Infection of wild queenfish with sea lice (*Lepeophtheirus spinifer* and *Caligus* spp.) in Australia,

with observations of skin rashes in fish from Gladstone Harbour, Queensland.

Dr. Ben Diggles

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Queensland

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- Shane Griffiths, CSIRO

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Northern Territory

- Kate Hutson, Will Macbeth, Lachlan Barnes, other field staff at Cardno Environmental Services, and INPEX Operations Australia Ltd
- NT Fisheries staff for assistance with field collections

Information from the NT was sourced from work undertaken for INPEX Operations Australia Pty Ltd in connection with the Ichthys LNG Project, and is based on data available at the time of preparation, from sources believed to be reliable and subject to the qualifications in the Disclaimer presented at the end of this talk.

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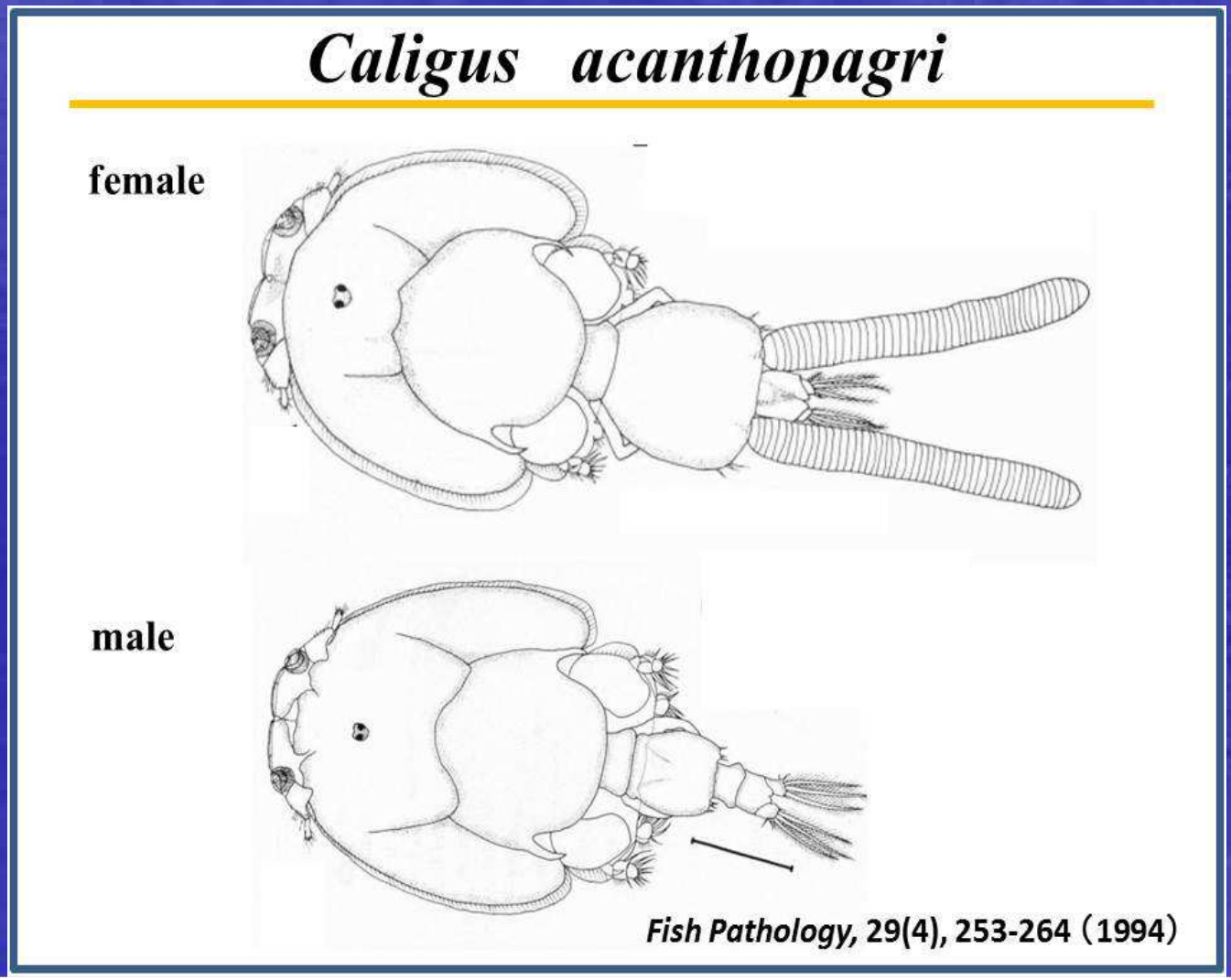
- Prof. Mike Kent, Oregon State University, USA
- Dr Mike Hine, France

"Sea lice" infections of finfish

- Crustacean ectoparasites of the fins and body surfaces of fishes.
- SubFamily Copepoda, Family Caligidae.
- In total around 37 genera and over 500 species worldwide
- Most common genera *Caligus* spp. (250+ species), and *Lepeophtheirus* spp. (150 + species)

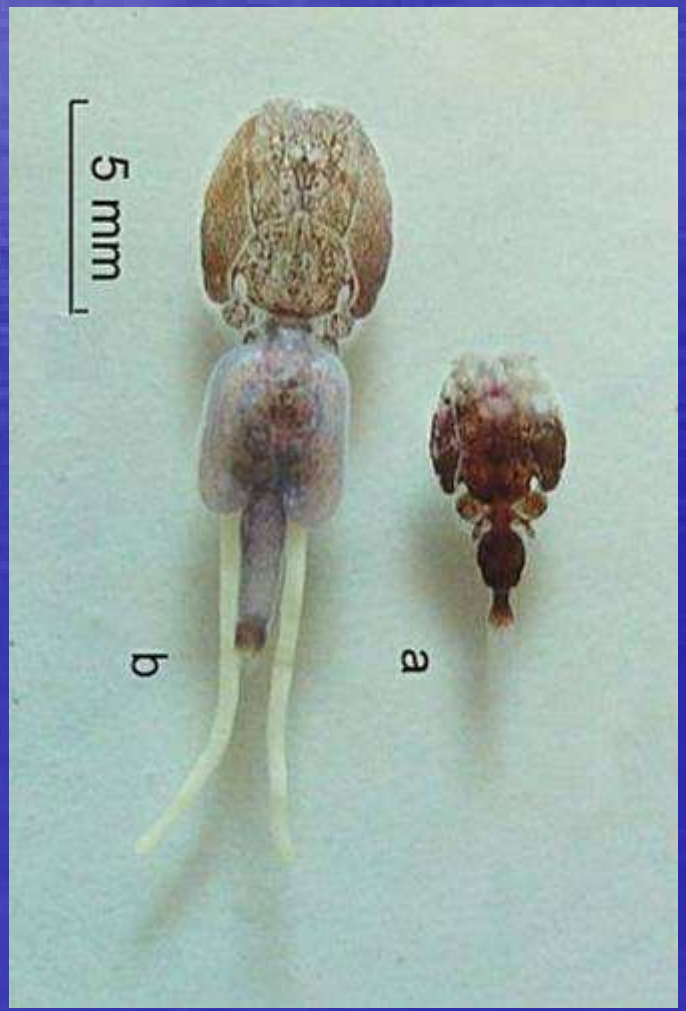
"Sea lice" infections of finfish

- *Caligus* spp.



"Sea lice" infections of finfish

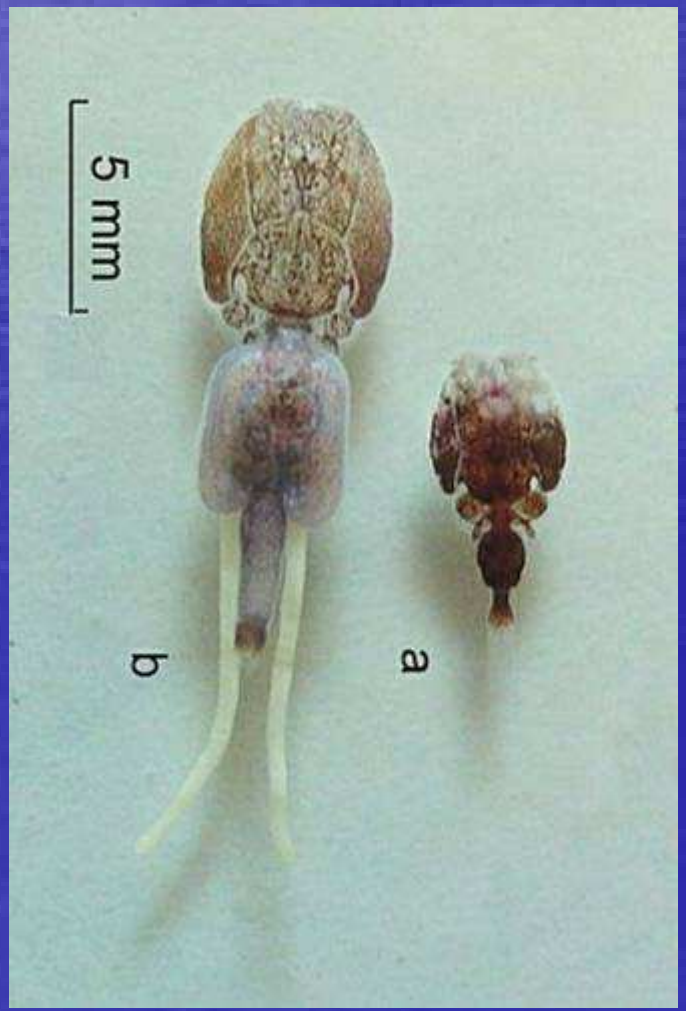
- *Lepeophtheirus* spp. (*L. salmonis*)



Pictures by Mark Costello and Ian Bricknell

"Sea lice" infections of finfish

- *Lepeophtheirus* spp. (*L. salmonis*)



Pictures by Mark Costello and Ian Bricknell

Caligid life cycle

- Egg → Nauplii (I and II) → Copepodid are planktonic larval stages (c. 1 week). Latter colonises host fish and moults into Chalimus
- Chalimus anchored to fishes body by a frontal filament, moulting to a pre-adult. These and adults cling to the body surface with prehensile appendages and are capable of free movement (though can be site specific).
- Adults feed on host mucous and blood. Can cause skin lesions when in large numbers.
- Generation time of 1-2 months with adult caligids living for around 6-7 months.

