
NB - Blocks BL will be submitted for brief decal prior to routine processing.
Eye bisected in toto block BI.

Blocks A, J, P, Q, AG and AH will require decalcification.

Microscopic:
1. A. Upper Boyne Barramundi 2: There is patchy congestion of the dermis and mild mononuclear leucocyte infiltrate. There is abundant clear space within scale pockets suggestive of oedema. A large cyst containing numerous Microsporidia is within the myocardium. There are scattered small foci of non suppurative to granulomatous myocarditis, occasionally centred on blood fluke eggs. Liver, spleen and kidney have markedly increased melanomacrophage centres. There is increased lymphoid tissue around splenic ellipsoids. The liver has mild portal infiltrate of mononuclear leukocytes. There is mild mural mixed gastritis. No infectious agents are seen. A few granulomas associated with moderate mixed enteritis are within the submucosa of the intestine. Some contain viable larvae. Monogenian flukes are associated with lamellar surfaces. There are a few cysts containing myxozoa at the base of filaments.
Organisms are around 2 microns in length, are elongate to pear shaped, and appear to have two polar capsules. No significant pathology is identified within body wall, eye or corpuscle or stannous.

2. B. Upper Boyne Barramundi 3: Skin changes are similar to those described for the previous Barramundi. There are large pigment deposits within peritoneal fat (ovarian atresia? melanomacrophage centres? Old inactive granulomas?). Many gill lamellae are fused by epithelial hyperplasia, oedema, and moderate mononuclear leucocyte infiltrate. Low numbers of gill flukes are present. Gastrointestinal, liver, kidney, spleen and heart changes are similar to those described for the previous fish. No significant pathology is identified within body wall. Deeper sections confirm the presence of true epidermal erosions.

3. C. Upper Boyne Bull Shark 4: There is segmental artefactual loss of epidermis however there does not appear to be true erosion. There is mild mononuclear infiltrate of the gastric mucosa. Within one section of skin is an area of erosion and hyperplasia in which there are several ciliate protozoa. These are 10 microns in length, oval, have a elongate to bent nucleus and a thin spined wall. A few similar organisms are associated with lamellar surfaces. A fluke is associated with the skin surface of one section. No significant pathology is identified within sections of kidney, body wall, liver, skeletal muscle, spleen, spiral valve or heart.

4. D. Boyne Upper Bull Shark 5: A fluke is associated with the skin surface. There are few foci of mixed leucocyte infiltrate throughout the pancreas. No infectious agents are seen. Low numbers of coccidian and a spironucleus-like organism are associated with the mucosal surface. There is mild mononuclear infiltrate in the mucosa. No significant pathology is identified within sections of body wall, gill, heart, kidney, liver or spleen.

5. E. Boyne Upper Barramundi 6: Gill changes are similar to Barramundi. The dermis has marked mixed leucocyte infiltrate associated with oedema and congestion. Mixed bacteria are clumped within mucous entrapped in folds of skin. There is increased clear space in scale pockets. There are segments of epidermal erosion and ulceration. There is mild to moderate mixed peritonitis associated with melanomacrophage centres and pigment laden granulomas. Heart, stomach, kidney, intestine and spleen changes are similar to those described for the previous Barramundi. No significant pathology is identified within the body wall.

6. F. Boyne Upper Bull Shark 7: There is mild to moderate non-suppurative gastritis associated with low numbers of coccidia-like organisms. Mild mononuclear leucocyte infiltrate is throughout the pancreas; no infectious agents are seen. There is mild splenic perisinusoidal histiocytosis. No significant pathology is identified within sections of kidney, liver, heart or skin.

7. G. Boyne Upper Bull Shark 8: The pancreas is similar to other bull sharks. It is associated with acinar loss. The stomach is similar to other sharks only more severe. Flukes are associated with the skin surface. There are patchy areas of epidermal hyperplasia, erosion, and hypodermal mononuclear leucocyte infiltration. A few aggregates of mononuclear leucocytes are within gill lamellae. No significant pathology is identified within sections of spleen, liver, kidney, heart or intestine.

8. H. Boyne Upper Catfish 9: There is erosion and hyperplasia of the epidermis over the fins. No significant pathology is identified within sections of eye, heart, hepatopancreas, stomach, intestine, kidney, or gill.

9. I. Boyne Upper Catfish 10: Monogenian flukes are embedded within the conjunctiva. There is mild mononuclear leucocyte infiltrate. There is moderate corneal oedema. The skin has segments of epidermal erosion. There are foci of early ulceration with underlying hyperdermal oedema and mild leucocyte infiltrate. The pancreas is mildly atrophic and hepatitic melanomacrophage centres are increased. Ectoparasites including flukes and potentially copepods are associated with the lamellae surfaces. No significant pathology is identified in sections of heart or spleen.

DIAGNOSIS:

BOYNE UPPER BARRAMUNDI 2, 3, 6:
- MILD-MARKED MIXED GASTRITIS AND ENTERITIS WITH FOCAL GRANULOMAS AND INTRALESIONAL DEGENERATE LARVAE (3 FISH).
- LAMELLAR DISTOMIASIS (3 FISH).
- MILD TO MODERATE SANGUINOCOLID MYOCARDITIS (3 FISH).
- EROSIVE TO ULCERATIVE DERMATITIS, AETIOLOGY UNKNOWN (3 FISH).
- LAMELLAR ENCYSTED MYXOZOA (2 FISH).
- MILD NONSUPPURATIVE PORTAL HEPATITIS (2 FISH).

UPPER BOYNE BULL SHARKS
- MILD-MODERATE NONSUPPURATIVE GASTRITIS, WITH INTRALESIONAL COCCIDIA +/- MUCOSO-FLAGELLATES (4 SHARKS).
- MILD TO MODERATE NONSUPPORATIVE PANCREATITIS (3 SHARKS).
- CUTANEOUS DISTOMIASIS (2 SHARKS).
- EROSIVE DERMATITIS (2 SHARKS) WITH INTRALESIONAL CILIATES (1 SHARK).

BOYNE UPPER CATFISH:
- MILD CONJUNCTIVAL DISTOMIASIS (ONE FISH).
- EROSIVE DERMATITIS (TWO FISH).
- LAMELLAR DISTOMIASIS (ONE FISH).

COMMENTS:
BOYNE UPPER BARRAMUNDI 2, 3, 6:
The findings in this group of barramundi are similar to those described in a previous report (lab number 12-4021148).

UPPER BOYNE BULL SHARKS:
In a theme similar to the barramundi, the sharks have consistent skin erosions and disease induced by parasites, of particular concern being the gastritis. The cause of the pancreatitis is not apparent. I suspect infectious aetiology, potentially viral, based on the nature of inflammation. None of the parasitic infections were very severe, so it is difficult to determine the significance without a baseline for this population (although it seems remarkable to find consistent changes among the study population).

BOYNE UPPER CATFISH: These fish also had skin erosions and ectoparasitism, although they seemed to have a lesser degree of internal parasitism.

Reported and checked by Michelle Dennis, Specialist Veterinary Pathologist

21/01/12 LANDOS, GLADSTONE PROJEC FISH 12-2755432

Appendix 4- Pathology Report 3- 12-3951675

DR MATT LANDOS LANDOS, GLADSTONE PROJECT FISH
FUTURE FISHERIES VETERINARY SERVICE PO BOX 364 LENNOX HEAD NSW 2478
Collected:15/02/12 **Lab Ref:**12-3951675
Collect Time:00:00 **Your Ref:**
Printed:10/04/12
BRI 2012 11474

Specimen: SPECIMENS X 9

Macroscopic: (SMW/DT)

1. A Trawl #2 Grinner: Multiple RS in blocks A to C.

2. B: Submitted with a single container are two 85 and 110mm in length sciaenid fish. 7RS of the smaller fish in blocks O to Q. 7RS of the larger fish in blocks R to T. Each of these sections will require decalcification.

3. Trawl 3 sciaenid 3: Multiple RS in blocks D and E.

4. Trawl 3 Sciaenid 4: Multiple RS in blocks F through to H.

5. E: Submitted within the same container are two prawns each measuring 110mm in length. Parasagittal section of the cephalothorax, bisected in blocks U to V. Tissue from the branchiostegal region in block W. 2TS of first and third abdominal segments in block X. 1LS of six abdominal sections in block Y. For the second prawn the cephalothorax is prepared into a parasagittal section, bisected in blocks V to AA. Branchiostegal tissue in block AB. 2TS of abdominal segment in block AC. 2TS of third abdominal segment in block AD. 1LS of six abdominal segment in block AE.

6. F: Submitted within one container are four scallops 3cm in diameter . Fish are removed from the shell, bisected and blocked in to, one each blocks AJ through AM.

7. G: This container contains a 8cm in length portion of a cephalothorax and one abdominal segment and one abdominal segment of a prawn. One parasagittal section of cephalothorax bisected in blocks AF to AG. RS of branchiostegal tissue in block AH. 2RS of abdominal segments in block AI. Blocks U to AI will require decalcification.

8. H Trawl 3 Sciaenid #8: Multiple RS in blocks I through to K.

9. I Trawl 2 flat fish #10: Multiple RS in blocks L through to N. Blocks J and L submitted for decal prior to processing.

Microscopic:

1. A Trawl #2 Grinner: There is segmental loss of epidermis covering the fin. There appears to be true necrosis in some exposed connective tissue elements. Cestodes are within the intestinal lumen. No significant pathology is identified within sections of gill, kidney, skin, body wall, stomach or pancreas.

2. B Sciaenid: The first fish has marked renal tubular myxosporidiosis. There is some associated glomerular injury and red blood cells within renal tubules. Multiple adult trematodes are associated with the intestinal surface. There is a mild non-suppurative infiltrate of the stomach. No significant pathology is identified within sections of brain, gill, heart, spleen, body wall or skin.

There is nearly diffuse presumptive artefactual loss of epidermis in the second fish. Some scale pockets, which are empty, contain sediment debris. There is mild renal tubular myxosporidiosis especially affecting the collecting ducts. Numerous approximately 5 micron diameter round to tear shaped protozoa are associated

with the mucosal surface of the gastric antrum. These are mildly PAS staining and do not have acid fast components. No significant pathology is identified within sections of gill by liver, pancreas or spleen.

3. Trawl 3 Sciaenid 3: There are low numbers of coccidia-like organisms associated with the intestinal mucosal surface and there is moderate mixed enteritis. There are multiple adult trematodes associated with the intestinal mucosal surface. No significant pathology is identified within sections of liver, stomach, gill or skin. The epidermis is mostly artefactually lost even when two deeper sections are evaluated.

4. Trawl 3 Sciaenid 4: There is a single granuloma within the wall of the stomach. Encysted cestodes are within the peritoneum. Intestinal changes are similar to fish 3. No significant pathology is identified within sections of gill, body wall, heart, liver, pancreas, kidney, ovary or stomach.

Deeper sections of the stomach reveal encysted larvae within granulomas No infectious agents are seen with ZN or PAS stains.

5. E Prawn: Several large larvae of multicellular organism consistent with fluke are encysted within the skeletal muscle just beneath the exoskeleton. Flukes are associated with lamellar surfaces. No significant pathology is identified within antenna gland, ovary, cephalothoracic ganglion, hepatopancreas, foregut, hindgut, or nerve cord.

For the second prawn sediment is often associated with the surface of the exoskeleton, especially where there are folds/pockets. There in infiltration of mononuclear cells around vessels in the anterior gut. No infectious agents are seen in this lesion. A cestode is encysted within the heptopancreas. There are areas where the exoskeleton appears degenerate with subjacent oedema, mild necrosis, and ulceration/hyperplasia of the integument. There is patchy leucocyte infiltrate. No infectious agents are seen. Many multicellular larvae appearing to be trematode larva are encysted within the nerve cord and adjacent soft tissue. There is sediment entrapped within the gill lamellae. No significant is identified within antenna gland, ovary, or hindgut.

6. F Scallop no.1: A few pockets of rod shaped bacteria are encapsulated within the mantle. In some areas of the mantle is patchy haemocyte infiltrates. The digestive diverticular has heavy gregarine protozoal infection, replacing most of the epithelium. Between 10 to 20 juvenile metazoa are within the mantle, gastrointestinal wall, digestive gland, adductor muscle, and ovary. No significant pathology is identified with in gill and nervous elements.
Scallop no.2: Lesions are similar to scallop no.1 only the insisted juvenile metazoa are in much greater numbers. There are a few parasitic ovoid to elliptical bodies within the interstitium of the digestive gland which are surrounded by intense haemocyte infiltrate.
Scallop no.3 has similar changes. There is moderate haemocyte infiltrate within the labial palp. Around 20 to 30 juvenile metazoa are throughout the tissues. No bacterial cysts are identified.
Scallop no.4 has similar changes with greater than 50 insisted juvenile metazoa throughout the tissues. Bacterial cysts are in the neural tissue and mantle.

7. G Prawn: The hepatopancreas has similar features to the second prawn. In one of the sections of abdominal segment is a large area of ulceration. The subjacent skeletal muscle has larked leucocyte infiltrate and spindle cell proliferation. The marginating degenerate exoskeleton is coated in necrotic debris and has mats of bacteria. No infectious agents are seen within the deeper tissues. No significant pathology is seen within sections of heart, ovary, antenna gland, cephalothoracic ganglion, eye or gill.

The bacteria within the ulcerated site are gram negative filamentous to rod shaped bacteria.

8. H Trawl 3 Sciaenid #8: There are several small granulomas within the wall of the stomach. Few coccidia are throughout the intestinal lamina propria. There are also low numbers of surface associated coccidia-like organisms as seen in the other fish. No significant pathology is identified within sections of body wall, kidney, gill, liver or pancreas.

Deeper sections reveal larvae encysted within stomach granulomas. No additional infectious agents are seen within the granulomas within ZN and PAS stains are used.

9. I Trawl 2 flat fish #10: There are segmental epidermal erosions and shallow ulcers marginated by moderate mononuclear leucocyte infiltration. No infectious agents are seen. A large fluke larva is insisted within the body wall. Cryptocaryon-like ciliate is insisted within the skin. Granulomas within the wall of the intestine are centred on larva. No significant pathology is identified within sections of liver, pancreas, or gill.

DIAGNOSIS:

GRINNER
MILD TO MODERATE EROSIVE DERMATITIS.
MILD INTESTINAL CESTODIASIS.

SCIAENID
MILD TO MODERATE MIXED ENTERITIS WITH COCCIDIA AND DISTOMIASIS. FOUR FISH)
MILD TO MODERATE MULTIFOCAL GRANULOMATOUS MURAL GASTRITIS WITH ENCYSTED METAZOAN LARVAE (TWO FISH).
RENAL TUBULAR MYXOSPORIDIOSIS (TWO FISH).
ENCYSTED PERITONEAL CESTODE LARVA (ONE FISH).
MILD-MODERATE NONSUPPURATIVE GASTRITIS (ONE FISH).
MILD GASTRIC PROTOZOAL PARASITISM (AETIOLOGY UNKNOWN) (ONE FISH).

FLAT FISH
MODERATE EROSIVE DERMATITIS.
FOCAL CRYPTOCARYON CYST.
MULTIFOCAL MILD GRANULOMATOUS MURAL ENTERITIS WITH INTRALESIONAL LARVA.

PRAWN
SEVERE ULCERATIVE DERMATITIS (2 PRAWNS).
ENCYSTED TREMATODE LARVAE, SKELETAL MUSCLE, NERVE CORDS, AND ASSOCIATED SOFT TISSUE (2 PRAWNS).
HEPATOPANCREATIC CESTODIAISIS (2 PRAWNS).
LAMELLAR DISTOMIAISIS (1 PRAWN).

SCALLOPS
ENCYSTED VISCERAL JUVENILE METAZOA MODERATE TO SEVERE (FOUR SCALLOPS).
GREGARINE COLONISATION OF DIGESTIVE GLANDS, SEVERE (FOUR SCALLOPS).
ENCYSTED GRAM NEGATIVE BACTERIA (THREE SCALLOPS).
INFLAMMATION OF DIGESTIVE GLANDS WITH INTERLESIONAL UNIDENTIFIED. PROTOZOA (TWO SCALLOPS).
STOMATITIS, MODERATE (TWO SCALLOPS).

