Port Survey for Introduced Marine Species.

STUDY SITE LOCATIONS

Within the harbour there are five port habitats that will be sampled both quantitatively and qualitatively:

1. Berths x 7
2. Marinas x 2
3. Piles x 4
4. Dredge Spoil x 2
5. Pristine areas x 4

The 2 dredge spoil grounds and 4 pristine areas have not yet been identified, these will be identified with CRIMP when sampling begins.

METHODOLOGY

All methods used are from the Centre for Research on Introduced Marine Pests, Technical report number 4, "Port surveys for introduced marine species – background considerations and sampling protocols".

BERTHS

There 7 berths within the harbour include:

1. Auckland Point Wharf (4 berths). We may need to sample two berths.
2. Clinton Coal wharf (2 berths)
3. Fishermans landing
4. South Trees wharf
5. Boyne wharf
6. Tug berths
7. Barney Point

The following sampling protocols will be performed at each of the seven berths:

Field Work

Quantitative sampling

1. Small cores- for cyst-forming species

- A sediment core is taken at each of the berths with a 200mm long plastic tube with a 25mm internal diameter.
- The corers are forced into the sediment to a depth that leaves the top 20-50mm of the tube unfilled.
- The top of the tube is capped with a bung before the core is withdrawn from the sediment.
- The lower end is capped with a bung after withdrawal to provide an air-tight seal.

- Three replicate cores are taken at each site.
- The cores are then placed upright in an insulated box and stored in the dark at 4^{o}C prior to fractionation and examination for cysts.

2. Large cores - for sampling benthic infauna

- A sediment sample for benthic infauna is sampled by using a tubular 0.025m^2 hand corer.
- The corer is placed in the sediment to a depth of 200-250mm then sealed with a rubber bung before being withdrawn from the sediment.
- Each core is transferred to a 1mm mesh bag and agitated underwater to remove fine sediment from the sample.
- The retained material is washed into a plastic bag and preserved in 7% seawater buffered formalin.
- A single core is taken within 1m of the base at each of the sample piles.
- A second core is taken at the end of the 50m transect run out perpendicular to the wharf from each of the sampled pile (giving a minimum of three inner and outer cores).

3. Sediment sample- for particle size analysis and organic content

- Approximately 100g of sediment is to be taken for analysis of grain size and organic content, from areas adjacent to large benthic infauna sampling cores.
- Samples are collected in sealable plastic bags and immediately frozen to stabilise organic content levels.

4. Traps – crab/shrimp

- Light-weight mesh-covered crab traps are deployed with shrimp traps.
- The crab traps are weighted with chain and deployed with surface buoys.
- The shrimp traps are attached to the crab trap tether and suspended from the wharf so that they are positioned just of the bottom.
- Traps are deployed in the late afternoon and recovered early the next morning.

5. Mesh drop net- for sampling zooplankton

- Using a 100µm mesh free-fall drop net, the net is weighted to achieve a fall rate of approximately 1m per second.
- The depth reached is monitored using a depth gauge attached to the frame of the net.
- The drop is allowed to fall from the surface to 0.5 –1.0m from the bottom before being stopped and closed when the cod end of the net sinks below the surface.
- One drop per site.
- On recovery the net is washed down and the retained plankton is preserved in 5% formalin.

6. Plankton sampling

- A vertical and horizontal tow of a small 20µm plankton net.
- Vertical tows- the net is allowed to sample both during descent and retrieval, several drops may be required to ensure adequate concentration of cells is obtained in each sample.
- Horizontal tows are carried out at a depth of approximately 2m below the surface.

- After the net is washed the retained samples are sealed in plankton jars and placed in an insulated container.
- Samples are to be returned to the laboratory immediately for incubation.

7. Poison Stations

- Rotenone is mixed with an approximately equal volume of seawater in a plastic bag.
- It is dispensed from the plastic bags immediately.
- Stations are sampled at slack water.
- Poisoned fish are collected by divers underwater and snorklers at the surface using hand nets.
- Sampled fish and bycatch are recorded and specimens are preserved in 10% seawater buffered solution.

8. Beach Seines

- Beach seines are used to sample nearshore fishes over sandy or muddy substrates.
- 25m seine net with 15 mm mesh size is used.
- The sampled fish and bycatch species are recorded and specimens are preserved in 10% seawater buffed formalin.