Caligids in Aquaculture

- Sea lice rarely occur in significant numbers or cause pathological effects on wild fish[1].
- But cause disease in the culture of salmonids, sparids, and groupers. The problems with sea lice in salmon culture are well known. Also emerging in culture of trevallies (Carangidae).
- Cruz-Lacierda et al. (2011) – oyster cracker dart (*Trachinotus blochii*) cultured in Philippines infected by up to 50 *Lepeophtheirus spinifer*.
- Infected fish became emaciated, and exhibited haemorrhagic lesions on the body surface.

1. Wooten et al. (1982). *Proc. Roy. Soc. Edinburgh (B)* 81: 185-197.

L. spinifer, pic. B. Diggles

Parasitic caligid copepods of farmed marine fishes in the Philippines

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ABSTRACT

Recently, heavy infestation of caligid copepods occurred among farmed rabbitfish *Siganus guttatus*, pompano *Trachinotus blochii* and sea bass *Lates calcarifer* in the Philippines. In *S. guttatus* broodstock, *Caligus epidemicus*, *Pseudocaligus umiartus* and *Lepeophtheirus sigani* concurrently caused severe erosion and hemorrhaging of the body surface, fins and eyes of affected fish occurring at 95.78%, 1.52% and 0.70% of the parasite load, respectively, and with associated mortality of the host fish. In marketable-sized *T. blochii*, *L. spinifer* caused body lesions that considerably reduced the market value of harvested fish. In *L. calcarifer* juveniles, infestation with *C. epidemicus* resulted to loss of appetite, lethargy and stunted growth of affected fish. Because of its pathogenicity, low host specificity and tolerance to brackish water, *C. epidemicus* poses the highest threat to farmed marine fish in the Philippines. *Lates calcarifer* and *T. blochii* are new host records for *C. epidemicus* and *L. spinifer*, respectively. This is also the first record of *L. spinifer* in the Philippines.

Key words: caligid, rabbitfish, pompano, sea bass, sea lice

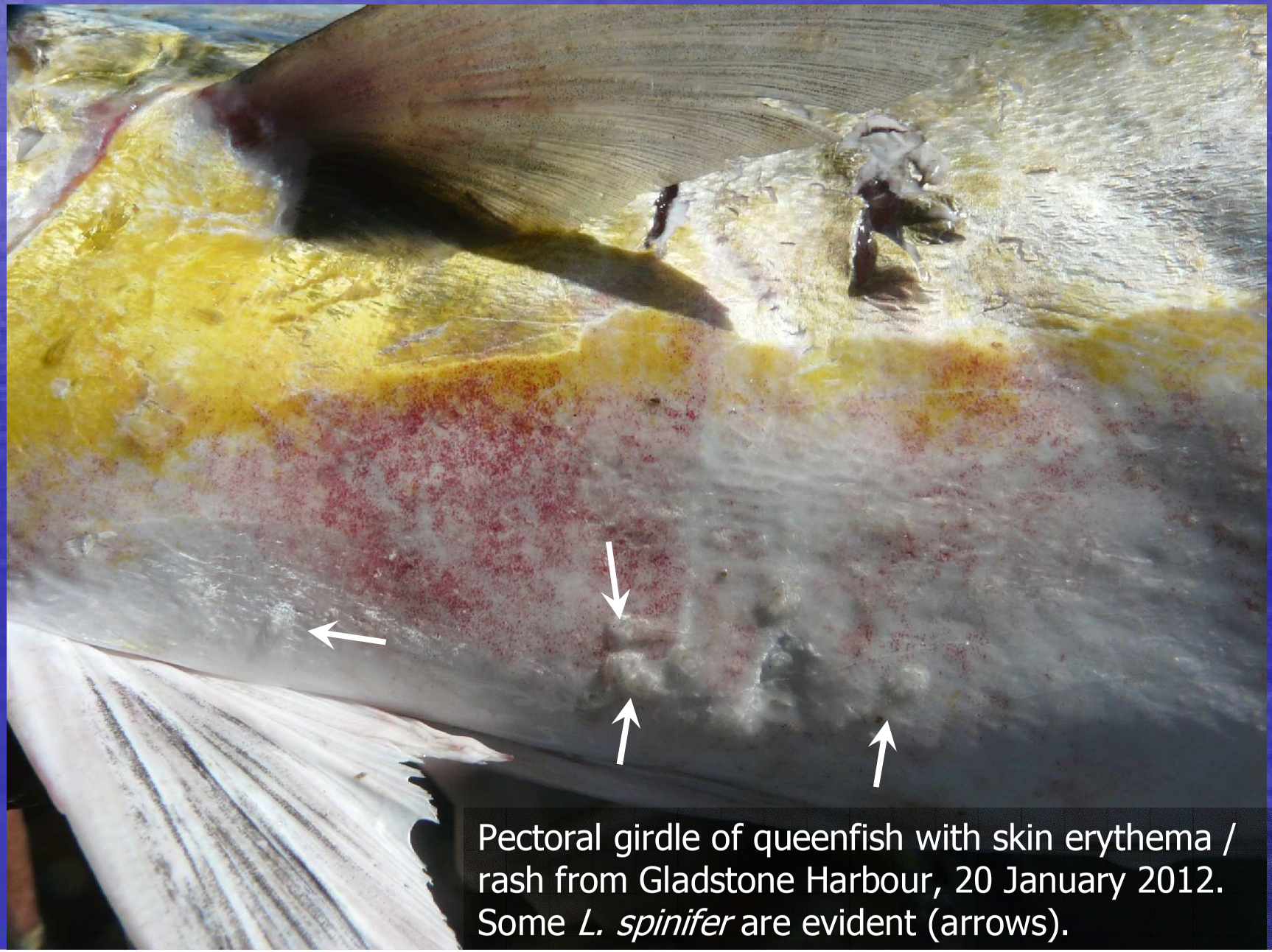
Lepeophtheirus spinifer in Australia

- *Lepeophtheirus spinifer* appears to have been first recorded in Australia on queenfish (*Scomberoides commersonnianus,* Family Carangidae) from Gladstone Harbour in QLD in January 2012.
- These fish (n = 27) were captured in gill nets at water temperatures of 24°C, 36 ppt salinity.
- 2/3 (n = 18 fish), had haemorrhagic lesions on the pectoral girdle resulting in skin erythema. Fish alive at time of examination.
- Prevalence of *L. spinifer* was 100%, with a mean intensity of 21.2 copepods/fish (range 4-46).
- Site specificity for the pectoral girdle area was noted.