GENERAL COMMENT:
In my opinion, it is unusual to observe this degree of parasitism, involving multiple types of parasites, affecting wild animal hosts, and simultaneously so many different species of wild animal hosts from a given area. I suspect that the host populations are subjected to factors causing immunosuppression (water quality, pollutants, etc), and/or factors which may facilitate parasite transmission (population density, environmental modification which causes re-suspension of ova/intermediate host, etc). The degree of described parasitism generally affecting the examined animals, while not necessarily lethal, would be expected to debilitate the host over time. I would expect corresponding higher mortality rates for the affected populations.

In keeping with previous accessions from this project, many animals also have an erosive to ulcerative dermatopathy, often, but not always, associated with ectoparasitism. Immunosuppression would also influence this lesion as would stress and general issues with water quality, as previously discussed. Multiple animals had sediment debris accumulated within anatomical surface folds/pockets suggesting that water quality could be very poor.

Reported and checked by Michelle Dennis, Specialist Veterinary Pathologist

/V01000/

Phoned at 6:00 pm on 09/03/2012 to 0437492863

15/02/12 LANDOS, GLADSTONE PROJEC FISH 12-3951675

Appendix 5- Pathology Report 4- 12-5251389

DR MATT LANDOS LANDOS GLADSTONE PROJECT, RODD'S 7
FUTURE FISHERIES VETERINARY SERVICE
PO BOX 364 LENNOX HEAD NSW 2478
D.O.B.:N/A **Sex:**U **Age:**unknown
Collected:25/01/12 **Lab Ref:**12-5251389
Collect Time:00:00 **Your Ref:**A - E
Printed:16/04/12
BRI 2012 26546

Specimen: SPECIMENS X 5

Macroscopic: (MB/DT)

1. A1, Whiting: The specimen consists of multiple pieces of organ tissue. The specimen is sampled across blocks A to C with blocks B and C submitted for decalcification prior to processing.

2. B2, Mullet: The specimen consists of multiple pieces of organ tissue sampled across blocks D, E and F. Block F has been submitted for decalcification prior to processing.

3. C3, Mullet: The specimen consists of multiple pieces of organ tissue. The specimen is sampled across blocks G, H and I. Blocks H and I are submitted for decalcification prior to processing.

4. D4, Mullet: The specimen consists of multiple organ tissue. The specimen is sampled across blocks J, K, L and M. Blocks K, L and M are submitted for decalcification prior to processing.

5. E5, Mullet: The specimen consists of multiple organ tissue. The specimen is sampled across blocks N, O, P and Q. Blocks P and Q are submitted for decalcification prior to processing.

FURTHER SPECIMENS (6 - 14) LAB NUMBERS 12-5260818 AND 12-2591766

Microscopic:

1. A1, Whiting: There are few small mineralised/inactive granulomas within the wall of the intestine. No infectious agents are seen within these using Gram and ZN stains. Several Myxozoan cysts are within the central nervous system associated with mild non-suppurative infiltrate. A few similar cysts are within body wall skeletal muscle. No significant pathology is identified within sections of kidney, spleen, pancreas, testicle or skin.

2. B2, Mullet: There is mild multifocal mixed inflammation within the liver, often adjacent to bile ducts. Active granulomas containing microsporidia are within the pancreas, associated with mild mixed leukocyte infiltrate. A few encysted metazoan larvae (suggestive of nematodes) are within the kidney. Low numbers of myxozoan cysts are within the kidney and body wall skeletal muscle. The gill has mild epitheliocystis. The skin appears artefactually lost. No significant pathology is identified within sections of intestine, spleen, testicle or heart.

3. C3, Mullet: Several encysted metazoan (suggestive of trematodes or cestodes) are within the heart. There is a fluke free within the lumen of the heart associated with marked villous endothelial hyperplasia and mixed inflammation. The liver is similar to B2 mullet but also has moderate peribiliary fibrosis. Low to moderate numbers of myxozoa cysts are within the wall of the intestine, kidney, CNS, and body wall. There is segmental architectural loss of skin. The gills have mild epitheliocystis and a few monogenian flukes, associated with mild epithelial hyperplasia. No significant pathology is identified within sections of spleen or pancreas.

4. D4, Mullet: A large adult nematode is within what appears to be a distended bile duct within the liver. The remaining liver has changes similar to those described in other fish. The heart is similar to Mullet C3. Low to moderate numbers of Myxozoa cysts are within the body wall, and nervous system. A few metazoan larvae

(consistent with nemotodes) are insisted within the body wall and kidney. No significant pathology is identified within sections of spleen or gill.

5. E5, Mullet: A single adult nematode is within the intestinal lumen. There are patchy areas of lamellar epithelial hyperplasia and fusion of lamellae. Moderate to high numbers of active myxozoa cysts are within the kidney, liver, spleen, intestine, pancreas, nervous system and body wall.

DIAGNOSIS:

WHITING:
MULTIFOCAL MURAL GRANULOMATOUS ENTERITIS, INACTIVE/MILD.
MYXOZOAN ENCEPHALITIS, MILD-MODERATE.

MULLET:
VISCERAL MYXOZOAN CYSTS/GRANULOMAS, MILD-MODERATE (4 FISH).
ENCYSTED RENAL METAZOAN LARVAE (SUSPECT NEMATODES), MILD-MODERATE (2 FISH).
ENCYSTED CARDIAC METAZOAN LARVAE (SUSPECT CESTODE/TREMATODE), MODERATE (2 FISH).
FOCAL NONSUPPURATIVE HEPATITIS, MILD-MODERATE (3 FISH) WITH INTRALESION NEMATODES (1 FISH).
PROLIFERATIVE BRANCHITIS, MILD (2 FISH), WITH INTRALESIONAL EPITHELIOCYSTIS (2 FISH) AND MONOGENEAN FLUKES (1 FISH).
VISCERAL MICROSPORIDAL CYTS/GRANULOMAS, MILD (1 FISH).

COMMENT:
The main abnormalities identified in these fish comprise a spectrum of parasitism which was overall mild-moderate. In my opinion, the degree of parasitism would seem excessive for that typically observed in healthy freeliving wild fish. The level of infection is such that if it were a farmed population of fish, mitigation strategies would be warranted.

Reported and checked by Dr Michelle Dennis, Specialist Veterinary Pathologist

25/01/12 LANDOS GLADSTONE PROJECT, ROD 12-5251389

APPENDIX 3- Gladstone Offshore Scallop Histology Report 12-5795283

PATHOLOGY REPORT 12-5795283

Report To: DR MATT LANDOS , GLADSTONE SCALLOPS
FUTURE FISHERIES VETERINARY SERVICE
PO BOX 7142 LENNOX HEAD NSW 2478 **Phone:** 0437492853
Collect Time:00:00 **Your Ref:**
Printed:27/07/12
Ref by/copy to: CC Drs: HUYNH.

Clinical notes: Ten (10) scallops collected from three locations off facing island.

Macroscopic: (LB/BK)

1. Labelled A: The specimen consists of a scallop and sampled blocks A to C.
2. Labelled B: The specimen consists of a scallop and sampled blocks D to F.
3. Labelled C: The specimen consists of a scallop and sampled blocks G to I.
4. Labelled D: The specimen consists of a scallop and sampled blocks J to L.
5. Labelled E: The specimen consists of a scallop and sampled blocks M to O.
6. Labelled F: The specimen consists of a scallop and sampled blocks P to R.
7. Labelled G: The specimen consists of a scallop and sampled blocks S to U.
8. Labelled H: The specimen consists of a scallop and sampled blocks V to X.

9. Labelled I: The specimen consists of a scallop and sampled blocks Y to AA.
10. Labelled J: The specimen consists of a scallop and sampled blocks AB to AD.

Microscopic:

1. Labelled A: The digestive gland epithelium are often distinctly vacuolated and contain many golden brown residual bodies. Low numbers of metazoan larvae are within the labial palp and are associated with minimal host response. No significant pathology is identified within sections of mantle, eyes, gonad, gill, adductor muscle or intestine.

2. Labelled B: The digestive gland is similar to the previous scallop. Low to moderate numbers of metazoan larvae are encysted within the submucosa adjacent to the labial palp. Moderate numbers of more developed metazoa are encysted within the gonad. No significant pathology is identified within sections of gill, muscle, intestine or mantle.

3. Labelled C: Low numbers of metazoan larva are encysted within the mantle, muscle, and gonad. No significant pathology is identified within sections of gill or intestine. The digestive gland is similar to previous scallops.

4. Labelled D: Low numbers of metazoan larvae are encysted in the gonad muscle. No significant pathology is identified within sections of gill, intestine, or eyes. Digestive gland is similar to the previous scallops.

5. Labelled E: Low numbers of metazoan larvae are encysted within the submucosa adjacent to labial palp, muscle, and gonad. No significant pathology is identified within the intestine, gill or eyes. The digestive gland is similar to previous scallops.

6. Labelled F: Low to moderate numbers of metazoan larvae are encysted within the gonad, muscle and digestive gland. The digestive gland is otherwise similar to previous scallops. No significant pathology is identified within gill, mantle or intestine.

7. Labelled G: Low to moderate numbers of encysted metazoan are within the labial palp muscle. Digestive gland is similar to previous scallops. There is mild haemocyte infiltrate associated with the parasites. No significant pathology is identified within sections of mantle, gill or intestine.

8. Labelled H: Digestive gland is similar to previous scallops. Low numbers of metazoan parasites are encysted within the labial palp and muscle. No significant pathology is identified within sections of intestine, gonads, gill or mantle.

9. Labelled I: Digestive gland is similar to previous scallops. Low numbers of metazoan larvae are encysted within the adductor muscle. No significant pathology is identified within sections of gill, gonad, intestine or mantle.

10. Labelled J: Numerous multicellular organisms are throughout most parenchymal organs and appear to entirely replace the gonad. They appear morphologically similar to scallop spat, including the presence of cilia and rudimentary organs. Low numbers of metazoan larvae are encysted within muscle, labial palp and digestive gland.

DIAGNOSIS: ENCYSTED METAZOA LARVAE, MILD TO MODERATE (ALL SCALLOPS).

COMMENT: Please compare results to relevant sampling group. I have the impression that the level of parasitism is lower in this population than the previously examined scallop sample.

I am unsure of the significance of the multicellular organism in scallop J. They appear to consist of offspring but I cannot rule out parasitism.
Please phone to discuss further.

Reported and checked by Dr Michelle Dennis, Specialist Veterinary

Pathologist.
/V00000/L50005

23/07/12 LANDOS, GLADSTONE SCALLOPS 12-5795283

APPENDIX 4: Reference site histology from Stanage Bay area 12-5487595

DR MATT LANDOS , GLADSTONE PROJECT FISH-STANAGE SAMPLES
FUTURE FISHERIES VETERINARY SERVICE
LENNOX HEAD NSW 2478 **Phone:**
Collected:19/04/12 **Lab Ref:**12-5487595
Collect Time:00:00 **Your Ref:**
Printed:25/06/12
Ref by/copy to: CC Drs: HUYNH.
BRI 2012 39694
Specimen: STANAGE BAY MULTIPLE SPECIMENS

Macroscopic: (LB/DN)

1. #1 19/4/12 barra site 1: The specimen consists of multiple samples of tissue. RS blocks A to E.

2. #2 19/4/12 barra site 1: The specimen consists of multiple portions of various organs. RS blocks F to I.

3. #3 19/4/12 barra site 1: The specimen consists of multiple samples of tissue. RS blocks J to M.

4. #4 19/4/12 barra site 1: The specimen consists of multiple portions of various organs. RS blocks N to P.

5. #5 19/4/12 barra site 1: The specimen consists of a single portion of tissue. 1RS bisected block Q.

6. #6 19/4/12 barra site 1: The specimen consists of multiple portions of various organs. RS blocks R to U.

7. #7 19/4/12 salmon site 1: The specimen consists of multiple portions of various organs. RS blocks V to X.

8. #8 19/4/12 forktail catfish site 3: The specimen consists of multiple portions of various organs. RS blocks Y and Z.

9. #9 19/4/12 catfish site 3: The specimen consists of multiple portions of various organs. RS blocks AA to AC.

10. #10 19/4/12 catfish site 3: The specimen consists of multiple portions of various organs. RS blocks AD to AG.

11. #11 19/4/12 little whaler shark site 3: The specimen consists of multiple portions of various organs. RS blocks AH to AJ.

12. #12 19/4/12 shark site 3: The specimen consists of multiple portions of various organs. RS blocks AK and AL.

13. #13 19/4/12 little whaler shark site 3: The specimen consists of multiple portions of various organs. RS blocks AM and AN.

14. #14 19/4/12 little whaler shark site 3: The specimen consists of multiple portions of various organs. RS blocks AO to AQ.

15. #15 19/4/12 little whaler shark site 3: The specimen consists of multiple portions of various organs. RS blocks AR and AS.

16. #16 19/4/12 queen fish site 2: The specimen consists of multiple portions of various organs. RS blocks AT to AW.

17. #17 ray: The specimen consists of multiple portions of various organs. RS blocks AX to BB.

18. #18 mullet: The specimen consists of multiple portions of various organs. RS blocks BC to BE.

19. #19 mullet: The specimen consists of multiple portions of various organs. RS blocks BF to BH.

20. #20 mullet: The specimen consists of multiple portions of various organs. RS blocks BI to BK.

21. #21 dart: The specimen consists of multiple portions of various organs. RS blocks BL to BO.

22. #22 mullet: The specimen consists of multiple portions of various organs. RS blocks BP to BS.

23. #23 mullet: The specimen consists of multiple portions of various organs. RS blocks BT to BV.

24. #24 21/4/12 mud crab: The specimen consists of multiple portions of various organs. RS blocks BW to BX.

25. #25 21/4/12 mud crab: The specimen consists of multiple portions of various organs. RS blocks BY to CC.

26. #26 21/4/12 mud crab: The specimen consists of multiple portions of various organs. RS blocks CD to CF.

27. #27 21/4/12 mud crab: The specimen consists of multiple portions of various organs. RS blocks CG to CJ.

28. #28 21/4/12 mud crab: The specimen consists of multiple portions of various organs. RS blocks CK to CN.

Microscopic:

1. #1 19/4/12 barra site 1: A few inactive granulomas and mild mixed leukocyte infiltrate is within the gastric submucosa. These are rarely associated with degenerate nematode larvae. Low numbers of monogenean flukes and myxosporidial cysts are within gill lamellae. Little host reaction is associated with their presence. No significant pathology is identified within sections of pyloric cecae, liver, spleen, pancreas, kidney, intestine, heart, skin and body wall.

2. #2 19/4/12 barra site 1: Rare inactive granulomas and very mild mixed leukocyte infiltrate is within the intestine. There are rare coccidia associated with epithelial surfaces of the intestinal mucosa. Moderate numbers of embryoinated eggs with thin pigmented cells are within the kidney, particularly within melanomacrophages centres, and are associated with chronic mild granulomatous response. These are consistent with sanguinocolid. Low to moderate numbers of similar eggs are associated mild to moderate lymphocytic to granulomatous myocarditis. Low numbers of similar eggs and associated granulomas are within the liver. The stomach is similar to that described for Sample 1. There are moderate to high numbers of myxosporidial cysts within gill lamellae. No significant pathology is seen within sections of skin, body wall or spleen.

3. #3 19/4/12 barra site 1: Low numbers of granulomas and mild mixed leukocyte infiltrate are within the intestinal submucosa. Some are centred on degenerate nematodes. Gastric changes are similar to other barramundi. There is mild nonsuppurative myocarditis associated with blood fluke eggs. These are in low numbers. Low numbers of blood fluke egg associated granulomas are within the kidney. Moderate numbers of blood fluke eggs are within filament vessels of the gill, some surrounded by nonsuppurative inflammation or fibroplasia. No significant pathology is identified within sections of skin, body wall, spleen, pancreas or liver.