9. Quadrat scraping

- 3 piles are selected at each berth, 10-15m apart, the first being located 10m from the end of the berth.
- At each pile, three 0.10m^2 quadrats are fixed to the surface at depths of -0.5, -3.0 and –7.0m from the surface.
- A video transect of each pile is made at high water from the high water mark down to the deepest exposed part of the pile.
- A scale and depth meter is attached to a rod so they fall within the field of view of the camera.
- Still photographs are to be taken of the 0.10m^2 quadrats
- Quantitative destructive sampling of the fouling/encrusting communities are made by carefully scraping the flora and fauna inside each of the three quadrats.
- These samples are then placed in a plastic bag.
- Samples are rough sorted into subsamples of representative fauna and preserved in either 90% alcohol or narcotised with isotonic $MgCl_2$ for at least one hour prior to formalin preservation.
- Representative flora is placed between sheets of herbarium paper.
- Remaining component of each sample is preserved in 7% seawater buffered formalin.

10. Environmental data – salinity, temperature and turbidity

- A submersible data logger is used to record depth profiles of salinity and water temperature in 1m increments from the surface to the bottom.
- Turbidity is measured with a secchi disk.
- Air temperature, cloud cover, sea state and wind speed should also be noted.

Qualitative sampling

IN AREAS WHERE THERE ARE BREAKWATERS, GROYNES, ROCKWALL FACINGS AND NATURAL ROCKY REEFS.

11. Visual surveys

- General visual surveys are carried out.
- In rocky areas where the depth is greater than 7m more detailed surveys are carried out.
- Three transect lines, 10- 15 m apart, are placed from high water to the base of the rocky areas.
- 0.10m^2 quadrats are placed at –0.5, -3.0 and –7.0m and both video and still photographs of transects are taken.
- Rocky areas with that are relatively shallow (<5m), a 50 m transect line is run along the rock wall and videoed.
- Paired 0.10m^2 quadrats (-0.5m and bottom) sampled at 5 randomly selected locations along the line.
- Still photographs are taken of the quadrats prior to sampling and qualitative sampling is carried out within the visual survey area.
- Samples are preserved as for piles.

12. Visual survey for crabs and target species.

- Divers swim the length of the wharf at several depths to provide complete visual coverage of the structure and adjacent bottom.
- Inner piles are surveyed when there are several piles.
- Visual surveys for Pacific seastar, macroalga, and the European fan worm are carried out by divers in rocky reef, wharf areas and over soft bottoms.

13. Visual surveys for epibenthos

- Visual surveys to locate and collect non-target, soft bottom epibenthic species are carried out at wharves where pile surveys occur.
- 50 m transect line is laid out perpendicular to the wharf, starting at the base of the pile.
- Transect line is marked every at intervals of 1,5 and 10m.
- If visability is adequate, the transect is videoed and epibenthos photographed and collected as appropriate.

Laboratory work

Quantitative sampling

1. Small cores- sediment and cyst identification.

- The top 60mm of sediment core is carefully extruded from the coring tube and stored at 4^oC in a sealed container prior to examination.

- Subsamples of each core sampled are mixed with filtered seawater to obtain a watery slurry.
- Subsamples of the slurry are then sonicated for 2 min to dislodge detritus particles.
- Sample is then screened through a 90µm sieve and panned to remove denser sand grains and larger detritus particles.
- Subsamples are then examined on wet-mount slides, using a compound light microscope, and cysts counted and identified
- 100 cysts are counted, where possible, for each sample.
- Suspected toxic species are photographed with a light microscope.

Cyst germination

- After sonication and size-fractionation of sediment subsamples, suspected toxic cysts are located and isolated by micro-pipette under a compound microscope and then washed twice in filtered seawater.
- Individual cysts are then placed into tissue culture wells containing 2ml of 75% filtered seawater with nutrients added to medium GPM of Loeblich (1975).
- Additional sediment incubations using subsamples from the 20 – 90 µm size fraction are carried out in Parafilm® sealed, sterile polystyrene petri-dishes containing 20ml of growth medium.
- Incubations are carried out at 20^{o}C at light intensity of 80µE $m^{-2}s^{-1}$ and are examined regularly for germinated cells.
- Actively swimming dinoflagellate cells from incubations are isolated by micro-pipette and washed in sterile growth medium prior to identification.

2. Large cores- for sampling benthic infauna

- Samples are sorted and identified.