L. spinifer – Gladstone Harbour

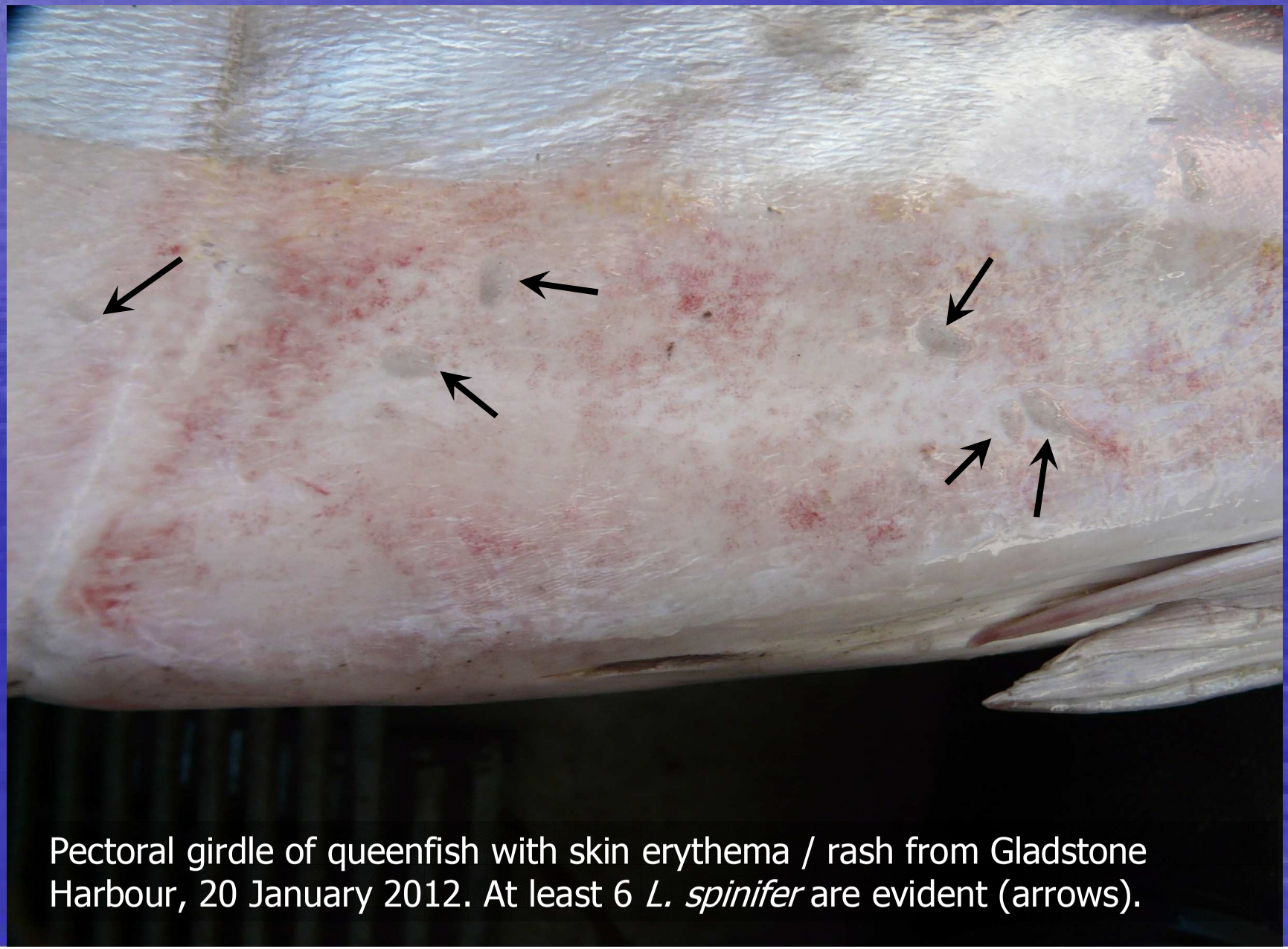
Queenfish with skin erythema / rash from Gladstone Harbour, 20 January 2012.

L. spinifer – Gladstone Harbour



Pectoral girdle of queenfish with skin erythema / rash from Gladstone Harbour, 20 January 2012. Some *L. spinifer* are evident (arrows).

L. spinifer – Gladstone Harbour



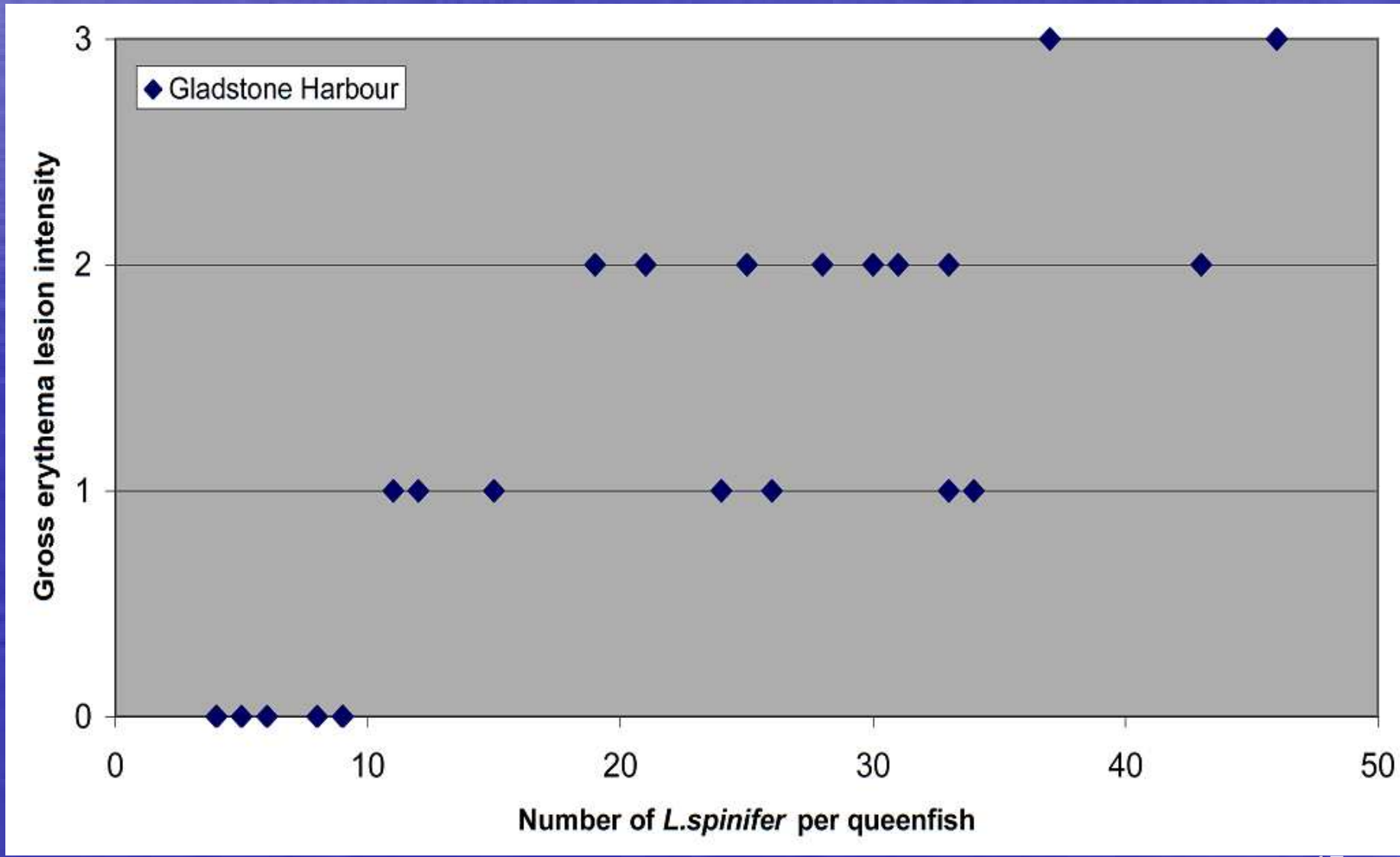
Pectoral girdle of queenfish with skin erythema / rash from Gladstone Harbour, 20 January 2012. At least 6 *L. spinifer* are evident (arrows).

L. spinifer – Gladstone Harbour

- Intensity of *L. spinifer* infection was compared against the extent of skin rashing by scoring lesion intensity as follows:

Lesion score	Gross lesion appearance	% coverage upon gross inspection
0	Erythema absent	No rash
1	Erythema visible	<25% of pectoral girdle with rash
2	Moderate erythema	25-50% of pectoral girdle with rash
3	Severe erythema	>50% of pectoral girdle with rash

L. spinifer – Gladstone Harbour



Is this normal for Queenfish ?

- Fishers in Gladstone Harbour had not seen rashed queenfish prior to mid-late 2011. (Personal communications, local commercial fishers, J. Wilhelm, Gladstone fishing guide).
- Charter fishers (Dave Donald Sportfishing) have captured in excess of 25,000 queenfish in north Queensland over the last 20 years. Guides in Hervey Bay (Andrew Chorley) 190 km to south also catch queenfish.
- CSIRO >300 queenfish Gulf of Carpentaria 2004-05. DEEDI >36 fish from Central QLD, mid 2012
- None of these people have ever observed queenfish with skin rashes like those sampled from Gladstone Harbour in January 2012.

Sea lice – Northern Territory

- Baseline data is available for sea lice (*L. spinifer* and *Caligus* spp.) infections in wild queenfish (n = 112) from the NT.
- Fish were sampled from several sites over the period of Aug 2012 - March 2014 using gill nets (water temps to 34°C, salinity 35 ppt).
- Sealice prevalence in NT 55.4%, mean intensity 3.58 sealice /fish (range 0-26).
- No seasonal or spatial variation evident (even during wet season), and no skin rashes observed on any fish.