4. #4 19/4/12 barra site 1: Kidney, intestine, heart, stomach and gill changes are similar to fish 3. Blood fluke egg numbers within the gill are lower however there are moderate numbers of myxosporidial cysts and low numbers of monogenean flukes. Moderate mononuclear leukocyte infiltrate and lamella epithelial hyperplasia is associated with these infections. No significant pathology is identified within sections of liver or pancreas.

5. #5 19/4/12 barra site 1: No significant pathology is identified within sections of skin or body wall.

6. #6 19/4/12 barra site 1: Very few myxosporidial cysts are monogenean flukes are associated with gill lamellae. These have little host reaction. Heart, intestinal and stomach changes are similar to those described for other barramundi. No significant pathology is identified within sections of spleen, kidney or liver.

7. #7 19/4/12 salmon site 1: Patchy moderate nonsuppurative infiltrate is within the superficial dermis. Low numbers of cestode larvae are encysted within the liver. No significant pathology is identified within sections of stomach, intestine, kidney, spleen, pyloric caeca, body wall, gill or heart.

8. #8 19/4/12 forktail catfish site 3: Moderate mixed leukocyte infiltrate is throughout the gastric submucosa. No infectious agents are seen. Low numbers of monogenean flukes are associated with lamellar surfaces. There is little host reaction to their presence. No significant pathology is identified within sections of liver, pancreas, intestine, skin, body wall, heart or kidney.

9. #9 19/4/12 catfish site 3: Very low numbers of ciliate protozoa are within renal collecting ducts. These are associated with little host reaction. No significant pathology is identified within sections of intestine, spleen, pancreas, stomach, liver, skin, body wall, heart or gill.

10. #10 19/4/12 catfish site 3: Similar ciliates are present within renal collecting ducts and are associated with mild to moderate epithelial hyperplasia. There is mild patchy nonsuppurative infiltrate within the gastric submucosa. No infectious agents are seen. A single monogenean fluke is associated with lamellar surfaces. No significant pathology is identified within sections of spleen, body wall, liver, pancreas, intestine, heart or skin.

11. #11 19/4/12 little whaler shark site 3: Low numbers of adult cestodes are within the intestinal lumen. Low numbers of coccidia-like organisms are within gastric epithelial cells. These are associated with moderate nonsuppurative infiltrate. No significant pathology is identified within sections of liver, skin body, lower kidney, heart, brain or gill.

12. #12 19/4/12 shark site 3: The intestine is similar to the previous shark. The gill has mild epitheliocystis and little associated host reaction. No significant pathology is identified within sections of spleen, stomach, liver, kidney, skin or body wall.

13. #13 19/4/12 little whaler shark site 3: There is mild biliary hyperplasia and zonal nonsuppurative infiltrate within the liver. There are segments of intestinal epithelium which have high numbers of coccidia-like organisms. This is associated with moderate epithelial necrosis and moderate nonsuppurative submucosal infiltrate. The stomach has mild patchy nonsuppurative leukocyte infiltrate. No infectious agents are seen. No significant pathology is identified within sections of skin, body wall, spleen, heart or gill.

14. #14 19/4/12 little whaler shark site 3: Gastric changes are similar to the previous shark. There is a single granuloma within the intestinal submucosa centred on an unidentifiable metazoan larvae. No significant pathology is identified within sections of kidney, spleen, pancreas, skin, body wall, liver, heart or gill.

15. #15 19/4/12 little whaler shark site 3: Low numbers of coccidia-like organisms are associated with the surface of intestinal epithelial cells. There is moderate nonsuppurative mucosal leukocyte infiltrate. No significant pathology is identified within sections of liver, pancreas, spleen, stomach, gill, heart or kidney.

16. #16 19/4/12 queen fish site 2: There is a regionally severe area of nonsuppurative infiltrate extending from the capsular surface into the parenchyma of the liver. No infectious agents are seen in this area.
There is marked eosinophilic infiltrate of the gastric mucosa and multifocally around large mesenteric blood vessels. No infectious agents are seen. The pyloric caecae have low numbers of coccidia-like organisms and little associated host response. There is a solitary caseogranuloma within the pyloric caecae mesentery. No significant pathology is identified within sections of intestine, kidney, gill, spleen or heart.

17. #17 ray: There are a few small foci of nonsuppurative inflammation throughout the pancreas. There is patchy mild nonsuppurative infiltrate within the gastric mucosa. No infectious agents are seen.
No significant pathology is identified within sections of gill, spleen, skin, body wall, liver, kidney, intestine, spiral valve, heart and unidentifiable hematopoietic organs.

18. #18 mullet: There are moderate numbers of myxozoal cysts within body wall muscles. There are scattered granulomas within the heart which contain degenerate unidentifiabl metazoan larvae. There is mild mixed intestinal mucosa lymphocyte infiltrate. No infectious agents are seen. Most of the integument is

artefactually lost. High numbers of unidentifiable metazoan larvae are encysted within the kidney. The gill has very low numbers of monogenean flukes and small foci of lamellar fusion due to epithelial hyperplasia. No significant pathology is identified within sections of spleen or liver.

19. #19 mullet: There are rare myxozoal cysts within the gill associated with little host reaction. The kidney contains moderate numbers of mineralised/inactive granulomas. There are few myxozoal cysts within the subcutaneous tissue and within myofibres of the body wall. Much of the skin has been artefactually lost. No significant pathology is identified within sections of intestine, liver, pancreas, spleen or heart.

20. #20 mullet: Low numbers of myxozoa cysts are present in tissue similar to that described for the previous mullet. There is mild peribiliary fibrosis. No significant pathology is identified within sections of gill integument, intestine, kidney, spleen, heart.

21. #21 dart: Rare profiles of adult flukes and cestodes are within pyloric caecae. Very low numbers of coccidia-like organisms are associated with enterocyte surfaces. There are moderate numbers of myxidia-like organisms within the intestinal mucosa. These are associated with mild nonsuppurative leukocyte infiltrate and epithelial degeneration. No significant pathology is identified within sections of spleen, liver, skin, body wall, pancreas, gill, kidney or heart.

22. #22 mullet: No significant pathology is identified within sections of skin, body wall, kidney, liver, fin, heart, gill, spleen or intestine.

23. #23 mullet: Low numbers of myxozoal cysts are present in various tissue as seen in other mullets. There is mild peribilliary fibrosis. No significant pathology is identified within sections of intestine, spleen, pancreas, stomach or heart.

24. #24 21/4/12 mud crab: There are rare bacculovirus-like inclusions within the nuclei of hepatopancreas epithelium. No significant pathology is identified within sections of integument, muscle, gill or eye.

25. #25 21/4/12 mud crab: There are segments where sediment is entrapped within crevices of the skin surface. One other section has a cuticle erosion which extends through epi/exocuticle into the superifical endocuticle. No infectious agents are seen in this area. Occasional hepatopancreas cells have cytoplasmic granules consistent with mineral. Some antenna glands are distended with necrotic debris. No significant pathology is identified within sections of gonad, eye, muscle, or gill.

26. #26 21/4/12 mud crab: There are moderate amounts of sediment entrapped within lamellar spaces at the tip of the gill. These contain moderate numbers of nematodes. Most hepatopancreatic cells contain granules consistent with mineral. No significant pathology is identified within sections of integument, muscle or eye.

27. #27 21/4/12 mud crab: A large crustacean is associated with the gill surface. There is congestion, necrosis, and haemorrhage in the adjacent gill. No significant pathology is identified within sections of hepatopancreas, integument, or muscle.

28. #28 21/4/12 mud crab: Most hepatopancreas cells have cytoplasmic granules consistent with mineral. No significant pathology is identified within sections of integument, muscle, gonad, eye or gill.

DIAGNOSIS:

BARRUMUNDI
MILD MIXED TO GRANULOMATOUS GASTRITIS/ENTERITIS WITH INTRALESIONAL LARVAL NEMATODES (4 FISH)
MILD-MARKED MULTIFOCAL BRANCHITIS WITH MONOGENEAN FLUKES AND OR MYXOSPORIDIAL CYSTS (4 FISH)
MILD-MODERATE VISCERAL AND LAMELLAR GRANULOMAS WITHIN INTRALESIONAL SANGUINOCOLID EGGS (4 FISH)
MILD ENTERIC COCCIDIOSIS (1 FISH)

CATFISH

MODERATE MIXED GASTRITIS (2 FISH)
MILD RENAL COLLECTING DUCT CILATE PROTOZOA (2 FISH)
MILD LAMELLAR DISTOMIASIS (1 FISH)

BULLSHARKS
MILD INTESTINAL CESTODIASIS (2 FISH)
MILD NONSUPPURATIVE GASTRITIS (3 FISH) WITH INTRALESIONAL COCCIDIA (1 FISH)
MODERATE-MARKED INTESTINAL COCCIDOSIS (2 FISH)
MILD LAMELLAR EPITHELIOCYSTIS (1 FISH)
MILD INTESTINAL LARVAL MIGRANS (1 FISH)
MILD ZONAL NONSUPPURATIVE HEPATITIS (1 FISH)

MULLET
MILD-MODERATE VISCERAL AND LAMELLAR MYXOZOAL CYSTS (4 FISH)
MILD VISCERAL GRANULOMATOUS DISEASE WITH ENCYSTED UNIDENTIFIABLE METAZOAN LARVAE (1 FISH)
MILD LAMELLAR DISTOMIASIS (1 FISH)

MUD CRAB
SUPERIFICAL (EXTERNAL) CUTICLE EROSIONS (1 CRAB)
MILD BRANCHITIS WITH INTRALESIONAL SEDIMENT AND NEMATODES (1 CRAB)
MODERATE LAMELLAR COPEPODIASIS (1 CRAB)
MILD HEPATOPANCREATIC BACCULOVIRUS-LIKE INCLUSIONS (1 CRAB)

COMMENT:
Given the urgency in which this report is needed, I am issuing without further comment. Please phone to discuss these results.

Reported and checked by Dr Michelle Dennis, Specialist Veterinary Pathologist

APPENDIX 5: Facing Island Gladstone Histology Results 12-2591766

DR MATT LANDOS LANDOS GLADSTONE PROJECT, FACING IS
FUTURE FISHERIES VETERINARY SERVICE PO BOX 364 LENNOX HEAD NSW 2478
Collected:22/02/12 **Lab Ref:**12-2591766
Printed:22/05/12
Ref by/copy to: CC Drs: HUYNH.
Specimen: FIVE SPECIMENS

Macroscopic: (MB/DT)

PREVIOUS SPECIMENS (1-9) LAB NUMBERS 12-5251389 AND 12-5260818

10. 10J, Queen Fish: The specimen consists of multiple organ tissue. The specimen is sampled across blocks AQ, AR, AS and AT. Block At is submitted for decalcification prior to processing.

11. 11K, Blubberlip bream: The specimen consists of multiple pieces of organ tissue. The specimen is sampled across blocks AU, AV, AW, AX, AY, AZ and BA. Blocks AV and BA are submitted for decalcification prior to processing. Please note an eye specimen was also received in this container however, this has not been blocks and has been returned to the container for pathologist to cut up.

(Further sections): A 4cm in diameter eye is submitted. It has a 2cm in length incision through the cornea. One sagittal section in block BQ. One parasagittal section in block BR (megacassette).

12. 12L, Shovelnose shark: The specimen consists of multiple organ tissue. The specimen is sampled across blocks BB, BC, BD and BE.

13. 13M, Blubberlip bream: The specimen consists of multiple organ tissue. The specimen is sampled across blocks BF, BG, BH and BI. Please note block BI has been submitted to decalcification prior to processing.

14. 14N, Bronze whaler: The specimen consists of multiple organ tissue. The specimen is sampled across blocks BJ, BK, BL, BM, BN, BO and BP.

Microscopic:

10. 10J, Queen Fish: Moderate numbers of nematode larva are encysted within the peritoneum, some associated with granulomatous inflammation. A few foci of granulomatous inflammation are within the body wall. No infectious agents are seen within these. There is severe mixed leukocyte infiltrate within the gastro-oesophageal mucosa. No significant pathology is identified within sections of spleen, testicle, kidney, liver, intestine or heart.

11. 11K, Blubberlip bream: There are moderate numbers of a coccidia associated with the apical surface of enterocytes. There is marked mixed enteritis. A metazoan larva, consistent with a cestode is encysted within the intestinal wall. A few adult trematodes are within the intestinal lumen. Several myxosporidial granulomas are within the epicardium. There is patchy moderate mixed epicarditis. No significant pathology is seen within the liver or pancreas.

The eye has a segment of corneal ulceration. The subjacent stroma is markedly expanded with oedema. There is mild neovascularisation and mild mixed leukocyte infiltrate of the corneal stroma. Similar mixed leukocyte infiltrate is within the iris and there is an anterior synechia. No infectious agents are seen.

12. 12L, Shovelnose shark: There are many subcutaneous/submucosal haemorrhages and marked oedema in the section of mucocutaneous junction. Eight profiles of a metazoan parasite, consistent with a trematode are within the wall of the oesophagus and are surrounded by intense mixed leucocyte infiltrate. No significant pathology is identified within spleen or heart.

13. 13M, Blubberlip bream: There is moderate mixed leukocyte infiltration of the intestinal mucosa. Similar inflammation is within pyloric cacae. Throughout the myocardium are several foci of mononuclear leukocyte infiltrate. There is a single small metazoan larva. The gill has multiple foci of lamellar fusion and cyst formation due to mononuclear leukocyte infiltrate and epithelial cell hyperplasia. No significant pathology is identified within sections of hepatopancreas, kidney or spleen.

14. 14N, Bronze whaler: Multiple metazoan larvae are encysted within the liver and are variably encased within granulomatous inflammation. There is moderate non-suppurative enteritis, however, much of the mucosa is artefactually lost to autolysis. A few metazoan larvae are encysted within the spleen. Low numbers of metazoan larvae are within the lumen of the branchial veins. There are severe segments of epidermal erosion and ulceration, associated with mixed leukocyte infiltrate of the dermis/subcutis and even underlying skeletal muscle of the body wall. Some of the effected muscle is necrotic. There is moderate mixed bacterial colonisation of superficial necrotic dermis. No other infectious agents are seen.

DIAGNOSIS:

Queenfish:
GRANULOMATOUS PERITONITIS WITH INTRALESIONAL METAZOAN LARVAE.
MILD GRANULOMATOUS MYOSITIS.
MARKED CHRONIC ACTIVE GASTRO-OESOPHAGITIS.

Blubberlip bream:
MODERATE INTESTINAL COCCIDIOSIS (1 FISH).
MODERATE MIXED ENTERITIS (1 FISH).
MILD INTESTINAL CESTODE CYTSTS (1 FISH).
MILD INTESTINAL DISTOMIASIS (1 FISH).
MILD GRANULOMATOUS EPICARDITIS WITH INTRALESIONAL MYXOSPORIDIA (1 FISH).
MARKED REGIONAL KERATITIS AND ANTERIOR UVEITIS (1 FISH).
MODERATE NONSUPPURATIVE MYOCARDITIS WITH INTRALESIONAL METAZOAN LARVAE (1 FISH).
MODERATE CHRONIC BRANCHITIS (1 FISH).

Shovelnose shark:
FOCAL MARKED CUTANEOUS HAEMORRHAGE.
SEVERE MURAL OESOPHAGITIS WITH INTRALESIONAL TREMATODE LARVAE.

Bronze whaler:
SEVERE ULCERATIVE DERMATITIS.
MODERATE GRANULOMATOUS HEPATITIS WITH INTRALESIONAL METAZOAN LARVAE.
MODERATE NONSUPPURATIVE ENTERITIS.
MILD SPLENIC METAZOAN LARVAE CYSTS.
MILD INTRAVENOUS BRANCHIAL METAZOAN LARVAE.