3. Sediment sample- for particle size analysis and organic content

- After samples are thawed, 25 g (dry weight) is removed for organic content analysis.
- Remaining sediment is then wet sieved through a 2mm mesh sieve and separated into <2mm and >2mm size fractions.
- Fractions and organic content sample are oven dried at 80^{o}C for 48 to 96 hours.
- The two fractions for particle size are analysed.
- The <2mm fraction is sieved through a stack of sieves and each fraction that is retained on each sieve is weighed.
- Sediments retained on the largest sieve includes all particles with a size >8mm.
- The individual sieves are then added to the dry weight of the <2mm fraction to give a total dry weight for the entire sample.
- The proportion of each component in the >2mm fraction is calculated as a percentage of the total dry sample.
- The <2mm fraction. The dry weight of the total <2mm fraction is measured to 0.01g and the whole sample, analysed using a Laser Particle Size Analyser to comply with the standards of the Marine Geophysical Laboratory, James Cook University, Queensland.
- This data is then combined with data from the analysis of the >2mm fraction.

Organic content

- 25g of dry, unsieved sediment is weighted in a crucible to the nearest 0.00001g and then ashed in a muffle furnace at 480oC for 4 hours.
- The crucible is then transferred to a dessicator and allowed to cool for 1 hour prior to being weighed.
- Difference between the net dry and net ash-free dry weight is then calculated.
- The difference is expressed as a percentage of the initial dry weight and represents the organic content in the sediment sample.

4. Traps – crab/shrimp

- Samples are identified and counted.

5. Mesh drop net- for sampling zooplankton

- Samples are identified and counted.

6. Poison Stations

- Samples are identified and counted.

7. Beach Seines

- Samples are identified and counted.

8. Quadrat scraping

- Samples are identified and counted.

9. Plankton sampling

- Net samples are diluted 1:1 with growth medium.
- Germanium dioxide (10mg l^{-1}) is added to inhibit overgrowth by diatoms and these enrichment cultures incubated as described for cysts above.
- Incubations are examined regularly by light microscopy, and single cells of suspected toxic species isolated by micro-pipette for further culture and toxicity testing.

Toxicity testing

Suspected species are tested for toxin production by High Perfomance Liquid Chromotography (HPLC) (Oshima *et al*. 1989)

MARINAS

The 2 marina sites within the harbour which include:

1. Private moorings
2. Commercial moorings

The following sampling protocols will be performed at the 2 marina sites, with variations to methodology depending on site characteristics, determined in consultation with CRIMP.

Field Work

Quantitative sampling

1. Small cores- for cyst-forming species

- Procedure as for berths.

2. Large cores – for sampling benthic fauna

- Procedure as for berths.

3. Sediment sample – for particle size analysis and organic content

- Procedure as for berths.

4. Traps – crab/shrimp

- Procedure as for berths.

5. Mesh drop net – for sampling zooplankton

- Procedure as for berths.

6. Plankton sampling

- Procedure as for berths.

7. Poison Stations

- Procedure as for berths.

8. Beach Seines

- Procedure as for berths.

9. Quadrat scraping

- Procedure as for berths.

10. Environmental data – salinity, temperature and turbidity

- Procedure as for berths.

Qualitative sampling

IN AREAS WHERE THERE ARE BREAKWATERS, GROYNES, ROCKWALL FACINGS AND NATURAL ROCKY REEFS.

11. Visual surveys

- Procedure as for berths.

12. Visual survey for crabs and target species.

- Procedure as for berths.

13. Visual surveys for epibenthos

- Procedure as for berths.

Laboratory work

Quantitative sampling

1. Small cores- sediment and cyst identification.

- Procedure as for berths.

2. Large cores- for sampling benthic infauna

- Procedure as for berths.

3. Sediment sample- for particle size analysis and organic content

- Procedure as for berths.

4. Traps – crab/shrimp

- Procedure as for berths.

5. Mesh drop net- for sampling zooplankton

- Procedure as for berths.

6. Poison Stations

- Procedure as for berths.

7. Beach Seines

- Procedure as for berths.

8. Quadrat scraping

- Procedure as for berths.

14. Plankton sampling

- Procedure as for berths.