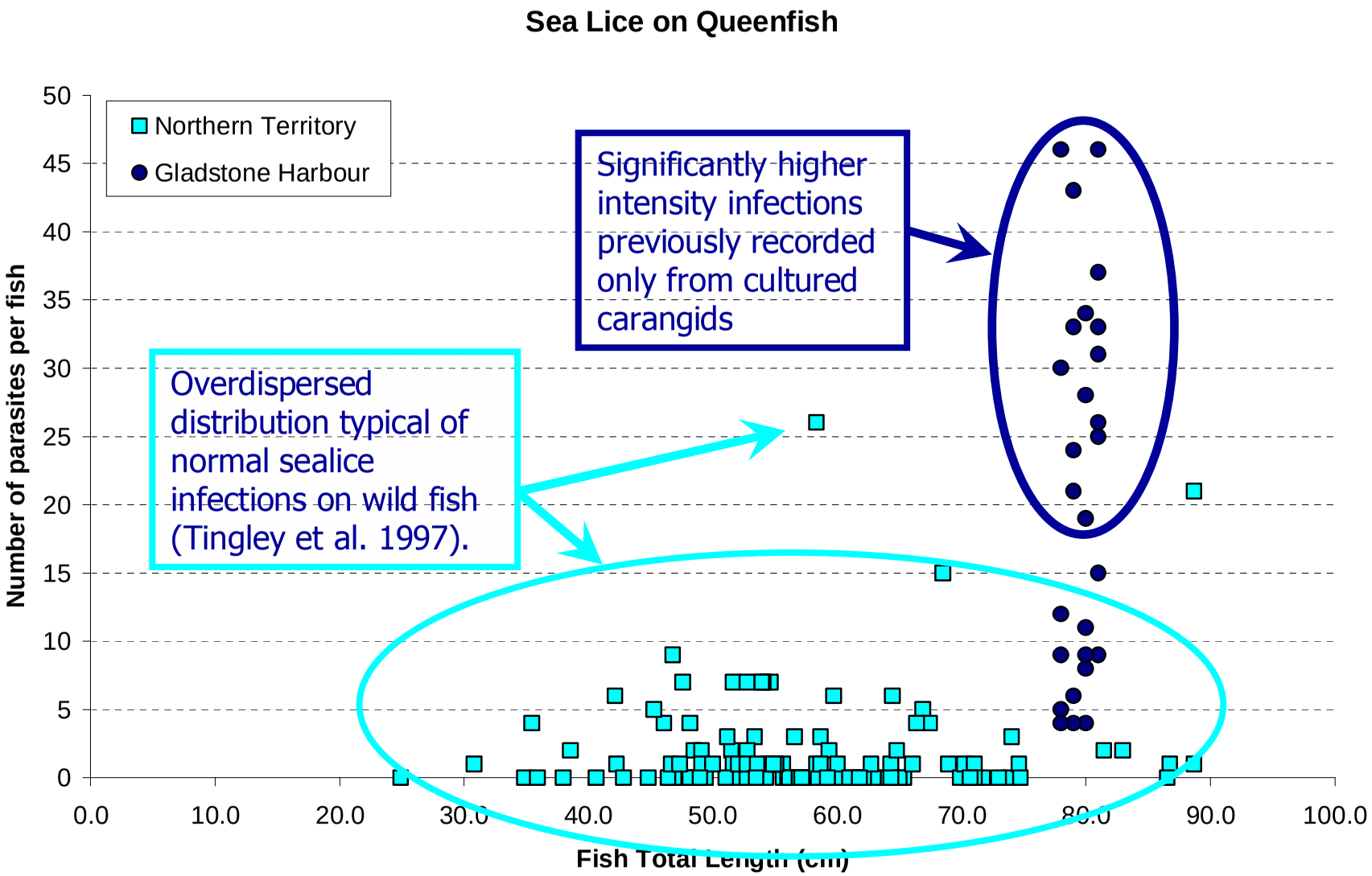
Sea lice on queenfish in Australia

Queenfish	Gladstone Harbour	All NT	Darwin Harbour	Bynoe Harbour	Adelaide River
No. sampled	27	112	85	25	2
mean length (TL, cm)	79.60	57.00	57.6	52.5	85.8
Range (TL, cm)	78-81	24.9 -88.7	30.8-88.6	24.9-71.7	82.9-88.7
SD	1.15	12.01	11.76	9.51	4.08
Sea lice					
Prevalence	100%	55.4%	60.0%	36.0%	100%
Mean intensity	21.2***	3.58	3.65	3.67	1.5
SD	13.72	4.56	4.86	3.12	0.71
Range	4-46	0-26	0-26	0-9	1-2
Lesion prevalence	66.60%	0%	0%	0%	0%
Mean lesion intensity	1.78	n/a	n/a	n/a	n/a

*** Significantly different at $P<0.001$ (ANCOVA, controlling for fish length)

- Note: Mean intensity of c. 4 sea lice per fish is typical of baseline infection levels of wild salmonids infected with *L. salmonis* (see Tingley et al. (1997). *ICES J. Mar. Sci.* 54: 1120-1128).

Sea lice on queenfish in Australia



Normal gillnet caught queenfish

Normal 86 cm TL queenfish caught by gill net in Adelaide River, NT, March 2013.

Normal gillnet caught queenfish

Pectoral girdle area of normal gill net caught queenfish from Darwin Harbour, March 2013.

Normal gillnet caught queenfish

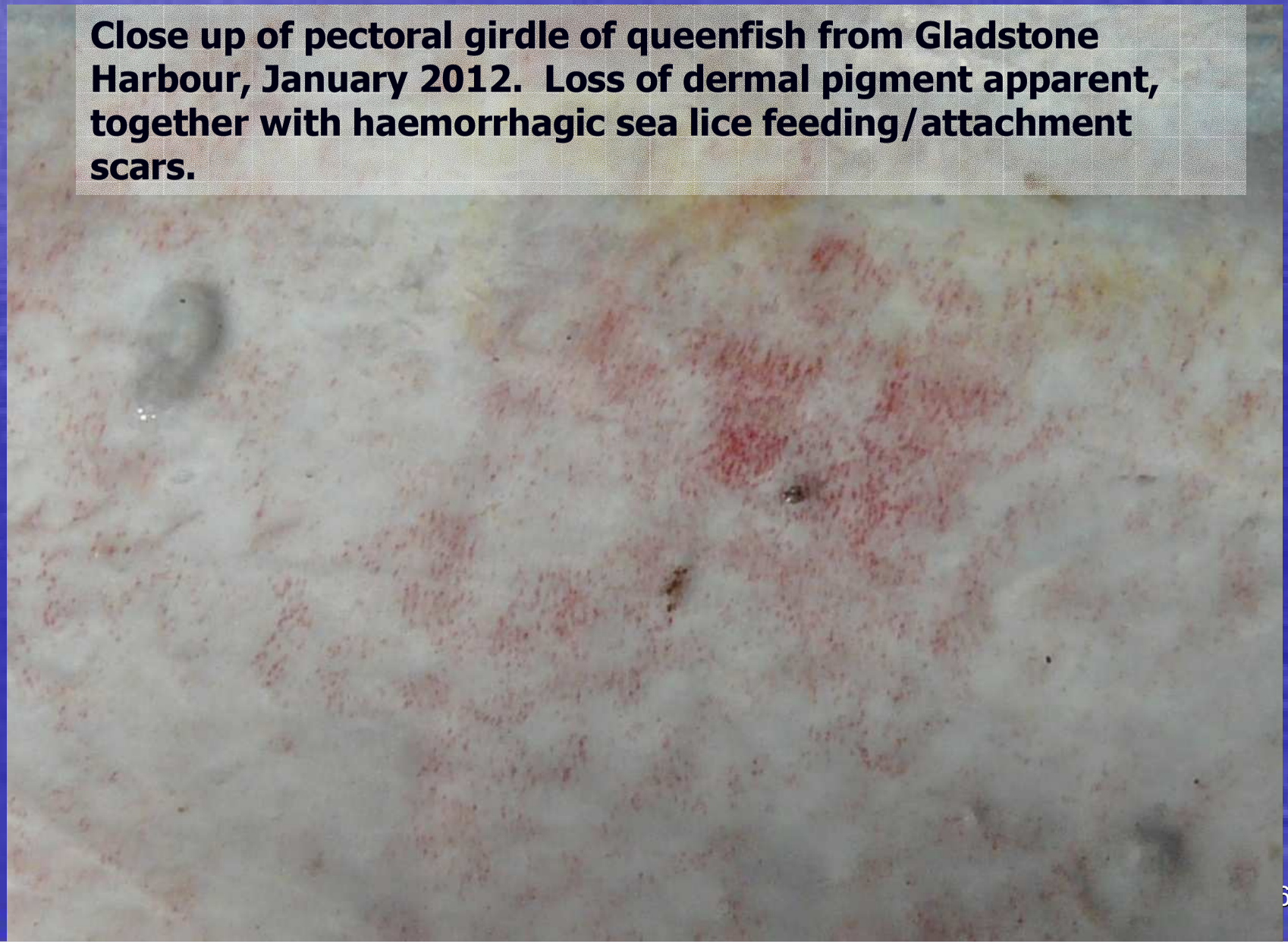
Pectoral girdle area of normal gill net caught queenfish from Darwin Harbour, March 2013.