COMMENT: The assortment of lesions is similar to those observed in other cohorts of fish sampled from this area, including parasitism and erosive/ulcerative skin lesions. The type of myositis observed in the queenfish is often a result of myxozoan or microsporidian infection. The branchitis described in the bream is often seen with ectoparasitism of the gill. Please contact me if there is any particular lesion for which you would like further comment.
Reported and checked by Dr Michelle Dennis, Specialist Veterinary Pathologist.
/V00000/

APPENDIX 6: Gladstone trawl histology results 12-5256385

DR MATT LANDOS LANDOS, GLADSTONE PROJECT FISH
FUTURE FISHERIES VETERINARY SERVICE PO BOX 364 LENNOX HEAD NSW 2478
Collected:17/04/12 **Lab Ref:**12-5256385
Printed:30/05/12

Ref by/copy to: CC Drs: HUYNH.
History: Samples collected from Gladstone region- a range of gross pathology signs described and photographed.
BRI 2012 28295
Specimen: SPECIMENS X 8

Macroscopic: (SMW/DN)

1. Sole: Sample consists of a 50 x 30 x 10mm portion of a head of a flat fish. There is a dark brown and friable cavity adjacent to the gill which contains what appears to be parasite. Three sections of gill in block A. 3RS of parasite and adjacent tissue in blocks B to C.

2. Jar labelled B: Sampled in blocks B through to H.

3. Jar labelled C: Sampled in blocks I through to K.

4. Jar labelled D: Sampled in blocks L through to P.

5. The first jar labelled E: Sampled in blocks Q to U.

6. The second jar labelled E: Sampled in blocks V and W.

7. Jar labelled F: Sampled in blocks X through to AC.

8. Jar labelled G: Sampled in blocks AD through to AJ.

Please note, there are eight jars received labelled A to G; however, two jars are labelled with letter E.

Microscopic:

1. Sole: A single nematode larva is encysted within the gill. Moderate to high numbers of myxozoan cysts are within skeletal muscle fibres of the body wall. A large microsporidial granuloma is within the spleen. Several cestode larvae are encysted within connective tissue of the body wall. A few granulomas are within the wall of the intestine and some of these contain nematode larvae. No significant pathology is identified within sections of liver, pancrease or gill.

2. Banana prawn: A single metazoan larvae is encysted within the hepatopancreas. No significant pathology is identified within gills, central nervous system, gonad, skeletal muscle or integument.

3. Herring: Much of the epidermis and scales have been artefactually lost, presumptively due to processing. However, there are patchy areas where the subcutis / dermis contains markedly increased mixed leucocytes suggestive of true epidermal erosion / ulceration. No significant pathology is identified within sections of brain, gill, kidney, heart, liver, pancreas, intestine or stomach.

4. Banana prawn: Several profiles of a metazoan larval parasite are throughout the hepatopancreas. No significant pathology is identified within sections of gonad, integument, central nervous system or skeletal muscle.

5and6. Barramundi (fish 5 and 6): There is marked mixed enteritis and several small granulomas are centred on variably viable metazoan

(apparently nematode) larvae. These are mostly localised to the submucosa. Small foci of mononuclear leucocytes are randomly throughout the liver. Low numbers of eggs consistent with sanguinicolid are through filament vessels. There is patchy lamellar congestion and oedema and occasional telangiectasis. Low to moderate numbers of similar eggs are throughout the myocardium, often surrounded by mixed to granulomatous
leucocyte infiltrate. There is a region of scarring within one section of body wall, suggestive of previous ulceration. The epidermis in both sections of skin has been artefactually lost. No significant pathology is identified within sections of spleen or kidney.

7. Barramundi (fish 7): High numbers of granulomas are throughout the kidney, some of these containing degenerate eggs consistent with blood fluke. There is mild mixed enteritis. No infectious agents are seen. A few myxozoan cysts are within gill lamellae. Low numbers of small granulomas occasionally contain in what appears to be degenerate blood fluke eggs are throughout they myocardium. There is loss of the epidermis. Scattered foci within the superficial muscle of the body wall are necrotic and are bordered by mononuclear leucocyte infiltration. Features of necrosis are acute. No significant pathology is seen within sections of liver, spleen or stomach.

8. Barramundi (fish 8): High numbers of granulomas containing nematode larvae are throughout the intestine and stomach wall. These are associated with diffuse mild to moderate mixed gastroenteritis. Low numbers of granulomas appearing to contain degenerate parasite eggs are within the kidney. Low numbers of myxozoan cysts are within gill lamellae. Low numbers of granulomas containing blood fluke eggs are within the heart. No significant pathology is identified within sections of gonads, spleen, liver, body wall or skin.

DIAGNOSIS:

SOLE
MILD LAMELLAR NEMATODE CYSTS.
MARKED INTRAMYOCYTIC MYXOZOAN CYSTS.
MARKED VISCERAL LARVAL CESTODIASIS.
MILD GRANULOMATOUS ENTERITIS WITH INTRALESIONAL NEMATODE LARVAE.

BANANA PRAWN
VISCERAL LARVAL METAZOANS (UNIDENTIFIED), MILD TO MARKED.

HERRING
FOCAL ACUTE ULCER.

BARRAMUNDI
GRANULOMATOUS MYOCARDITIS, BRANCHITIS, AND NEPHRITIS WITH INTRALESIONAL SANGUINICOLID EGGS, MILD TO MODERATE (3 FISH).
GRANULOMATOUS AND MIXED ENTERITIS WITH INTRALESIONAL NEMATODE LARVAE, MARKED (2 FISH).
FOCAL AUCTE MUSCLE NECROSIS (POTENTIALLY ULCER RELATED) (1 FISH).
LAMELLAR MYXOZOAN CYSTS, MILD (2 FISH).

COMMENTS: Histological findings are consistent those previously reported for other cohorts evaluated by this project. Please contact for further discussion on any of the described findings.

Reported by Dr Michelle Dennis, Specialist Veterinary Pathologist

APPENDIX 7: 7 Mile Gladstone histopathology results 12-5260818

DR MATT LANDOS LANDOS GLADSTONE PROJECT, 7 MILE ESTUARY
FUTURE FISHERIES VETERINARY SERVICE PO BOX 364 LENNOX HEAD NSW 2478
Collected:29/02/12 **Lab Ref:**12-5260818
Printed:17/04/12
Ref by/copy to: CC Drs: HUYNH.

Specimen: SPECIMENS X 4

Macroscopic: (MB/DT)

PREVIOUS SPECIMENS (1-5) LAB NUMBER 12-5251389

6. F6, Oyster cracker: The specimen consists of multiple organ tissue. The specimen is sampled across blocks R, S, T, U, V, W and X. Blocks W and X are submitted for decalcification prior to processing.

7. G7, Oyster cracker: The specimen consists of multiple organ tissue. The specimen is sampled across blocks Y, Z, AA, AB, AC and AD. Blocks AC and AD are submitted for decalcification prior to processing.

8. H8, Oyster cracker: The specimen consists of multiple pieces of organ tissue. The specimen is sampled across blocks AE, AF, AG, AH, AI, AJ and AK. Please note blocks AJ and AK are submitted for decalcification prior to processing. Please note a shell was also received in the specimen container but this has not been blocked.

9. 9I, Barramundi: The specimen consists of multiple organ tissue. The specimen is sampled across blocks AL, AM, AN, AO and AP. Blocks AO and AP are submitted for decalcification prior to processing.

FURTHER SPECIMENS (10 - 14) LAB NUMBER 12-2591766

Microscopic:

6. F6, Oyster cracker: The spleen has prominent stroma and ellipsoids. Moderate to marked numbers of granulomas are throughout the kidney. These are active and many centrally contain spherical to elongate basophilic structures. Some have a clear halo suggestive of cell wall. They stain intensely with PAS, and not with Von Kossa or Grocott. Moderate numbers of adult nematodes are embedded throughout the intestinal mucosa. Moderate non-suppurative infiltrate is present. High numbers of protozoa organisms are associated with apical enterocyte cytoplasm. These range from oval to elongate, are up to 10 microns in diameter, have deep basophilic cytoplasm with poorly defined internal structures and occasionally are somewhat segmented. Some are clearly attached to enterocytes. There are foci of enterocyte erosion. One section of intestine has an area of ulceration with fibroplasia. Moderate numbers of granulomas similar to those described within the kidney are within the heart. In the sections of skin from the fin area, there are multiple organisms consistent with a crustacean associated with the skin surface. There is marked erosion to ulceration association associated with the presence and moderate mixed inflammation in the dermis. Low numbers of granulomas similar to those of the kidney are in the gill. No significant pathology is seen within the hepatopancreas or gill.

7. G7, Oyster cracker: Low to moderate numbers of coccidia are throughout the intestinal mucosal surface, sometimes associated with blunting and fusion of mucosal folds. There is moderately increased mononuclear leukocytes. Low numbers of protozoa similar to those described for F6 are also present within the intestinal mucosa. Moderate numbers of adult cestodes are within the intestinal lumen and attached to the mucosa. The spleen is similar to F6. The heart and kidney are similar to F6, only more severely affected. There is marked peribiliary fibrosis in the liver. The skin in the fin are is similar to that described for F6. No significant pathology is seen within the gill.

8. H8, Oyster cracker: Low numbers of granulomas similar to those described in the other oyster crackers are within the heart and kidney. There are also low numbers of encysted metazoan larvae within the peritoneum, suggestive of cestodes. Low numbers of protozoa similar to those seen in the other fish are associated with

enterocyte surfaces. There is only mild inflammation associated. The skin in the fin area is similar to that of other fish. No significant pathology is seen within sections of spleen, liver or pancreas.

9. 9I, Barramundi: Splenic, renal, and hepatic melanomacrophage centres are markedly increased. High numbers of granulomas are throughout the intestinal submucosa. Some contain encysted metazoa suggestive of nematodes. There is marked non-suppurative enteritis. Large numbers of granulomas are throughout the heart, and low to moderate throughout the gill, and most are centred on what appear to be degenerate eggs. The gill has low numbers of myxozoan cysts. There is diffuse loss, presumptive artefactual, of the epidermis. Three additional sections taken deeper from the skin sample fail to reveal epidermis.

DIAGNOSIS:

OYSTER CRACKERS:
SEVERE ULCERATIVE DERMATITIS WITH INTRALESIONAL COPEPODS (THREE FISH).

MILD TO MARKED ENTERITIS (THREE FISH) WITH INTERLESIONAL NEMATODES (ONE FISH), UNIDENTIFIED PROTOZOA (THREE FISH), COCCIDIA (ONE FISH), AND CESTODES (ONE FISH).

RENAL, MYOCARDIAL, AND BRANCHIAL GRANULOMAS, MODERATE, WITH INTRALESIONAL ORGANSIMS CONSISTENT WITH MESOMYCETOZOEA OR FUNGUS (THREE FISH).

BARRAMUNDI:
MARKED NON-SUPPURATIVE ENTERITIS WITH INTERLESIONAL NEMATODES.

MYOCARDIAL AND BRANCHIAL SANGUINOCOLOID GRANULOMAS, MODERATE-MARKED.

MILD LAMELLAR MYXOZOAN CYSTS.

COMMENT:
OYSTER CRACKERS: All evaluated fish are affected by a similar spectrum of lesions. These fit with the theme of parasitism and erosive skin disease identified in other species evaluated by this project. In my opinion, the degree of skin and intestinal lesions is sufficient to notably impact the host's health. Moreover, the assortment of lesions is suggestive of immunosuppressive factors which seem to be impacting a population of fish as a whole.

BARRAMUNDI: The assortment of findings is similar to those previously described for barramundi evaluated by this project.

Reported and checked by Dr Michelle Dennis, Specialist Veterinary Pathologist.
/V00000/M88320/D541704

29/02/12 LANDOS GLADSTONE PROJECT, 7 M 12-5260818

APPENDIX 8: Gladstone mud crab histopathology results 12-5311480

DR MATT LANDOS LANDOS, GLADSTONE PROJECT FISH
FUTURE FISHERIES VETERINARY SERVICE
PO BOX 364 LENNOX HEAD NSW 2478
Collected:19/04/12 **Lab Ref:**12-5311480
Printed:20/06/12
Ref by/copy to: CC Drs: HUYNH.
BRI 2012 29220
Specimen: VARIOUS SPECIMENS

Clinical notes: Varying degrees of carapace lesions and claw lesions. Increased parasite loading.

Macroscopic: (SMW/DN)

Fifteen pots are received labelled A thorough to O.
The jar labelled A is sampled in blocks A through to D.
The jar labelled B is sampled in blocks E through to H.
The jar labelled C is sampled in blocks I through to L.
The jar labelled D is sampled in blocks M through to P.
The jar labelled E is sampled in blocks Q through to T.
The jar labelled F is sampled in blocks U through to X.
The jar labelled G is sampled in blocks Y through to AB.
The jar labelled H is sampled in blocks AC through to AF.
The jar labelled I is sampled in blocks AG through to AJ.
The jar labelled J is sampled in blocks AK through to AN.
The jar labelled K is sampled in blocks AO through to AR.
The jar labelled L is sampled in blocks AS through to AV.
The jar labelled M is sampled in blocks AW through to AZ.
The jar labelled N is sampled in blocks BA through to BD.
The jar labelled O is sampled in blocks BE through to BG.
Please note in all cases, the shell, eyeball, claw and gills have been treated for decalcification prior to processing.

Microscopic:

Crab A. There are segments of debris/sediment associated with the epicuticle surface. There are foci where the epicuticle has been lost and the underlying exocuticle is fragmented. In one of the sections carapace has an erosion which extends into deep endocuticle. This is marginated by haemocyte infiltrate. The sediment is clumped in the lysed edges of endocuticle. No infectious agents are seen. Epithelium beneath the eroded sites appears reactive. No features of epithelial injury are identified. The epithelium of the hepatopancreas frequently have apical calcium spherules, consistent with premolt stage. Some of the epithelial cells have acidophilic nuclei, also consistent with premolt stage. No significant pathology is identified within sections of gills, heart, foregut, and midgut.

Crab B. Cuticle changes are similar to those described for crab A. Hepatopancreas cells occasionally contain rhomboid bright eosinophilic cytoplasmic inclusion bodies. No cellular degeneration is associated with the crystals. There are hepatopancreas cells that show premolt associated changes as those described for crab A. A significant proportion of hepatopancreas cells have large cytoplasmic vacuoles. No cellular necrosis is identified. There are increased RI cells within the connective tissue of the mid gut. Abundant sediment is entrapped between gill lamellae. There are low to moderate numbers of ciliate protozoa and nematode larvae associated with lamellar surfaces. No significant pathology is identified within sections of gonad.

Crab C. The integument sections similar to those described for other crabs. They occasionally have moderate haemocyte infiltrate at the margins of ulcers. Some are colonised by clusters of gram negative variety shaped bacteria. No fungal agents are seen with PAS stains. Hepatopancreas changes are similar to crab B only much

more milder. Gill changes are similar to crab B. There is heavy colonisation of the lamellae surfaces by nematodes, ciliates, zones of consolidation with sediments, and a few large copepods. No significant pathology is seen within sections of heart gonad or gut.

Crab D. The hepatopancreas changes are similar to crab A. Gills are similar to the other crabs. Accumulation of parasites is most severe adjacent to where the gills are coated in sediment. No significant pathology is identified within sections of integument, forgut, gonad heart, muscle, or eye.

Crab E. The hepatopancreas changes are similar to crab C. Gill changes are similar to other crabs. No significant pathology is identified within sections of integument, gonad, or gut.

Crab F. In one of the sections of integument there is a cleft just beneath the exocuticle formed by loss of endocuticle. It is filled with eosinophilic globular material and runs parallel with the skin surface. The marginating endocuticle is darkened. The subjacent muscle has mild haemocyte infiltrate. Hepatopancreas cells often have small eosinophilic intranuclear inclusions. No features of cellular degeneration are associated with these. There are many swollen/distinctively vacuolated hepatopancreas cells are seen with other crabs. Gills are similar to those of other crabs. No significant pathology is identified within sections of gonad, foregut, or heart.