PILES (CHANNEL MARKERS)

The 4 piles within the harbour include:
1. Pile opposite Clinton Coal wharf
2. Pile opposite Auckland Creek wharf
3. Pile opposite South Trees wharf
4. Pile opposite Fisherman's landing

Field Work

Quantitative sampling

The following sampling protocols will be performed at the 4 piles:

1. Small cores- for cyst-forming species

- Procedure as for berths.

2. Large cores – for sampling benthic fauna

- Three 0.025m^2 cores are taken within 2m of the pile and at least 2m away from each other.
- A video transect is then run along each line and at 5 random distances along the transect, paired cores, 1m on either side of the line, are taken from the centre of a 0.10m^2 quadrats. Each quadrat is photographed prior to coring.

3. Sediment sample – for particle size analysis and organic content

- Procedure as for berths.

4. Traps – crab/shrimp

- Procedure as for berths.

5. Mesh drop net – for sampling zooplankton

- Procedure as for berths.

6. Plankton sampling

- Procedure as for berths.

7. Poison Stations

- Procedure as for berths.

8. Beach Seines

- Procedure as for berths.

9. Quadrat scraping

- Procedure as for berths.

10.Environmental data – salinity, temperature and turbidity

- Procedure as for berths.

Qualitative sampling

IN AREAS WHERE THERE ARE BREAKWATERS, GROYNES, ROCKWALL FACINGS AND NATURAL ROCKY REEFS.

11. Visual surveys

- Procedure as for berths.

12. Visual survey for crabs and target species.

- Procedure as for berths.

13. Visual surveys for epibenthos

- Procedure as for berths.

Laboratory work

Quantitative sampling

1. Small cores- sediment and cyst identification.

- Procedure as for berths.

2. Large cores- for sampling benthic infauna

- Procedure as for berths.

3. Sediment sample- for particle size analysis and organic content

- Procedure as for berths.

4. Traps – crab/shrimp

- Procedure as for berths.

5. Mesh drop net- for sampling zooplankton

- Procedure as for berths.

6. Poison Stations

- Procedure as for berths.

7. Beach Seines

- Procedure as for berths.

8. Quadrat scraping

- Procedure as for berths.

9. Plankton sampling

- Procedure as for berths.

DREDGE SPOIL

Field Work

Quantitative sampling

The following sampling protocols will be performed at the 2 dredge spoil grounds:

1. Small cores- for cyst-forming species

- Procedure as for berths.

3. Large cores – for sampling benthic fauna

- Procedure as for berths.

3. Sediment sample – for particle size analysis and organic content

- Procedure as for berths.

4. Environmental data – salinity, temperature and turbidity

- Procedure as for berths.

Qualitative sampling

IN AREAS WHERE THERE ARE BREAKWATERS, GROYNES, ROCKWALL FACINGS AND NATURAL ROCKY REEFS.

5. Visual surveys for epibenthos

- A video transect is recorded along a 50m marked transect line laid across the bottom.
- 35mm still photographs and sample collection are completed where appropriate.

Laboratory work

Quantitative sampling

1. Small cores- sediment and cyst identification.

- Procedure as for berths.

2. Large cores- for sampling benthic infauna

- Procedure as for berths.

3. Sediment sample- for particle size analysis and organic content

- Procedure as for berths.

PRISTINE AREAS

The following sampling protocols will be performed at 4 pristine areas:

Field Work

Quantitative sampling

1. Environmental data – salinity, temperature and turbidity

- Procedure as for berths.

Qualitative sampling

2. Visual surveys for epibenthos

- A video transect is recorded along a 50m marked transect line laid across the bottom.
- 35mm still photographs and sample collection are completed where appropriate.

REPORTING

Survey data will be entered into CEM databases and processed using methodology developed for other programmes run at the CEM.

A voucher specimen from each species will be retained, and one individual sent to CSIRO Division of Fisheries in Hobart.

REFERENCES

Hewitt, L. 1960. Port surveys for introduced marine species:background considerations and sampling protocols. CSIRO Division of Fisheries, Tasmania.

Loeblich, A.R. 1975. A seawater medium for dinoflagellates and the nutrition of *Cachinina niei*. J. Phycol. 11, 80-86.

Oshima, Y.K. Sugino, and T.Yasumoto. 1989. Latest advances in HPLC analysis of paralytic shellfish toxins. In: S. Natori, K. Hashimoto and Y.Uneno, eds, Mycotoxins and Phycotoxins 1988, Elseiver Science Publishing Co., New York, pp 319-326.