Normal gillnet caught queenfish

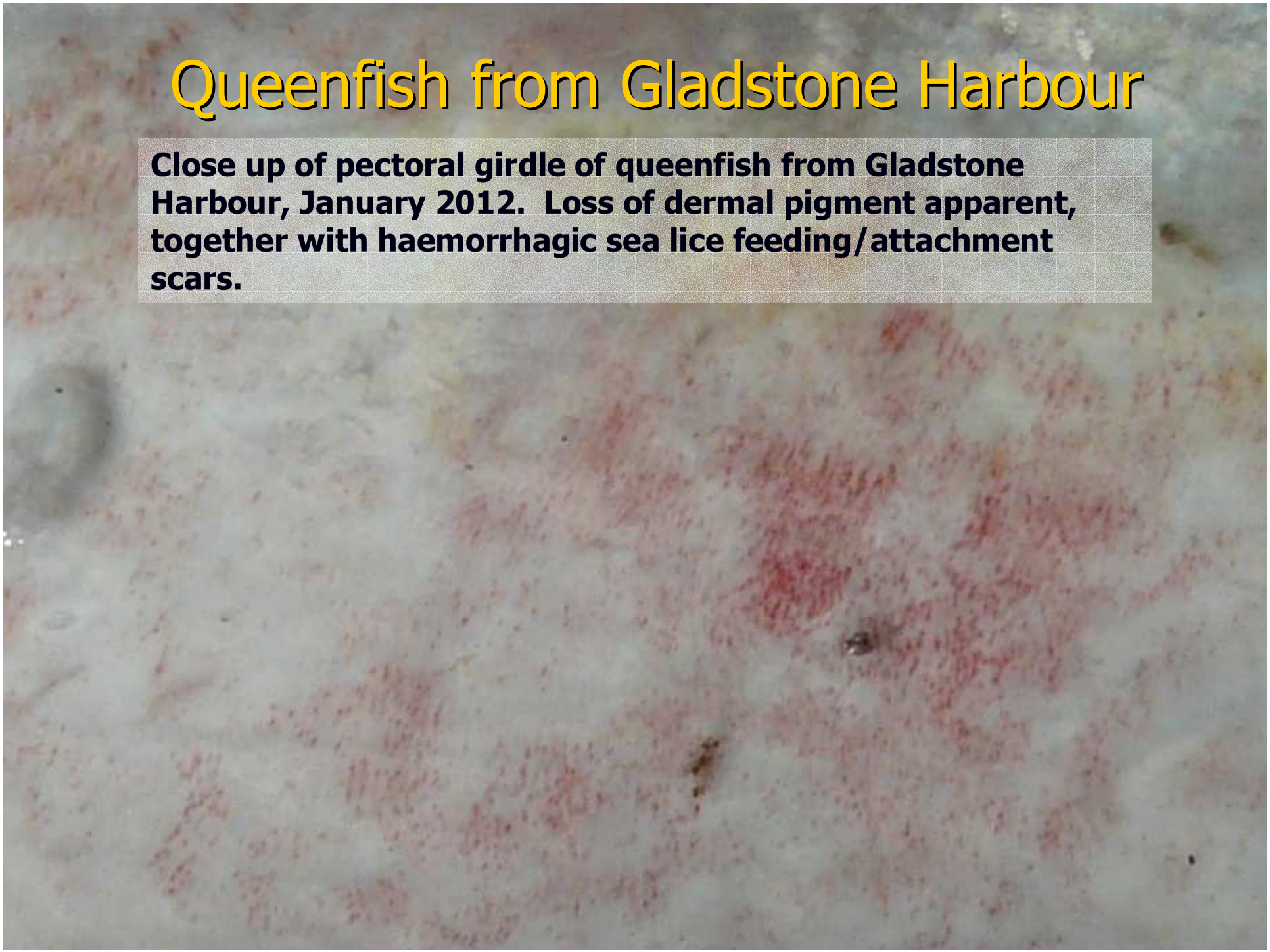
Large net caught Queenfish taken in Brunei Darussalam in June 2012. Note absence of skin erythema.

Queenfish from Gladstone Harbour

Close up of pectoral girdle of queenfish from Gladstone Harbour, January 2012. Loss of dermal pigment apparent, together with haemorrhagic sea lice feeding/attachment scars.



Queenfish from Gladstone Harbour

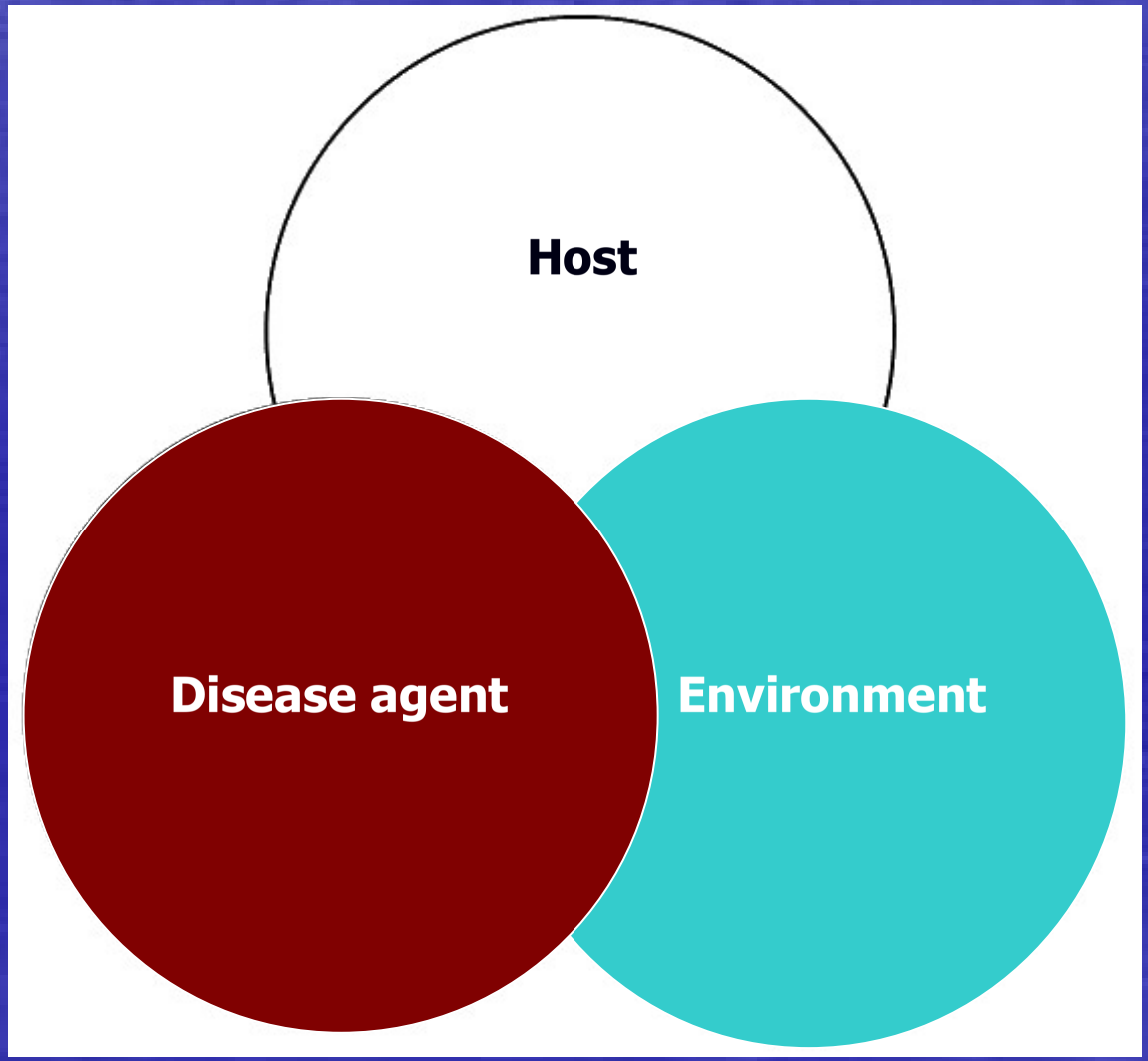


Close up of pectoral girdle of queenfish from Gladstone Harbour, January 2012. Loss of dermal pigment apparent, together with haemorrhagic sea lice feeding/attachment scars.