Crab G: Integument changes are similar to those described for crab F only the endocuticle clefted areas are much larger. There is some irregular indentation along the edges of the marginating cuticle. The overlying epicuticle and exocuticle are intact. No infectious agents are seen. Gill changes are similar to other crabs, including the marked presence of sediment, nematodes, and ciliates. No significant pathology is identified within sections of skeletal muscle gonad heart or gut.

Crab H: Some sections of integument appear to have artefactual loss of exo and epicuticle. Gill tips have changes similar to those described for other crabs. Severely effected areas appear somewhat necrotic. No significant pathology is seen within sections of gonad and hepatopancreas.

Crab I: There are multiple ulcers which extend through the upper cuticle into deep endocuticle. These are coated in sediment and contain colonies of filamentous bacteria. Moderate numbers of granulomas are throughout the gut interstitium. Some of these contain round shaped bacteria. Similar granulomas are within the hepatopancreas. The hepatic pancreatic cells often have large numbers of basophilic granules, consistent with mineral and premolt stage. Gills are similar to other crabs. There is a remarkable amount of sediment between lamellae. No significant pathology is seen within sections to the gonad.

Crab J: Gills are similar to that described for other crabs. There is a section of large copepod associated with gill surfaces. No significant pathology is identified within sections of integument, gonad, heptopancreas, or gut.

Crab K: Bacculovirus-like inclusion bodies are within nuclei of some hepatopancreas cells. These are associated with mild cellular swelling/degeneration. Gill changes are similar to those described for other crab only more mild; nematodes and celiates are present. No significant pathology is identified within sections of integument, heart or gut.

Crab L: Most hepatopancreas cells are vacuolated and contain granules consistent with minerals (consistent with pre-moult stage). Very low numbers of inactive granulomas are throughout the interstitium of the hepatopancreas. Gills are similar to other crabs. No significant pathology is identified within sections of integument, eye, gut or heart.

Crab M: In one of the integument sections there is nearly a full thickness ulcer which expands all layers of the cuticle. Sediment c oats this area and the endocuticle is markedly distorted. No infectious agents are seen. Gills are similar to other crabs only fewer parasites are present. There is a great amount of sediment within the lamellae. No significant pathology is identified within the sections of gonad, hepatopancreas, gut or heart.

Crab N: One of the sections of integument has a shallow ulcer which extends into superficial endocuticle. Aggregates of sediment are within the exposed cuticle. Some sections have segments of epi/exocuticle erosion. There are multiple sites of foregut cuticle
erosion/degeneration in some areas progressing to ulceration. Clusters of gram negative rods colonise eroded/ulcerated areas of the integument and foregut. No significant pathology is identified within sections of hepatopancreas, gonad, gut. The gill is similar to other crabs only the degree of parasitism is much lower.

Crab O: There are primary endocuticle lesions, similar to those described for Crab F, in multiple sections. No associated infectious agents are seen. The overlying epi/exoccuticles are mostly intact. The hepatopancreas cells frequently have cytoplasmic granules consistent with mineral or are markedly vacuolated. The gill has mild change similar to those described in other crabs including the presence of moderate numbers of nematodes. No significant pathology is identified within sections of gonad or heart.

DIAGNOSIS:
MULTIFOCAL SHELL ULCERS (9 CRABS).
MILD-SEVERE LAMELLAR PARASITISM (13 CRABS).
MILD-MODERATE CHRONIC VISCERAL GRANULOMAS, AETIOLOGY UNKNOWN (2 CRABS)
BACCULOVIRUS ASSOCIATED HEPATOPANCREATIC DEGENERATION, MILD (1 CRAB).

COMMENT: The integument changes were the most remarkable in this population of crabs. The majority of affected crabs have erosions/ulcers that either are limited to the external layers, or span all layers of the cuticle. Three crabs had lesions limited to the endocuticle, similar to that described by LE Anderson et al (Vol. 43: 233239, 2000). This is not consistent with lesions caused by external infections or trauma and is thought to involve non-infectious aetiology, potentially related to environmental conditions. It is possible that these progress to ulcerated lesions involving the epi/exocuticles, so the number of crabs with primary endocuticle disease may be underestimated. A minority of crabs with ulcers had intralesional bacteria. I am interpreting these as opportunists.

Many of the crabs had hepatopancreas changes thought to coincide with various stages of premoult/moult. I am unsure how this relates to environmental conditions or to concurrent integument lesions.
The interlamellar spaces of the gills of most crabs, particularly at the gill tips, were filled with sediment. Often within the sediment was a mixture of parasites including copepods, nematodes, and cilates. These appeared to be opportunists limited to areas of sediment accumulation. I am unsure if this is a common finding in mud crabs, but it appeared that respiration would be difficult in areas coated with sediment, albeit that the underlying tissue did not appear damaged.

Very few crabs had incidental lesions also described by LE Anderson et al, including the visceral granulomas and bacculovirus-like inclusion bodies.

Reported and checked by Michelle Dennis, Specialist Veterinary Pathologist
/V01000/

APPENDIX 9: Facing Island Coral, Gladstone histology results

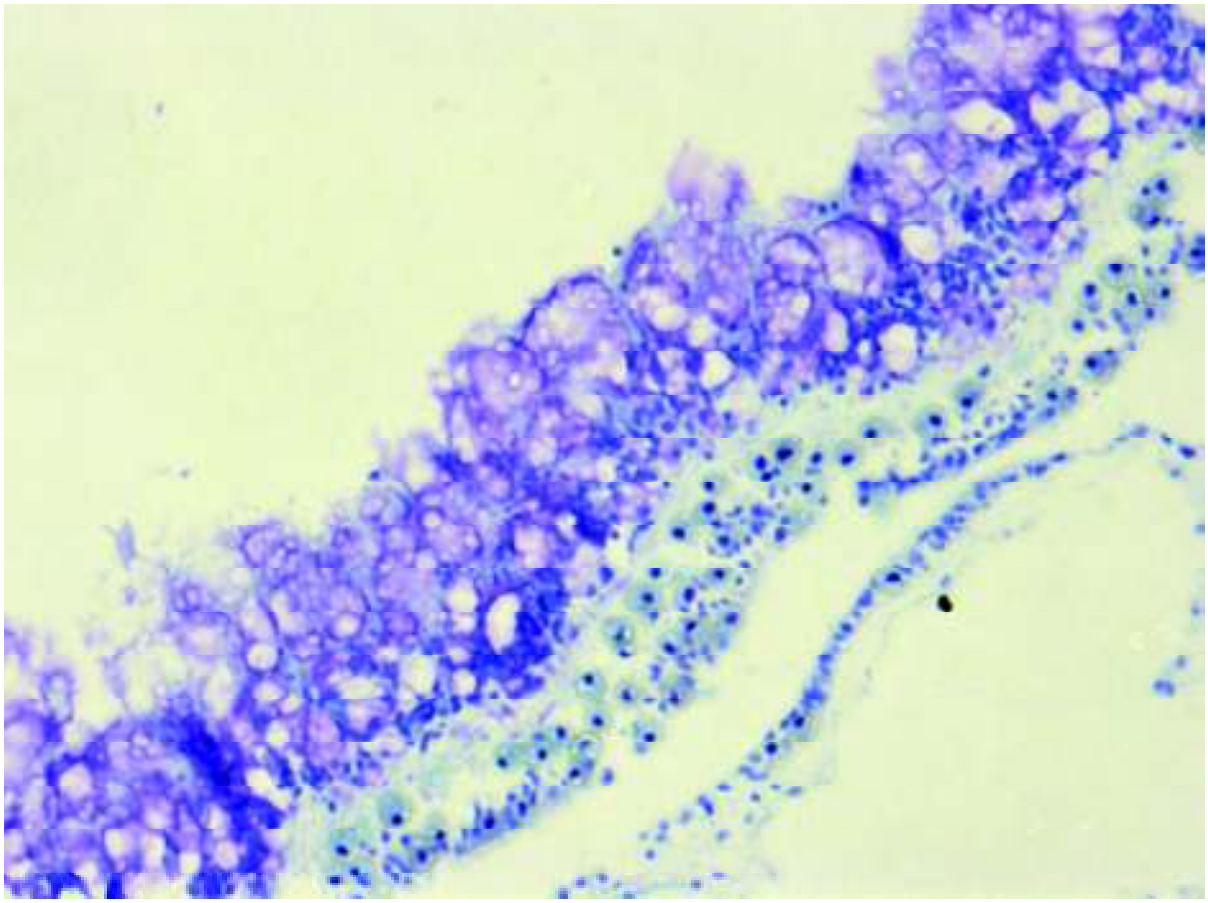
Coral collected from Facing Island samples on 22/02/2012

Pathology undertaken at AIMS, Townsville.

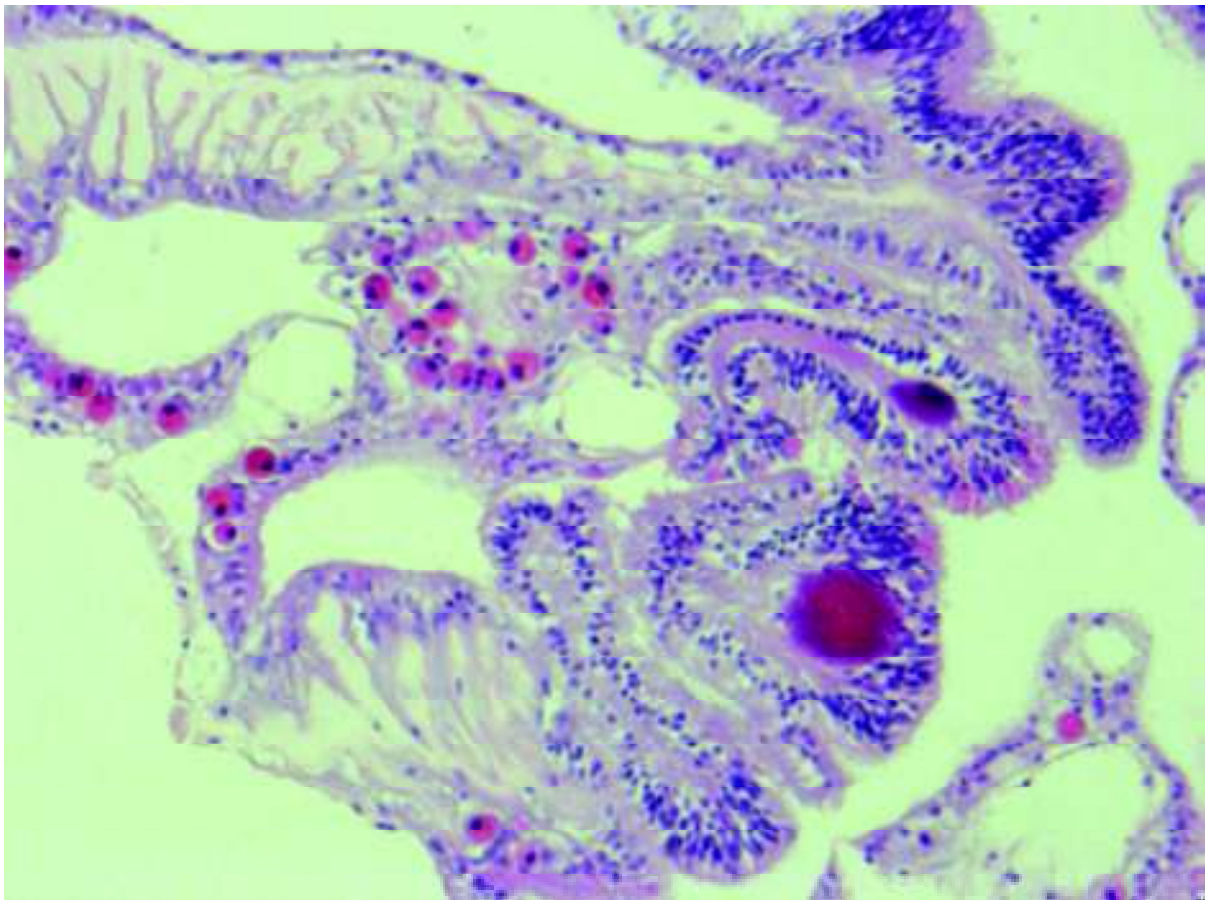
Acropora sp. post-formalin fixation

Pocillopora sp. post formalin fixation

Coral with lesions- fresh sample appearance

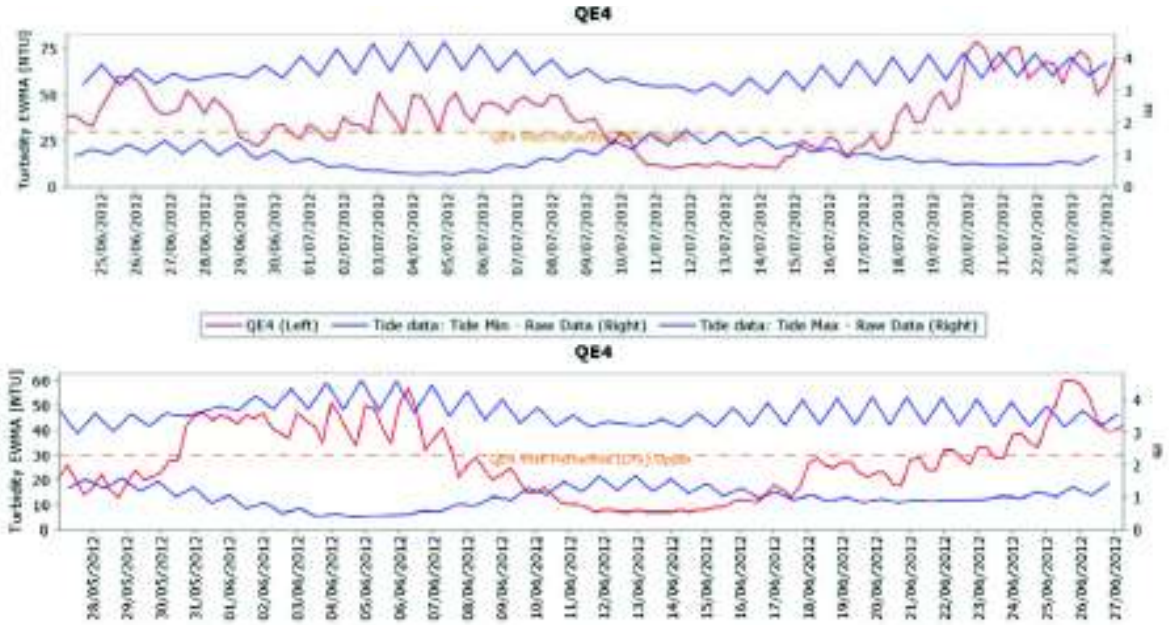


Proliferation of mucous cells on surface of *Acropora* sp.

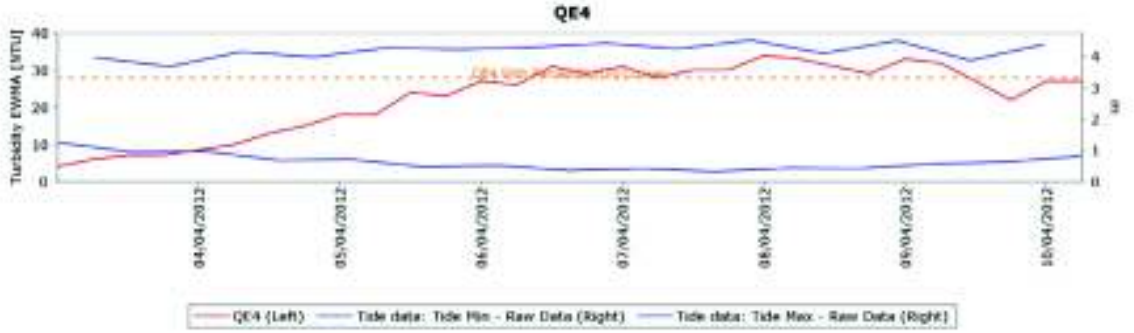


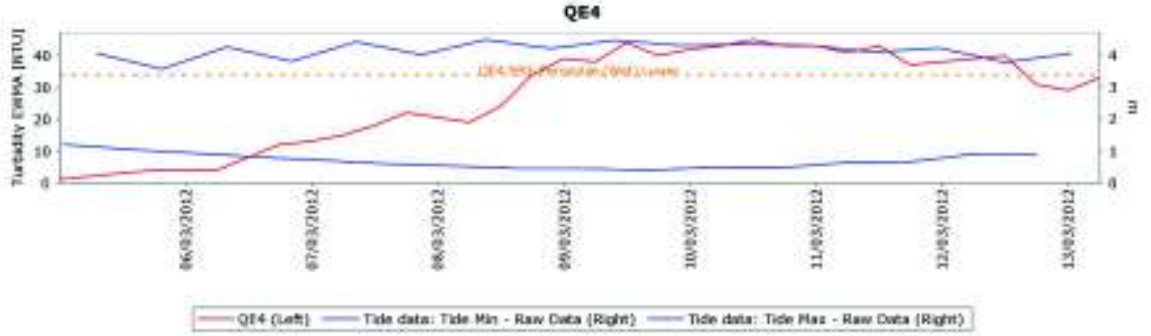
Bacterial colonies in *Pocilliopora* sp.

APPENDIX 10- Monitored turbidity exceedances of 99th percentile from GPC



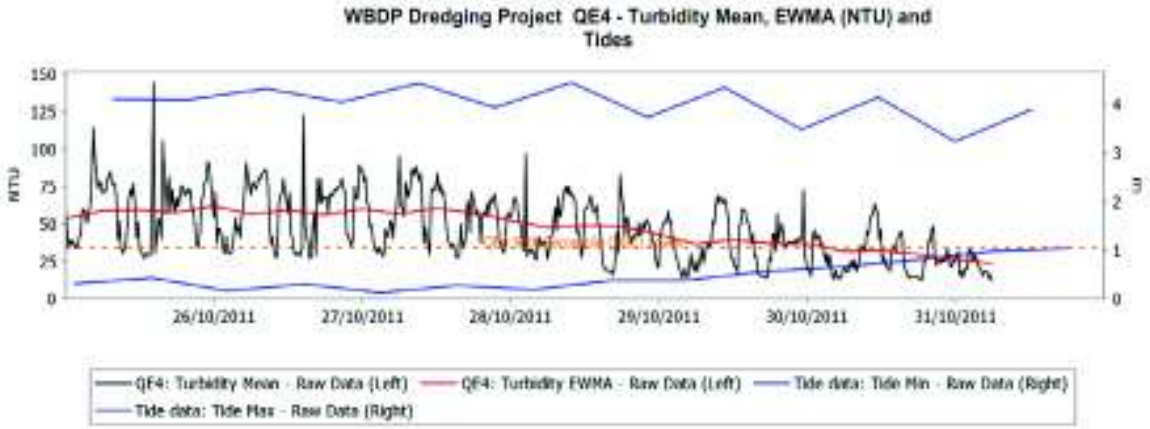

QE4
Turbidity EWMA [NTU]
m
01/05/2012
02/05/2012
03/05/2012
04/05/2012
05/05/2012
06/05/2012
07/05/2012
08/05/2012
09/05/2012
10/05/2012
11/05/2012
12/05/2012
13/05/2012
14/05/2012
15/05/2012
16/05/2012
17/05/2012
18/05/2012
19/05/2012
20/05/2012
21/05/2012
22/05/2012
23/05/2012
24/05/2012
25/05/2012
26/05/2012
27/05/2012
28/05/2012
29/05/2012
30/05/2012

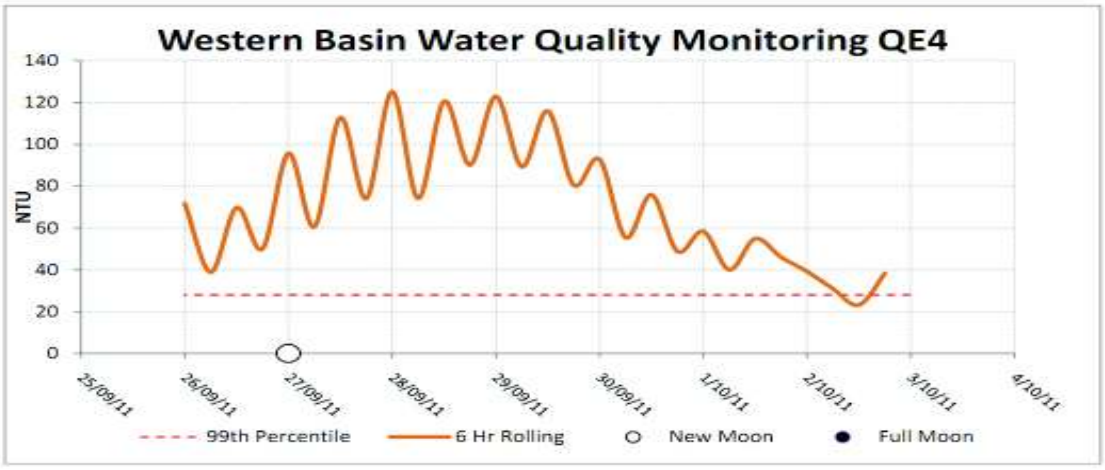

QE4
Turbidity EWMA [NTU]
m
04/04/2012
05/04/2012
06/04/2012
07/04/2012
08/04/2012
09/04/2012
10/04/2012
QE4 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


QE4
Turbidity EWMA [NTU]
m
06/03/2012
07/03/2012
08/03/2012
09/03/2012
10/03/2012
11/03/2012
12/03/2012
13/03/2012
QE4 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

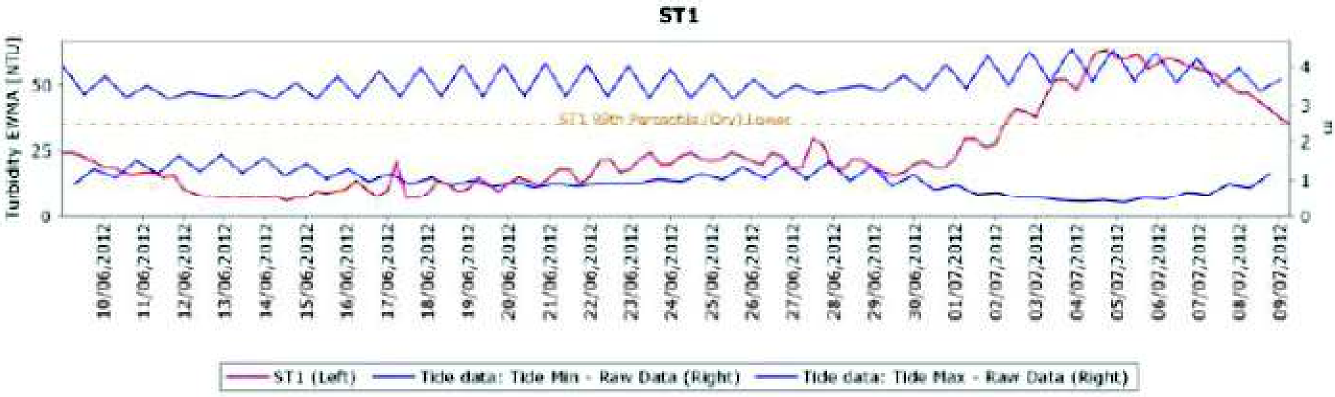

QE4
Turbidity EWMA [NTU]
m
06/02/2012
07/02/2012
08/02/2012
09/02/2012
10/02/2012
11/02/2012
12/02/2012
13/02/2012
QE4 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

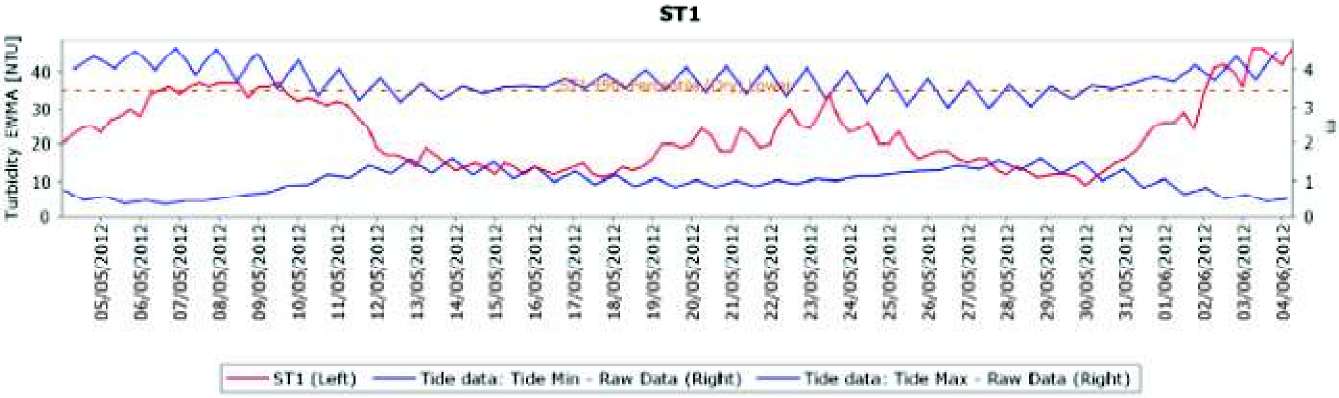

QE4
Turbidity EWMA [NTU]
m
12/01/2012
13/01/2012
14/01/2012
15/01/2012
16/01/2012
17/01/2012
18/01/2012
19/01/2012
QE4 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

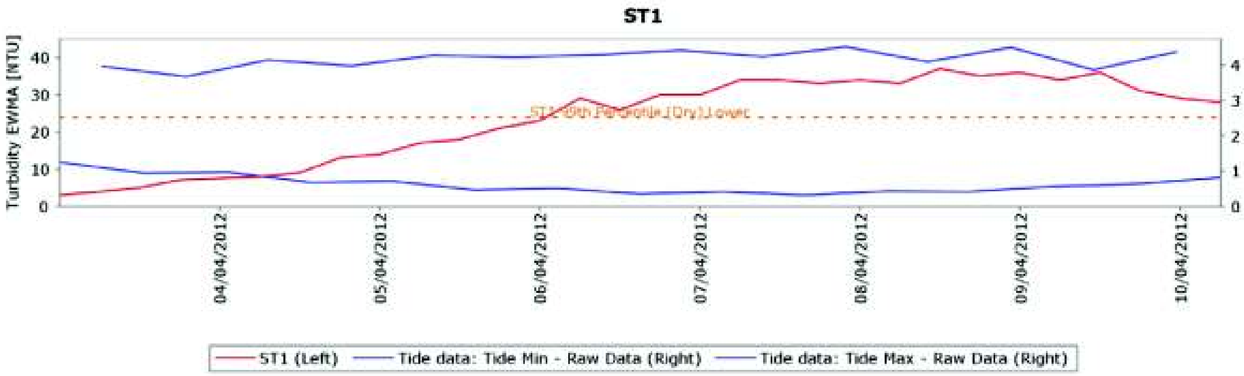

WBDP Dredging Project QE4 - Turbidity Mean, EWMA (NTU) and Tides
NTU
m
150
125
100
75
50
25
0
4
3
2
1
0
26/10/2011
27/10/2011
28/10/2011
29/10/2011
30/10/2011
31/10/2011
QE4: Turbidity Mean - Raw Data (Left)
QE4: Turbidity EWMA - Raw Data (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

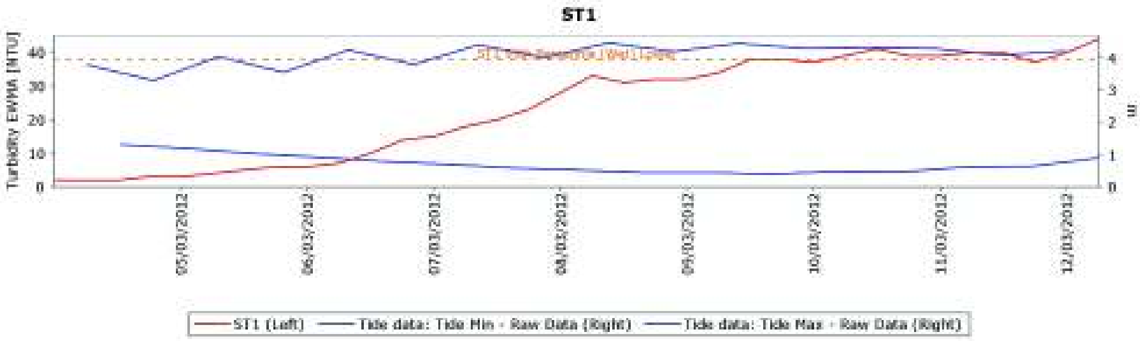

Western Basin Water Quality Monitoring QE4
NTU
140
120
100
80
60
40
20
0
25/09/11
26/09/11
27/09/11
28/09/11
29/09/11
30/09/11
1/10/11
2/10/11
3/10/11
4/10/11
99th Percentile
6 Hr Rolling
New Moon
Full Moon

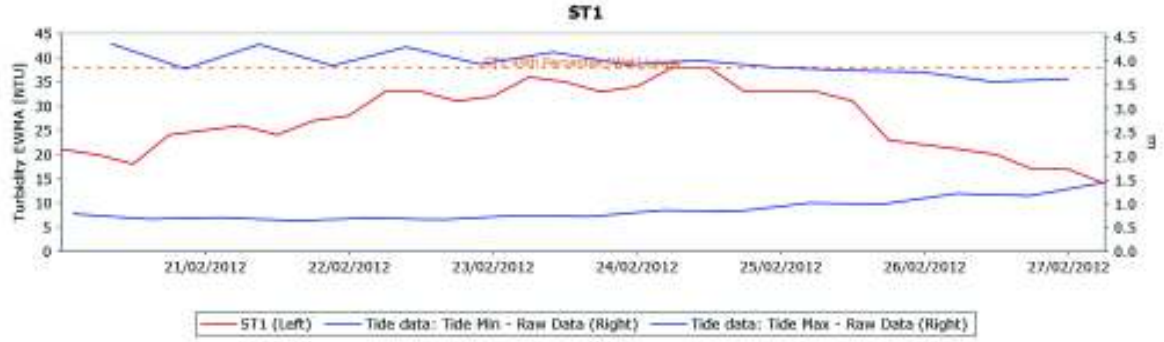

ST1
Turbidity EWMA [NTU]
m
50
25
0
4
3
2
1
0
25/06/2012
26/06/2012
27/06/2012
28/06/2012
29/06/2012
30/06/2012
01/07/2012
02/07/2012
03/07/2012
04/07/2012
05/07/2012
06/07/2012
07/07/2012
08/07/2012
09/07/2012
10/07/2012
11/07/2012
12/07/2012
13/07/2012
14/07/2012
15/07/2012
16/07/2012
17/07/2012
18/07/2012
19/07/2012
20/07/2012
21/07/2012
22/07/2012
23/07/2012
24/07/2012
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


ST1
Turbidity EWMA [NTU]
ST1 99th Percentile (Dry) Lower
m
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


ST1
Turbidity EWMA [NTU]
ST1 99th Percentile (Dry) Lower
m
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


ST1
Turbidity EWMA [NTU]
ST1 99th Percentile (Dry) Lower
m
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


ST1
Turbidity EWMA [NTU]
m
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


ST1
Turbidity EWMA [NTU]
m
21/02/2012
22/02/2012
23/02/2012
24/02/2012
25/02/2012
26/02/2012
27/02/2012
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