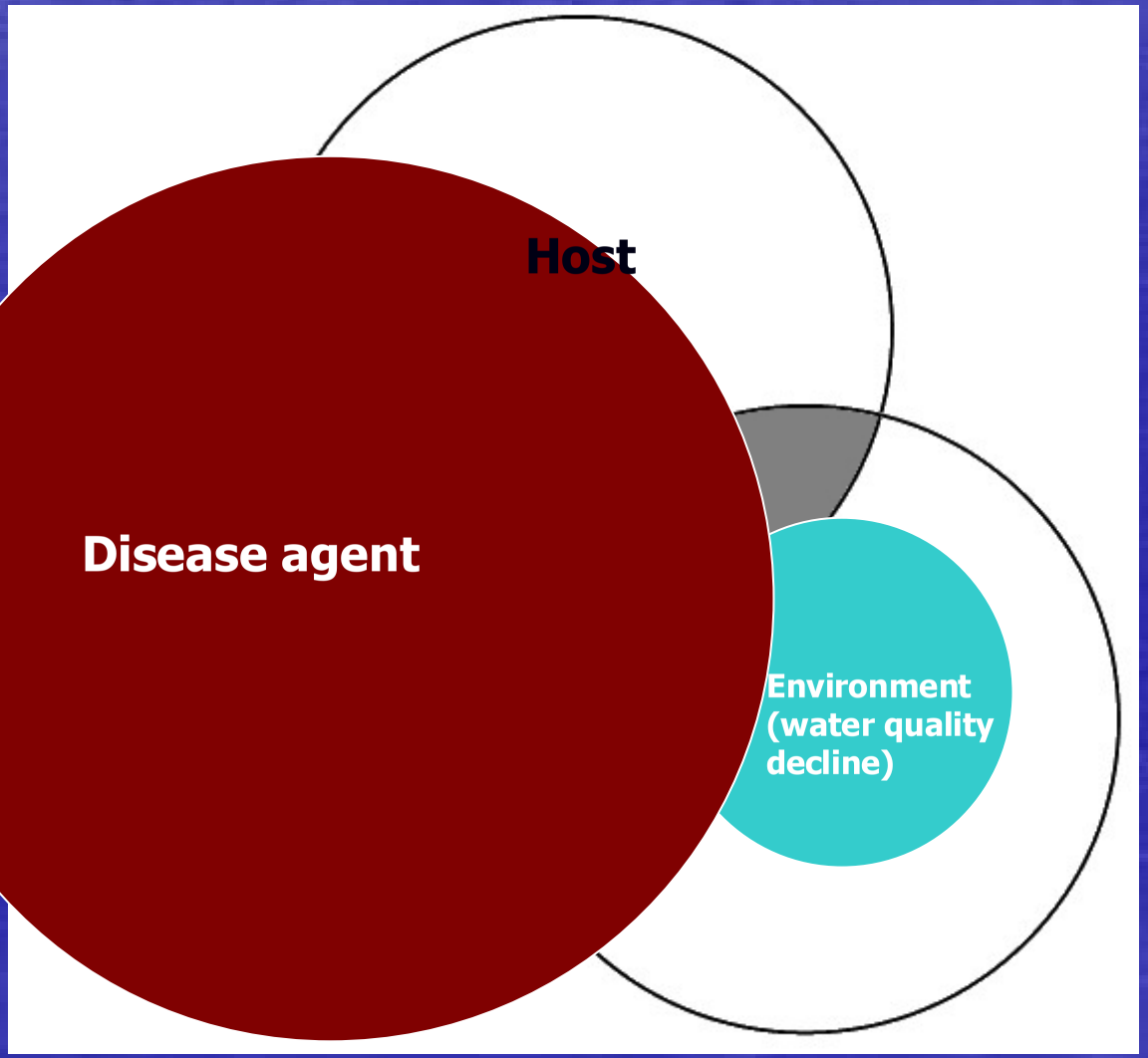
Haemorrhagic lesions on queenfish

- Pectoral girdle rashes have not been reported on queenfish in other areas of Australia.
- These lesions not reported on queenies in Gladstone Harbour prior to mid/late 2011. Lesions only associated with high numbers of sea lice.
- Why ?
- One risk factor – spatial and temporal correlation of lesions with start of dredge spoil dumping at Gladstone (but not in Darwin Harbour).

Water quality - an environmental factor influencing host/parasite interactions



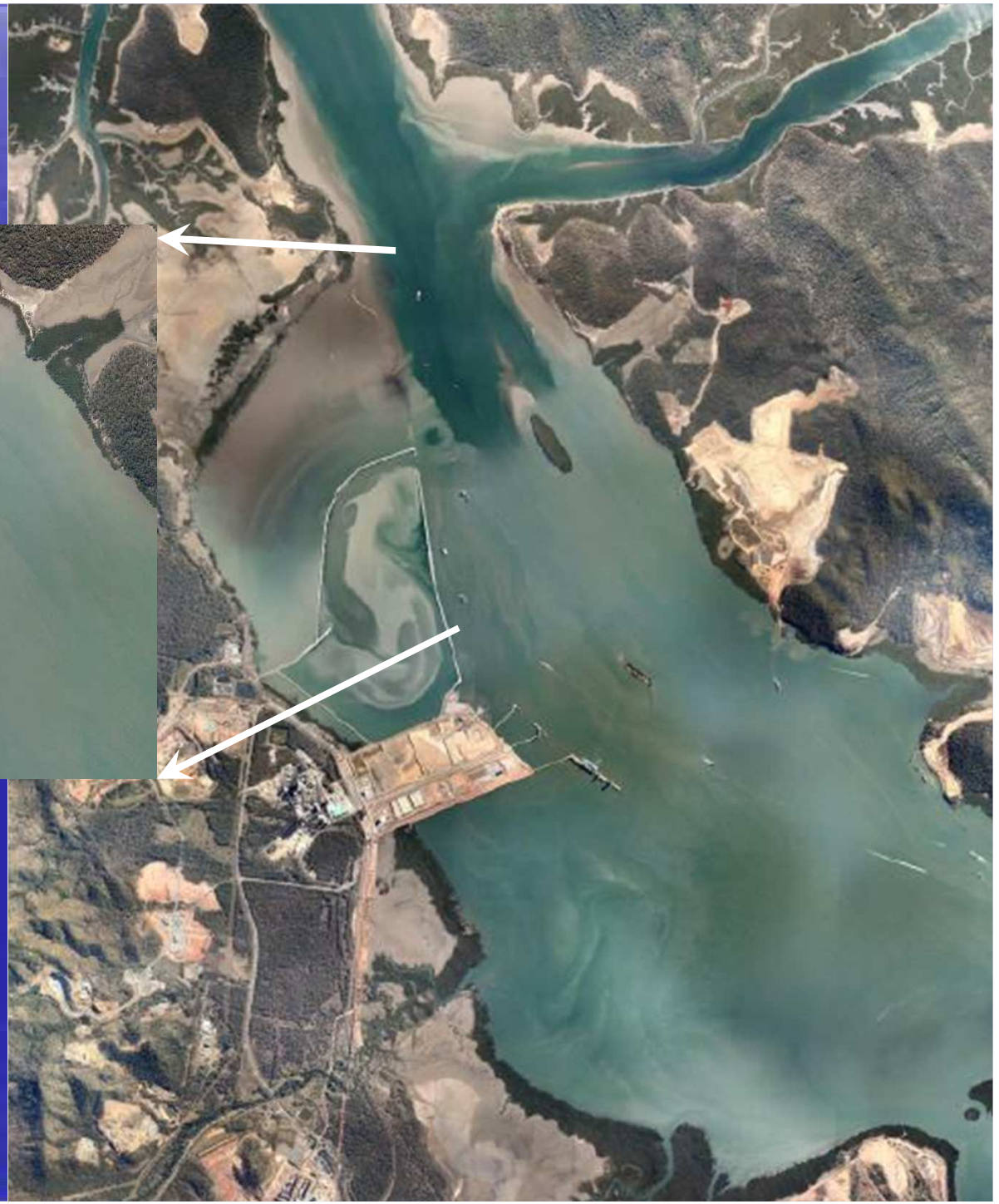
Water quality - an environmental factor influencing host/parasite interactions



Water quality - an environmental factor influencing host/parasite interactions

A major dredging project began in Gladstone Harbour in early 2011.
Photo date 27 September 2011.