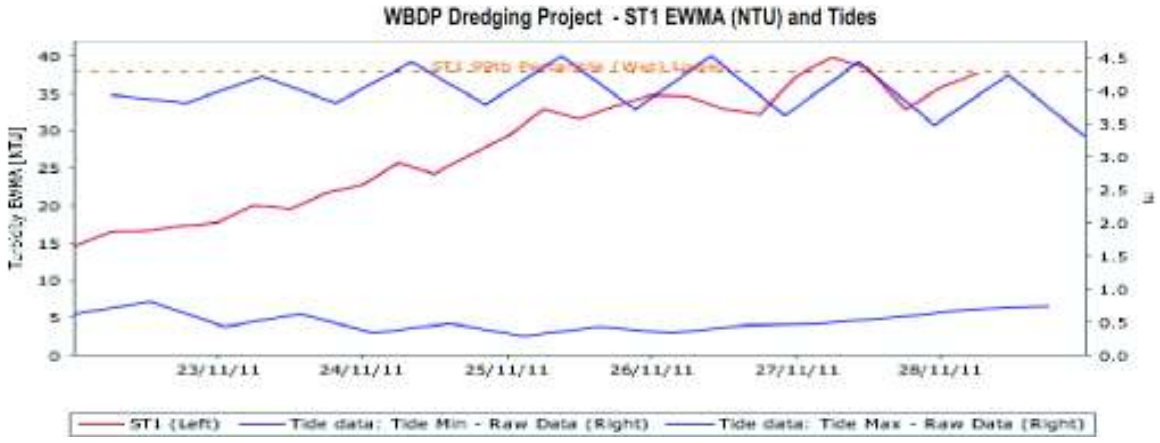

ST1
Turbidity EWMA [NTU]
m
19/01/2012
20/01/2012
21/01/2012
22/01/2012
23/01/2012
24/01/2012
25/01/2012
26/01/2012
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

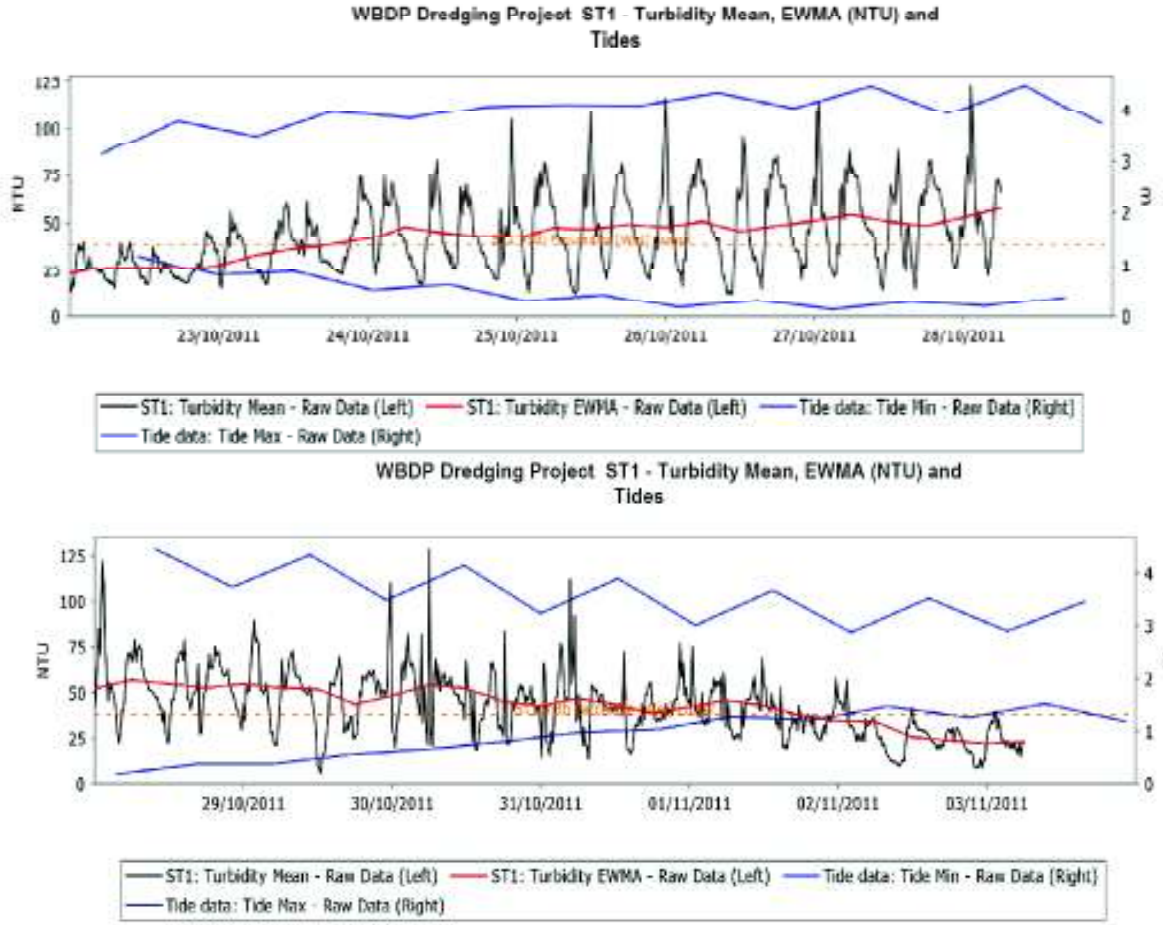

ST1
Turbidity EWMA [NTU]
m
11/01/2012
12/01/2012
13/01/2012
14/01/2012
15/01/2012
16/01/2012
17/01/2012
18/01/2012
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

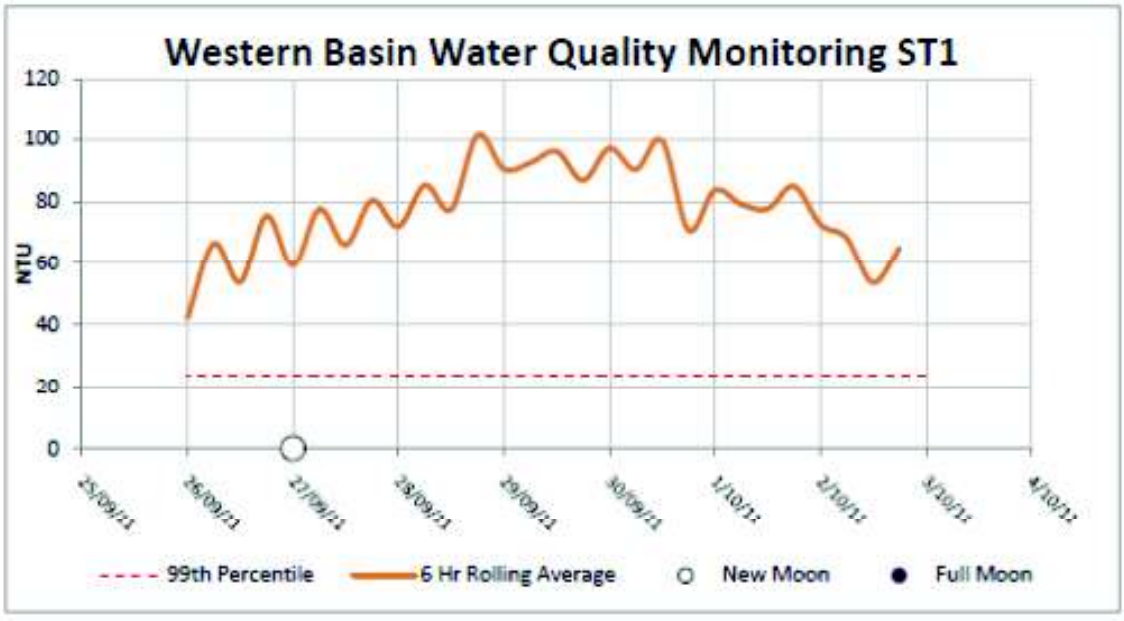

ST1
Turbidity EWMA [NTU]
m
05/01/2012
06/01/2012
07/01/2012
08/01/2012
09/01/2012
10/01/2012
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)

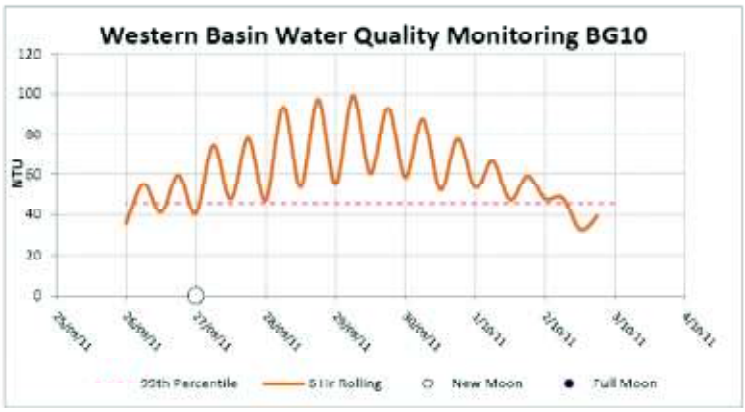

ST1
Turbidity EWMA [NTU]
25/12/2011 26/12/2011 27/12/2011 28/12/2011 29/12/2011 30/12/2011 31/12/2011 01/01/2012 02/01/2012 03/01/2012
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


ST1
Turbidity EWMA [NTU]
16/12/2011 17/12/2011 18/12/2011 19/12/2011 20/12/2011 21/12/2011 22/12/2011 23/12/2011
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


WBDP Dredging Project - ST1 EWMA (NTU) and Tides
Turbidity EWMA [NTU]
23/11/11 24/11/11 25/11/11 26/11/11 27/11/11 28/11/11
ST1 (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


WBDP Dredging Project ST1 - Turbidity Mean, EWMA (NTU) and Tides
NTU
125
100
75
50
25
0
4
3
2
1
0
23/10/2011
24/10/2011
25/10/2011
26/10/2011
27/10/2011
28/10/2011
ST1: Turbidity Mean - Raw Data (Left)
ST1: Turbidity EWMA - Raw Data (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)
WBDP Dredging Project ST1 - Turbidity Mean, EWMA (NTU) and Tides
NTU
125
100
75
50
25
0
4
3
2
1
0
29/10/2011
30/10/2011
31/10/2011
01/11/2011
02/11/2011
03/11/2011
ST1: Turbidity Mean - Raw Data (Left)
ST1: Turbidity EWMA - Raw Data (Left)
Tide data: Tide Min - Raw Data (Right)
Tide data: Tide Max - Raw Data (Right)


Western Basin Water Quality Monitoring ST1
NTU
120
100
80
60
40
20
0
25/09/11
26/09/11
27/09/11
28/09/11
29/09/11
30/09/11
1/10/11
2/10/11
3/10/11
4/10/11
99th Percentile
6 Hr Rolling Average
New Moon
Full Moon



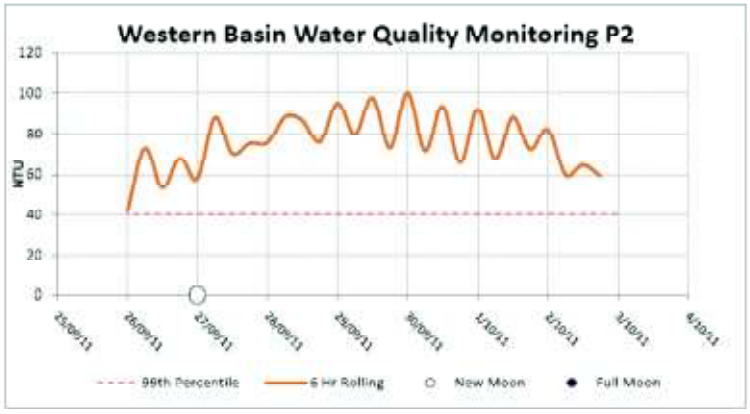
Monitoring Point ID: BG10	
Maximum 99th Percentile Turbidity (NTU)	
56 (Wet)	46 (Dry)

'Wet' season is from 20 November to 31 March inclusive. The remainder of the year is taken to be the 'Dry' season.

A Water Quality Management Plan (WQMP) has been developed for the Western Basin Dredging and Disposal Project (WBDDP) to manage the impacts of turbidity generated by the Western Basin dredging and offshore disposal activities.

This WQMP sees the implementation of a water quality monitoring program that manages water quality within zones of predicted high, medium and low impact, with water quality triggers based on background conditions and predicted sediment plume loading from dredging.

Monitoring sites have been nominated in each designated impact zone and where practical have been situated over or near sensitive receptor locations within those zones. This will enable the measurement and management of any potential dredging and disposal impacts to those receptors.



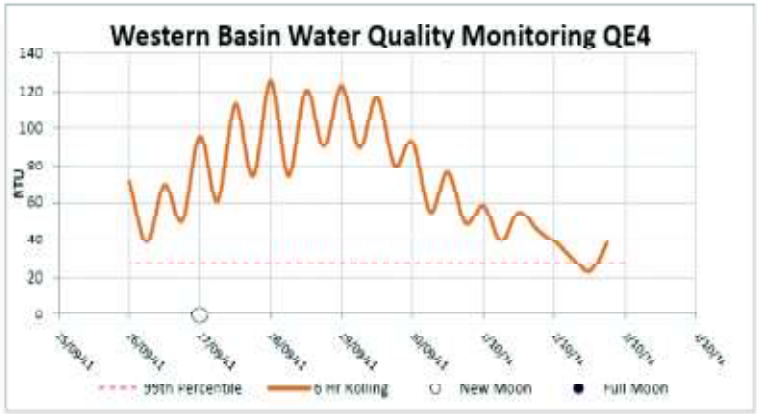
Monitoring Point ID: P2	
Maximum 99th Percentile Turbidity (NTU)	
69 (Wet)	40 (Dry)

'Wet' season is from 20 November to 31 March inclusive. The remainder of the year is taken to be the 'Dry' season.

A Water Quality Management Plan (WQMP) has been developed for the Western Basin Dredging and Disposal Project (WBDDP) to manage the impacts of turbidity generated by the Western Basin dredging and offshore disposal activities.

This WQMP sees the implementation of a water quality monitoring program that manages water quality within zones of predicted high, medium and low impact, with water quality triggers based on background conditions and predicted sediment plume loading from dredging.

Monitoring sites have been nominated in each designated impact zone and where practical have been situated over or near sensitive receptor locations within those zones. This will enable the measurement and management of any potential dredging and disposal impacts to those receptors.



Monitoring Point ID: QE4	
Maximum 99th Percentile Turbidity (NTU)	
34 (Wet)	28 (Dry)

'Wet' season is from 20 November to 31 March inclusive. The remainder of the year is taken to be the 'Dry' season.

A Water Quality Management Plan (WQMP) has been developed for the Western Basin Dredging and Disposal Project (WBDDP) to manage the impacts of turbidity generated by the Western Basin dredging and offshore disposal activities.

This WQMP sees the implementation of a water quality monitoring program that manages water quality within zones of predicted high, medium and low impact, with water quality triggers based on background conditions and predicted sediment plume loading from dredging.

Monitoring sites have been nominated in each designated impact zone and where practical have been situated over or near sensitive receptor locations within those zones. This will enable the measurement and management of any potential dredging and disposal impacts to those receptors.

Monitoring Point ID: ST1	
Maximum 99th Percentile Turbidity (NTU)	
38 (Wet)	24 (Dry)
'Wet' season is from 20 November to 31 March inclusive. The remainder of the year is taken to be the 'Dry' season.	

A Water Quality Management Plan (WQMP) has been developed for the Western Basin Dredging and Disposal Project (WBDDP) to manage the impacts of turbidity generated by the Western Basin dredging and offshore disposal activities.

This WQMP sees the implementation of a water quality monitoring program that manages water quality within zones of predicted high, medium and low impact, with water quality triggers based on background conditions and predicted sediment plume loading from dredging.

Monitoring sites have been nominated in each designated impact zone and where practical have been situated over or near sensitive receptor locations within those zones. This will enable the measurement and management of any potential dredging and disposal impacts to those receptors.

APPENDIX 11: Cross reference for all samples, all sites: pathology reports and proportion of animals with gross signs of disease.