Water quality

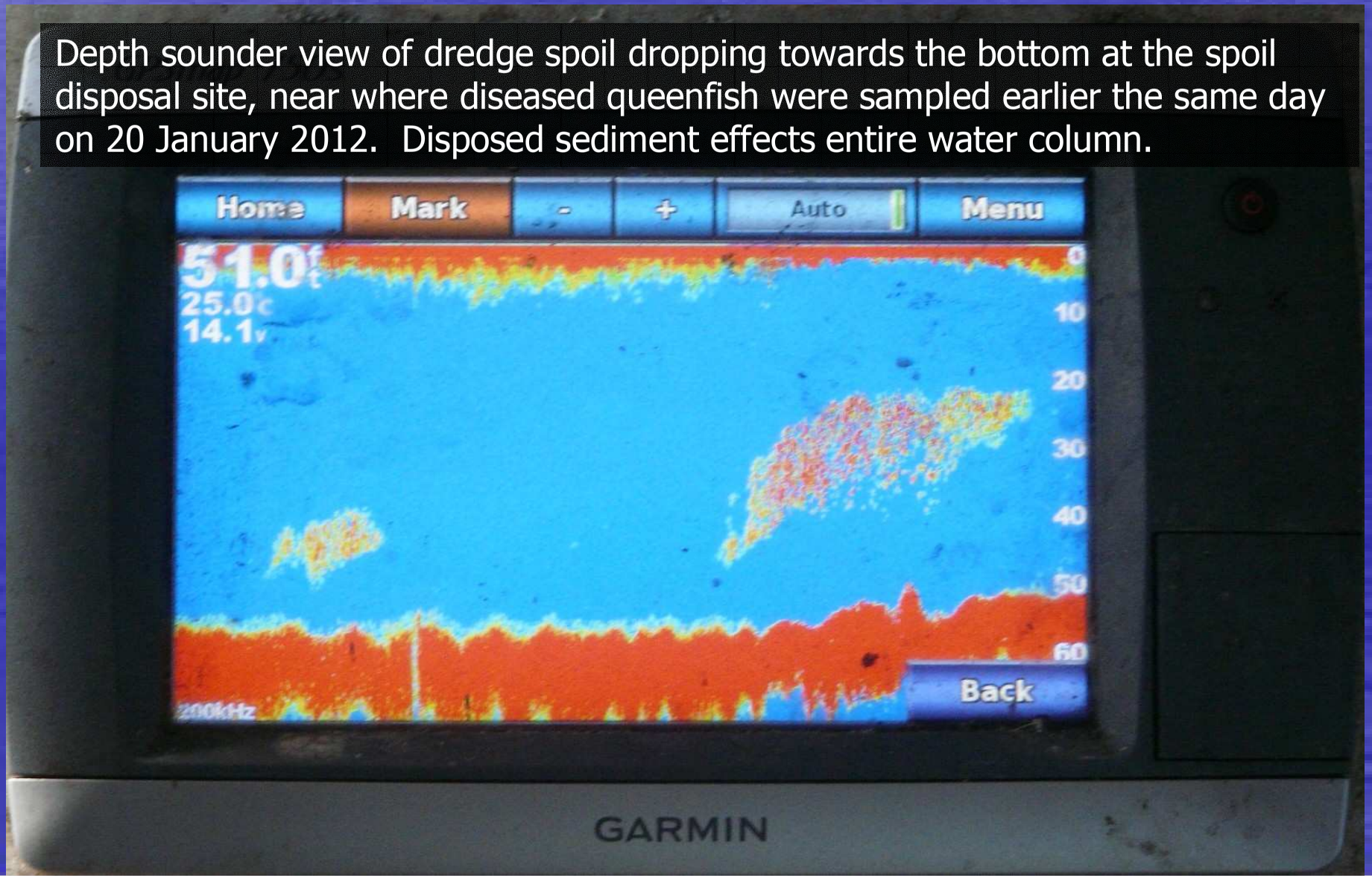


Satellite pictures of Gladstone Harbour, 11th July 2011. (photos courtesy of Nearmap)

Auckland Inlet – 28th September 2011 (dry season).

Site of fish hatchery water intake. Staff were changing filter bags every day, 10 times usual rate. Filters clogging and turning black, smelling of sulphides. The hatchery also recorded transient pH spikes to 9, which they presumed was from lime overdosing to control PASS reactivity in dredge spoil.

Dredge spoil disposal – exposes entire water column to sediment



Depth sounder view of dredge spoil dropping towards the bottom at the spoil disposal site, near where diseased queenfish were sampled earlier the same day on 20 January 2012. Disposed sediment effects entire water column.

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Aquatic Toxicology 54 (2001) 231–247

AQUATIC TOXICOLOGY

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Histological, growth and 7-ethoxyresorufin *O*-deethylase (EROD) activity responses of greenback flounder *Rhombosolea tapirina* to contaminated marine sediment and diet

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Abstract

Pathological abnormalities and mixed function oxygenase (MFO) enzyme changes are frequently used as indicators of anthropogenic contaminant exposure and effect. However, there is a paucity of research investigating the effects of contaminated sediment on native Australian benthic teleosts. As part of an ecotoxicological assessment of contaminated marine sediments in northern Tasmania, CYP1A induction, histological and growth response of the greenback flounder, *Rhombosolea tapirina*, exposed to contaminated marine sediments were examined. Hatchery reared flounder were exposed to reference sediment, contaminated sediment or contaminated sediment and diet for 6 weeks. CYP1A induction, using the ethoxyresorufin-*O*-deethylase (EROD) assay, and the histological and growth response in the flounder were examined on cessation of the exposure trial. Significant differences were found between treatments in histological, growth and EROD response. Exposure to contaminated sediment and diet elicited a multi-organ histological response, principally partial and total epidermal erosion and multifocal necrosis of the liver. The prevalence of total epidermal erosion was greatest with exposure to disturbed contaminated sediment (66.65 ± 16.65%). The prevalence of multifocal necrosis of the liver was greatest with exposure to contaminanted sediment and diet (66.65 ± 16.65%). Growth reduction, measured as percentage growth inhibition, was evident in flounder exposed to contaminated sediment and diet (18.2 ± 11.99%). Additionally, exposure to contaminated sediment and diet elicited elevated induction of the EROD liver detoxification enzyme (139.65 ± 24.22 pmol/min/mg protein) compared to exposure to contaminated sediment and non-contaminated diet (6.25 ± 0.81 pmol/min/mg) indicating the presence and potential bioavailability of xenobiotics via food. Further, more inhibited growth and histological alteration associated with exposure to contaminated sediment and diet suggest contaminants in Deceitful Cove sediment are cytotoxic. © 2001 Elsevier Science B.V. All rights reserved.

Epidermal erosion can occur due to direct exposure of fish to contaminated sediment (e.g. Mondon et al. (2001) *Aquat. Toxicol.* 54: 231-247).

- Dredging and/or spoil disposal increases exposure of aquatic animals to contaminants that may occur in fine sediments (e.g. 4 fold increase in metals in corals Esselmont et al. (2004) *J. Mar. Syst.* 52: 51-64).

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Journal of Marine Systems 52 (2004) 51–64

JOURNAL OF MARINE SYSTEMS

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Coral record of harbour dredging: Townsville, Australia

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Abstract

Concentrations of Ba/Ca, Cu/Ca, Sr/Ca and Zn/Ca were measured by secondary ion mass spectrometry in a branching coral, *Pocillopora damicornis*, from a coral reef receiving suspended sediments from Townsville Harbour dredging activities. The Sr/Ca record correlated with observed sea surface temperatures (SST), which allowed pulse inclusions of barium, copper and zinc to be dated and compared with various environmental records.

Copper and zinc concentrations in this coral skeleton correlated with short exposures to labile metals in resuspended fine sediment. The biota–sediment accumulation factor (BSAF) indicated that concentrations of these metals in the coral skeleton were lower than the labile fraction in the sediment (BSAF = 0.15 and 0.18, respectively). Distortions to the timing and amplitude of pulse-records in the coral record may have been caused by seasonal variation in skeleton growth.

High barium concentrations in the coral skeleton recorded during a dry period corresponded with weather events that resuspended fine, inner-shelf sediment, specifically local winds interacting with swells driven by offshore trade winds, and dredging of the harbour's access channel. This coral has recorded entry of resuspended fine sediment into the reef.

Irregularities in the strontium paleothermometer were similar to the solar radiation profile, which preceded variation of seawater temperature by about a month. When coral records were temporally adjusted to fit variation in solar radiation, the correlation between strontium and solar radiation was greater than the strontium–temperature correlation used to date the coral.

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METAL POLLUTION-INDUCED IMMUNOMODULATION IN FISH

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Abstract. The exact relationship between disease incidence in aquatic organisms and environmental pollution is not well defined. A number of mechanisms by which aquatic pollutants may act to increase disease incidence in fish have been speculated, many suggesting immunosuppression as a link in the etiology of disease among fishes in highly contaminated areas. This article will review the effects of metal pollutants on the immune responses of fish by examining *in vitro* and *in vivo* laboratory studies carried out since 1980. It will also describe how those alterations may be responsible for pollution-associated diseases in directly exposed fish. While a large number of environmental contaminants represent aquatic pollutants of concern (e.g. polycyclic aromatic hydrocarbons, chlorinated organics, and pesticides), heavy metals were selected as the pollutants for this review because of their: (a) prevalence in polluted aquatic environments; (b) immunotoxic potential in mammalian systems; (c) ability to induce tumors in exposed rodents; and (d) their overall toxicity in a variety of species. It can be concluded that a number of heavy metal pollutants shown to be immunotoxic in mammalian systems, including cadmium, chromium, copper, lead, manganese, nickel, and zinc, also alter immunoregulatory functions in a variety of fish species. These alterations may ultimately lead to increased host susceptibility to infectious and malignant diseases in fish inhabiting heavy metal-contaminated waters.

Keywords. Metals, Immunomodulation, Fish, Pollution

- Exposure to sediment alone can increase stress
- Some sediment contaminants (e.g. metals) are potent immuno-suppressants for fish at levels found in Gladstone (e.g. Zelikoff (1993). *Ann. Rev. Fish Dis*. 1993: 305-325).

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Effects of cortisol implants on the susceptibility and the histopathology of the responses of naive coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae)

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[2] Institute for Aquaculture Research, Simon Fraser University, Burnaby, British Columbia, Canada V5A 1S6

ABSTRACT: The effects of hydrocortisol implants on the susceptibility of naive coho salmon *Oncorhynchus kisutch* to infection with the economically important marine ectoparasitic copepod *Lepeophtheirus salmonis* was investigated under laboratory conditions. Cortisol-implanted coho salmon were more susceptible to infection than the control coho salmon. Copepods were lost from the gills of the control coho salmon by 10 d post-infection, and only a few remained on the body and fins at 20 d post-infection. Copepods were retained on the gills, fins and body of the cortisol-implanted coho salmon over the 20 d studied. Histological sections of control coho gills and fins revealed well developed epithelial hyperplasias and inflammatory responses to the presence of *L. salmonis*. The magnitude of the inflammatory response and the development of epithelial hyperplasia was suppressed in the cortisol-implanted coho. These data support the hypothesis that non-specific host defence mechanisms are important in resistance of coho salmon to infection with *L. salmonis*.