Normal= absence of visible external lesions, and absence of signs of inappetance (tucked up abdomen)

Abnormal= Any visible external lesion including: change in skin colour; erosion of fins/skin/eye, change in eyes, visible signs of inappetance (emaciated body condition of tucked up abdomen)

N- Necropsy

H- Histology

M- Microbiology

A- Lugol's iodine preserved water sample for algal analysis

Table 10: Date, location, animal type, number sampled, number with gross signs of disease, laboratory reference for sampled animals

Date of sample collection	Location	Aquatic Animal type (lesion description if present, no comment denotes an absence of externally visible lesions)	Number of animals examined by eye	Sample processed	Pathology case number
20/01/2012 Jamie and Nathan Govt Observers on board	Hummocky 1 Falzon gill net	Hawkesbill turtle (thin, fluid around heart and free in coelom) freshly dead.	1	1 x NH	JCU 12-179
		Barramundi	1	-	
		Giant trevally	1	-	
		Blue salmon	4	-	
		Stripey	1	-	
		Sickle fish	2	-	
		Yellowfin whiting	1	-	
		Eagle ray	1	-	
		Milk shark	2	-	
		Cat shark	1	-	
		Lemon shark	1	-	
		Whitespot shovelnose	3	-	
		Black tip reef shark (2/7 dorsal skin erosion between dorsal fins associated with attached parasites (30+ in/around lesion)	7	1 x NHM	12-4021148
		Hammerhead shark	1	-	
20/1/2012	Seal rocks-Falzon gill net "queenies"	Queenfish (18/27 queensfish had abnormal red skin lesions, associated with the ectoparasite *Lepeophtheirus spinifer.*	27	6 x NH	12-4021148

		Weasel shark (haemorrhagic lesions near pelvic fins, adjacent copepod attachment sites and extensive haemorrhagic lesions on ventral surface)	2	1 x NH	12-4021148
		Milk shark (haemorrhagic lesions under fins and mouth)	1	-	
20/1/2012	Trawl 1 - Wise	Scaienid (5/300 abnormal with haemorrhagic skin lesions)	300		
		Ghost grinner (18/78 red skin lesions, frayed fins and head/mouth superficial haemorrhagic ulcerations)	78		
		Gotcha crab (*Charybdis* sp.)	82		
		Tannum tiger prawn (6/117 ulceration of carapace and epipenaeon parasites)	117		
		Coral prawn (4/47 with epipenaeon)	47		
20/01/2012	Trawl 2- Wise	Scaienid (2/291 red skin lesions associated with scale loss and tail fin damage)	291	1 x NH	12-3951675
		Ghost grinner (15/32 haemorrhagic skin lesions)	47	1 x NH	12-3951675
		Gotcha crab (*Charybdis* sp.)	225		
		Tannum tiger prawn (1/320 with carapace lesions, 7/320 with epipenaeon parasites)	320		
		Coral prawn	27		
		Banana prawn (1/170 with eroded pleopod and pigmentation, 1/170 with epipenaeon)	170		
		Sole (haemorrhage on ventral skin surface and head)	1	1 x NH	12-3951675
20/01/2012	Trawl 3- Wise	Sciaenid (5/300 skin fin red lesions)	300	3 x NH	12-3951675
		Ghost grinner	25		
		Gotcha crab (*Charybdis* sp.)	133		
		Tannum tiger prawn (2/22 with shell lesions)	22	2 x NH	12-3951675
		Coral prawn	12		
		Banana prawn (1/190 shell lesion and 1/190 with	190	1 x NH	12-3951675

		epipenaeon)			
		Scallop- rough shelled	4	4 x NH	12-3951675
20/01/2012	Trawl 4- Wise	Coachwhip trevally	7		
		Squid	10		
20/01/2012	Trawl 5- Wise	Coachwhip trevally	2		
		Yakka	7		
		Squid	13		
		Pipefish	2		
21/01/2012	Boyne 1 upper-Falzon gill net	Barramundi (Gill copepod-*Lernanthropus* spp.) (6/10 had lesions including clear peritoneal effusion, splenomegaly, petechial haemorrhage on liver, lethargic, empty GIT, tucked up abdominal contour, recovering eye lesions, skin erythema(redness), white patches in gills)	10	2 x NHM	12-2755432
		Bull shark (1/1 ventral erythema, erosion on dorsum between 1st and 2nd dorsal fins associated with infestations of monogenean *Dermophthirius* sp.. No gross internal lesions observed)	1	1 x NHM	12-2755432
		Mullet (5/28 mild reddening in fins exacerbated by stress)	28		
		Scatophagus sp.	2		
		Silver biddy	4		
		Shortbeak garfish	2		
		Winter whiting	2		
21/01/2012	Upper Boyne Site 2- Falzon gill net	Barramundi (6/8 had a range of red skin lesions from superficial to deep ulcers, multifocal pale gill lesions, tucked up abdomens, peritoneal effusion, rock stuck in one skin lesion)	8	1 x NHM	12-2755432
		Bull shark (3/3 multifocal depigmented ulcerated skin lesion on dorsum between dorsal fins, and on lateral surface associated with *Dermophthirius* sp.. Live sharks rapidly turn red after removal from water.	3	3 x NHM	12-2755432
	Cast net	Forktail catfish (One fish had red fins and	2	2 x NHM	12-2752432

		haemorrhage around sclera of eyes and mouth, the other was severely emaciated but had ingesta in its gut.			
	Cast net	Yellowfin bream	6		
	Cast net	Silver biddy	6		
	Cast net	Mullet	7		
	Cast net	Whiting	4		
21/01/2012	Lower Boyne- Falzon- gill net	Barramundi (1/1 red lesion behind pectoral fin, and red ventral abdomen, low level of abdominal fat, 40ml + abdominal clear effusion, splenomegaly, shrunken dark liver with large amount of pigmented areas, tucked up abdomen	1		
22/01/2012	Benaraby bridge Boyne river- Falzon- gill net	Barramundi (13/14 superficial red ventral skin, multifocal pale areas on gill, recovered eye lesion, tucked up abdomen, some healing skin lesions with hyperpigmentation, some gill monogeneans, one fish had bilateral exopthalmus, one had corneal damage and rupture, one had corneal ulceration and rupture, oedema on surface of liver, 2 fish gills examined under microscope- moderate to high levels of monogenean flukes, moderate to high level of suspect intra-lamellar myxozoa, suspect blood fluke eggs)	14	6 x N 5 x H 2 x gill biopsy fresh examination	12-4021148
		Spotted Scatophagus	1		
23/01/2012	Calliope river boat ramp- Roberts	Oysters (large numbers of dead rock oysters which were yet to be colonised by other biota)	>300		
23/01/2012	Grahams Creek Lower- Roberts- crab pot	Mud crab (1/14 with severe shell disease)	14		
23/01/2012	Friends Point- Roberts- crab pot	Mud crab (16/72 with minor to severe shell lesions, most consistent with description by Andersen and Norton of shell disease)	72	5 x NH	12-5311480
24/01/2012	Colosseum- Robson- crab	Mud crab (12/33 with shell lesions, most	33	5 x NH	

	pot	consistent with description by Andersen and Norton of shell disease)			
25/01/2012	7 Mile- Putmin-gill net	Whiting (1/45 skin ulcer	45	1 x NHM	12-5251389
		Mullet (4/145 skin ulcers and redness in skin)	145	4 x NHM	12-5251389
		Blue salmon	2		
		Queenfish	2		
		Whitespot shovelnose shark (1/1 skin lesions associated with ectoparasites)	1	1 x N	
25/01/2012	Turkey Beach-Hodgetts crab pot	Mud crab (3/45 rust spot lesions)	45		
		Bream	7		
		Estuary cod	1		
15/02/2012 Mud crab shell lesion prevalence study	Narrows-Badger creek-crab pot	Mud crab (4/7 shell lesions)	7		
	Narrows-Mosquito Creek- crab pot	Mud crab (6/11 shell lesions)	11		
	Narrows-Deception 1-crab pot	Mud crab (9/17 shell lesions)	17		
	Narrows-Deception 2 and 3- crab pot	Mud crab (4/14 shell lesions)	14		
	Narrows- 3rd creek Deception- crab pot	Mud crab (6/10 shell lesions)	10		
	Narrows- crab pot	Mud crab (6/11 shell lesions)	11		
	Colosseum 16-23- crab pot	Mud crab (10/25 shell lesions)	25		
	Colosseum 25-32- crab pot	Mud crab (19/36 shell lesions)	36	5 x NH	12-5311480
	Colosseum 33-39- crab pot	Mud crab (6/18 shell lesions)	18		
	Colosseum 40-54- crab pot	Mud crab (7/20 shell lesions)	20		
	Colosseum 55-65- crab pot	Mud crab (5/40 shell lesions)	40		
16/02/2012 Mud crab shell lesion prevalence study	Enfield Creek Upper and branch 2- crab pot	Mud crab (28/61 shell lesions)	61	5 x NH	12-5311480
	Nutmeg Creek-crab pot	Mud crab (24/36 shell lesions)	36		

	Barge Landing- crab pot	Mud crab (23/25 shell lesions)	25		
	Flying Fox River Wpt 7- crab pot	Mud crab (17/47 shell lesions)	47		
	Adjacent dredges Wpt 71- crab pot	Mud crab (10/17 shell lesions)	17		
	Grahams Creek Wpt 72- crab pot	Mud crab (6/15 shell lesions)	15		
	Grahams Creek Wpt 73- crab pot	Mud crab (5/8 shell lesions)	8		
22/02/2012	Facing Island- Falzon- gill net	Queenfish (2/6 red tail, gut lesions consistent with heavy parasitic load)	6	1 x NH	12-5291766
		Shovelnose shark (peritoneal effusion, red claspers and skin lesions)	1	1 x NH	12-5291766
		Bronze whaler (1/1 skin ulcers some full thickness, skin erythema)	1	1 x NH	12-5291766
		Blubberlip bream (2/2 skin erythema, bilateral corneal opacity, evidence of internal parasitism, and multi-focal gill damage)	2	2 x NH	12-5291766
		Black tip whaler (2/3 skin erythema)	3		
		Black jewfish (1/1 bilateral scleral erythema, skin erythema)	1		
		2 x hard Coral species (*Acropora* sp and *Pocillopora* sp._ (4/4 discolouration, multifocal extensive white lesions)	4	1 x NH	Coral Pathology image report
		Whitespot shovel nose shark	1		
22/02/2012	Sable chief rocks- Falzon- gill net	Black tip whaler (9/13 skin erythema)	13		
		Scallop hammerhead (1/1 skin erythema)	1		
		Weasel shark (1/1 skin erythema)	1		
		Bronze whaler (1/3 skin ulceration and erythema)	3		
		Whaler shark (4/4 skin erythema 1/4 kin ulceration)	4		
		School shark	1		
		Slatey bream (1/1 erythema around vent)	1		
		Batfish	1		
23/02/2012	Algae site 1	Algae	1	1 x A	

	(Curtis Is adjacent dredging) 25mm plankton net				
23/02/2012	Algae site 2 (Calliope river mouth) 25mm plankton net	Algae	1	1 x A	
	Algae site 3 (Bridge) 25mm plankton net	Algae	1	1 x A	
	Algae site 4- (Colosseum) 25mm plankton net	Algae	1	1 x A	
	Algae site 5- 25mm plankton net	Algae	1	1 x A	
	Algae site 6- 25mm plankton net	Algae	1	1 x A	
	Algae site 7- (Auckland creek) 25mm plankton net	Algae	1	1 x A	
24/02/2012	Algae site 8- Benaraby bridge- 25mm plankton net	Algae	1	1 x A	
24/02/2012	Benaraby bridge- Mercer gill net	Barramundi	1	1 x NM Fresh gill preparations (normal)	
24/02/2012	Boyne upper- Mercer- gill net	Barramundi (1/1 large lateral dermal ulceration with haemorrhage, hook lesion in mouth, free fluid in abdomen, effusion within serosa of liver, gill biopsy high level hennegya sp., suspect sanguillicolid eggs in gill)	1	1 x NM Fresh gill preparation	
27/02/2012	Harbour trawl 1- Wise	Sole (1/1 skin erythema and ulceration)	1	1x NH	12-5256385
		Banana prawn (2 out of ~5kg carapace lesion caudal to eye)	5kg (avg 12-14cm length)	1 x NH	12-5256385
		Herring (1/1 erythema on jaw)	1	1 x NH	12-5256385
		Tannum tiger prawn	8kg		
		Scaienid (soapy jewfish)	154		
		Scaienid (grunter)	178		
		Black sting ray	1		
27/02/2012	Harbour trawl 2- Wise	Ghost grinner (1/1 tail fin erosion and erythema)	1	1 x N	

		Banana prawn (5 prawns out of 4.2 kg had carapace lesions)	4.2kg	1 x NH	12-5256385
		Tannum tiger prawn (3 prawns out of 3kg had epipenaeon parasites deforming their carapace)	3kg		
		Scaienid (soapy jewfish)(5/124 with intraocular lesions- suspect retinal detachment, one with tail fin erosion and erythema)	124	1 x NH	
		Scaienid (grunter)	144		
27/02/2012	Curtis Island- McMillan 1- gill net	Barramundi (1/2 multifocal white lesions in gill)	2	2 x NH	12-5256385
	Curtis Island- McMillan 2- gill net	Barramundi (1/1 proliferative intestinal mucosa and erythema)	1	1 x NH	12-5256385
28/02/2012	7 Mile- crab pot prevalence study	Mud crab (44/134 shell lesions)	134		
29/02/2012	7 Mile- Otto - gill net	Oyster cracker fish (3/3 bilateral distal pectoral fin erosion and erythema, 3 suspect caligid skin parasites, parasitic worms in intestine)	3	3 x NHM	12-5260818
		Barramundi (1/4 skin erythema ventral abdomen, multifocal white patches in gill)	4	1 x NHM	12-5260818
		Forktail catfish (2/2 three and eight suspect caligid sp. parasites on skin. Skin colour normal)	2		
		Threadfin salmon	2		
		Eagle ray	1		
		Scallophead hammerhead shark (1/1 skin erythema)	1		
		Blacktip whaler shark (2/4 skin erythema)	4		
		Giant herring	1		
		Whitespot shovelnose shark	2		
19/04/2012	Stanage 1- Holland- gill net	Barramundi (1/75 with 1 x 1.5cm focal skin ulcer- rest of skin normal)	75	5 x NH (inc. fish with skin lesion)	12-5487595
		King salmon (red lesion on flank suspect net trauma)	1	1x NH	12-5487595
19/04/2012	Stanage 2- Holland- gill net	Barramundi	1		
		Queenfish (nil visible ectoparasites, nil external gross lesions)	1	1 x NH	12-5487595

	Stanage 3- Holland- gill net	King salmon	9		
		Barramundi	1		
		Forktail catfish (nil external gross lesions)	13	2 x NH	12-5487595
		Blue salmon	3		
		Flat salmon	4		
		Whaler shark (5/5 from 1-4 suspect caligid sp on skin. No discolouration or erosion present)	5	5 x NH	12-5487595
20/4/2012	Stanage 4- Holland- gill net	Stingaree (1x1 skin erythema(suspect acute bruising on ventral surface)	1	1 x NH	12-5487595
		Dart (no gross external lesions)	7	1 x NH	12-5487595
		Giant herring	6		
		Squid	1		
20/04/2012	Stanage 5- Holland- gill net	Flat salmon	1		
		Mullet(1/5 skin ulcer(acute damage suspected) on dorsal caudal peduncle)	5	5 x NH	12-5487595
		Forktail catfish (2/9 fish had one suspet caligid sp parasite. All skin was grossly normal in colour and as not visibly ulcerated)	9		
		Grunter	1		
		Dog mackeral	1		
21/04/2012	Stanage 6- crab pots- Glanford	Mud crab (21/153 with shell lesions)	153	5 x H	12-5487595
	Seaward of ocean spoil dumping ground- trawl 1	Saucer scallop	5	5 x H	12-5795283
	Seaward of ocean spoil dumping ground- trawl 2	Saucer scallop	5	5 x H	12-5795283