- Implantation of cortisol resulted in immune suppression and heavier sea lice infections in controlled experiments (Johnson and Albright 1992).
- Thus, increased sea lice levels in wild fish are potentially a clinical sign of stress/ immuno-suppression.

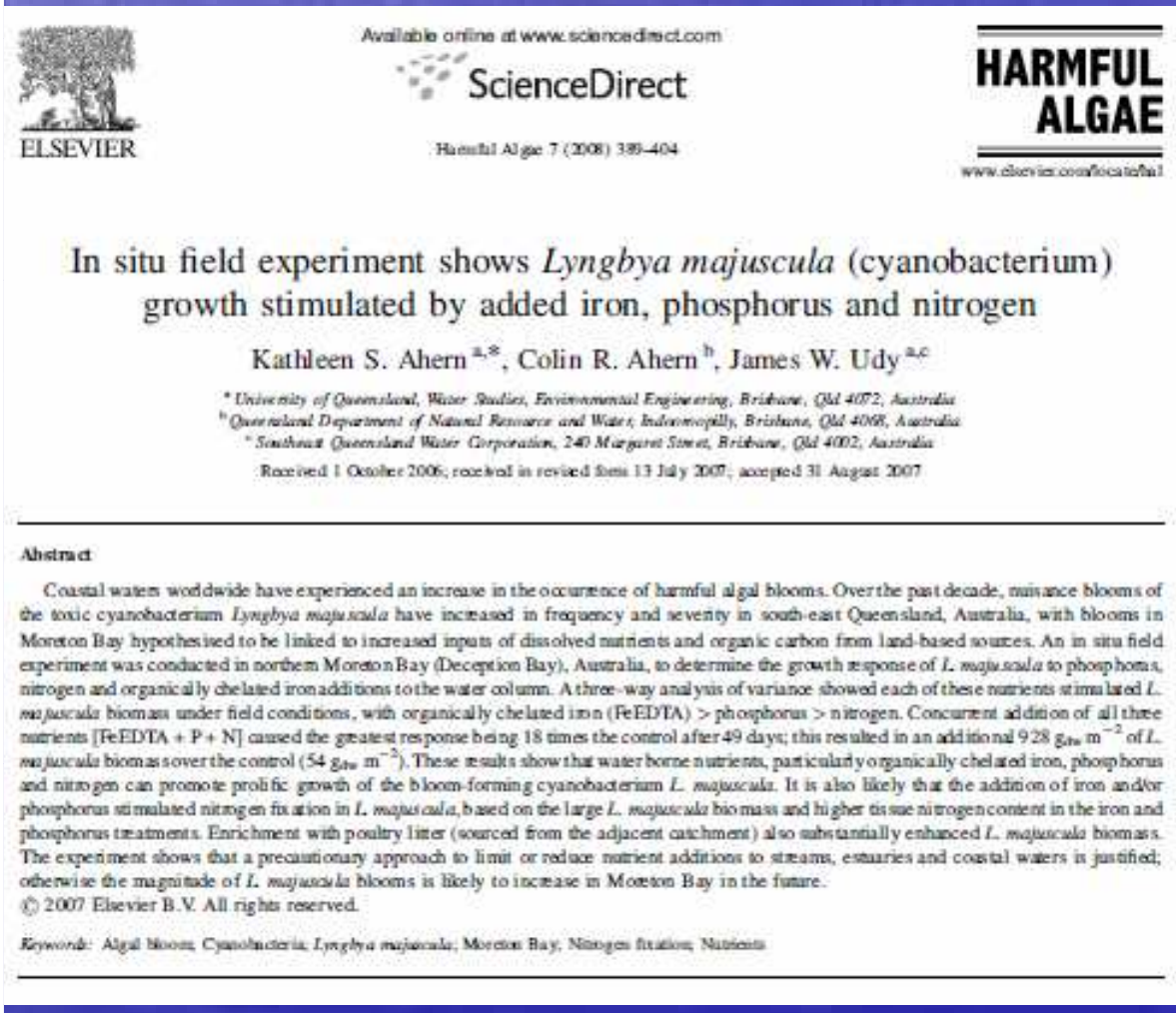
Another risk factor: Toxic algal bloom

- A toxic cyanobacterial bloom (*Lyngbya majuscula* = fire weed) was detected during independent (non govt.) water sampling (ID confirmed by Gustaff Hallegraeff, Univ. of Tasmania).
- Cases of rashes/dermatitis were reported by people working on the water in Gladstone Harbour at the time. These highly likely due to exposure to *Lyngbya majuscula* (Gustaff Hallegraeff, pers. comm., June 2012).

Another risk factor: Toxic algal bloom

Rash on the lower leg of Dr B. Diggles, 21 January 2012, after being in the water for around 1 hour during field sampling in the Boyne River, Gladstone Harbour.

Another risk factor: Toxic algal bloom



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Harmful Algae 7 (2008) 389–404

HARMFUL ALGAE

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In situ field experiment shows *Lyngbya majuscula* (cyanobacterium) growth stimulated by added iron, phosphorus and nitrogen

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Abstract

Coastal waters worldwide have experienced an increase in the occurrence of harmful algal blooms. Over the past decade, nuisance blooms of the toxic cyanobacterium *Lyngbya majuscula* have increased in frequency and severity in south-east Queensland, Australia, with blooms in Moreton Bay hypothesised to be linked to increased inputs of dissolved nutrients and organic carbon from land-based sources. An in situ field experiment was conducted in northern Moreton Bay (Deception Bay), Australia, to determine the growth response of *L. majuscula* to phosphorus, nitrogen and organically chelated iron additions to the water column. A three-way analysis of variance showed each of these nutrients stimulated *L. majuscula* biomass under field conditions, with organically chelated iron (FeEDTA) > phosphorus > nitrogen. Concurrent addition of all three nutrients [FeEDTA + P + N] caused the greatest response being 18 times the control after 49 days; this resulted in an additional 928 $g_{dw}\ m^{-2}$ of *L. majuscula* biomass over the control (54 $g_{dw}\ m^{-2}$). These results show that waterborne nutrients, particularly organically chelated iron, phosphorus and nitrogen can promote prolific growth of the bloom-forming cyanobacterium *L. majuscula*. It is also likely that the addition of iron and/or phosphorus stimulated nitrogen fixation in *L. majuscula*, based on the large *L. majuscula* biomass and higher tissue nitrogen content in the iron and phosphorus treatments. Enrichment with poultry litter (sourced from the adjacent catchment) also substantially enhanced *L. majuscula* biomass. The experiment shows that a precautionary approach to limit or reduce nutrient additions to streams, estuaries and coastal waters is justified; otherwise the magnitude of *L. majuscula* blooms is likely to increase in Moreton Bay in the future.

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Keywords: Algal bloom; Cyanobacteria; *Lyngbya majuscula*; Moreton Bay; Nitrogen fixation; Nutrients

- Dissolved iron and other organic nutrients required to initiate *Lyngbya* blooms (Ahern et al. (2008) *Harmful Algae* 7: 389-404).
- Land use (incl. dredging) which disturbs acid sulphate soils can stimulate *Lyngbya* blooms[2].

2. http://www.environment.gov.au/water/topics/acid-sulfate-soils/about-ass.html

Another risk factor: Toxic algal bloom

- Does exposure to *Lyngbya* toxins stress queenfish and/or predispose queenfish with heavy sea lice infections to dermatitis like symptoms ?

Risk Factor ? – Flooding

- River flow events occurred in January 2011 throughout east coast Queensland (not just Gladstone area).
- Normal turbidity and salinity levels regained (and evident in satellite photos) by May/June 2011.
- Sampling of Gladstone queenfish occurred 10 km offshore in full strength seawater January 2012, 12 months after river flow events.
- River flow events are therefore very unlikely to be a significant risk factor for rashes in these queenfish.

In summary……

- Dredging and spoil disposal activity that disturbs acid sulphate soils can stimulate *Lyngbya* blooms. Acute and chronic effects on fish health are possible as a result of reduced water quality.
- Chronic: Dredge spoil disposal can erode the epidermal layers, raise stress levels, predisposing fish to increased levels of sea lice infection (immunosuppression due to metals ?).
- Acute: Rashes/erythema in queenfish become evident only once a certain threshold of sea lice numbers is reached (c. >10 copepods/fish).
- Rashes due to haemorrhages arising from penetration by sea lice appendages and mouthparts into areas of epidermal erosion.

Unfortunately……

- Measures of baseline fish health from Gladstone Harbour are not available - baseline aquatic animal health and algal monitoring were not done prior to the development. Transient effects around start of the dredging project are likely to have been missed.
- Correlation does not necessarily prove causation – more data needed, but the informative testing window for the baseline condition has long passed.
- These unfortunate events show the importance of incorporating baseline aquatic animal health studies into future port related industrial developments in Australia.

Questions ?

Any other insights as to possible contributing factors ?

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World record 16 kg queenfish from Darwin Harbour, June 2012
Photo Steve Starling