Ecological effects due to contamination of sediments with copper-based antifoulants (Part 2)

D.P. O'Brien, S.L. Simpson & D.A. Spadaro

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Project No. 2009/218

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Dr. Dominic O'Brien
Aquaculture, Management & Development P/L,
31, Mortimer Ave, Hobart. Tasmania, 7000
Email: domob@mpx.com.au

Dr Stuart Simpson & David Spadaro
Centre for Environmental Contaminants Research (CECR), CSIRO Land and Water
Private Mail Bag 7, Bangor, NSW 2234, Australia
Email: Stuart.Simpson@csiro.au
David.Spadaro@csiro.au,

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Investigators: Dr Dominic O'Brien, Dr Stuart Simpson & David Spadaro

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

ISBN: 978-0-646-55253-8

Acknowledgements

This project was made possible through funding from the Fisheries Research and Development Corporation.

Hempel (Australia) was also a cash contributor to the project. In-kind support was also provided by the Tasmanian Salmonid Growers Association, Tassal Ltd., Huon Aquaculture Company P/L, and CSIRO Land and Water.

Graham Woods of the Tasmanian Department of Primary Industries and Water (DPIW), Graeme Batley and Anthony Chariton (of CSIRO Land and Water) are thanked for inputs into the assessment approach.

Ian Hamilton and Chad Jarolimek (CSIRO Land and Water) are thanked for contributions to the sample analyses and tests. We would like to thanks Joel Davis of ANSTO for undertaking SEM and x-ray microanalysis of the paint particles. We thank Graeme Batley and two other anonymous internal reviewers for comments on the report.

We would also like to thank Cameron Dalgleish and Craig Selkirk of Tassal Ltd for their input and help across all areas of both this project and the accompanying FRDC project 2008/226.

Non-Technical Summary

2009/218	Ecological effects due to contamination of sediments with copper-based antifoulants (Part 2).

PRINCIPAL INVESTIGATOR: Dr. Dominic O'Brien

ADDRESS: Aquaculture, Management & Development P/L,
31, Mortimer Avenue,
Hobart. Tasmania, 7000

TELEPHONE: 03 62283167

OBJECTIVES

To provide information on the bioavailability and potential sub-lethal or chronic effects of copper-based anti-fouling paints contaminated sediments, using the following approaches.

1. Direct measures of bioavailability through measuring bio-accumulation of copper by the benthic bivalve, *Tellina deltoidalis*,

2. Sub-lethal and chronic toxicity tests using the three benthic organisms, amphipod (*Melita.plumulosa*), copepod (*Nitocra spinipes*) and bivalve (*Tellina deltoidalis*).

NON TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

The overall study (comprising FRDC Projects; 2007/246, 2008/226, and 2009/218) has provided a clearer understanding of the nature (including bio-availability and toxicity) of the copper in the sediments below fish farms that is derived from the Cu(I)O antifouling paints used on the fish cage nets. This information has been provided to Tasmanian and Federal regulatory authorities, including the Australian Pesticides and Veterinary Medicines Authority (APVMA), Environmental Protection Authority-TAS (EPA) and Department of Primary Industry, Parks, Water and Environment (DPIPWE) who share responsibility for the administration and licensing of the use of anti-foulings in the Tasmanian salmonid farming industry. This data is specifically provided as part of the Environmental Assessment as required by the APVMA for the registration of anti-fouling paint. As such the study provides additional data on environmental exposure and effect of the paint and this information then forms part of the Environment Risk Assessment required as part of the overall process.

Data provided in the present study indicates that although the copper levels in the sediments beneath the fish farms were unlikely to cause acute toxicity to benthic invertebrates (confirming observations in 2008/226), the higher of these levels could lead to either bio-accumulation and/or chronic toxicity in some faunal species. It appears however, that these effects are seen at levels of total recoverable copper (TR-Cu) that exceed both the no effects (ISQG-low) and higher trigger level (ISQG-high) used under the present Sediment Quality guidelines (ISQG). The results indicate that measurement of TR-Cu may not be in itself be the most appropriate indicator of the toxicity (bio-availability) of the copper in the sediments and the present study now suggests that alternate measures of bio-availability such as dilute acid extractable copper (AE-Cu) and TR-Cu levels in the <63um fraction of the sediments might provide better estimates of the level of bio-availability of the paint copper.

As stated in FRDC project 2008/226, these results are now being incorporated into the Risk Assessment process employed by the APVMA in order to determine the limits associated with the environmentally sustainable use of Cu(I)O anti-fouling paint on fish farm nets as part of the requirements for its' registration.

Essentially, the information from the two studies and the ongoing monitoring surveys provides more confidence for the Industry, DPIPWE and the APVMA on the appropriate management protocols that are required at both depositional and scouring sites (also referred to as High and Low Organic sediment sites) to prevent the copper paint from causing significant changes in the sediments in and around fish pens.

The present study is the third in a continuum of research projects aimed at defining the sustainable level of use of copper based antifouling paints in the salmonid industry in Tasmania. Ultimately this data will provide the environmental requirements for registration of the product for the industry and will also be able to set Cu-paint specific guidelines for use in the industry. The two previous studies were:

2007/246	A review of the ecological impacts of selected antibiotics and antifoulants currently used in the Tasmanian salmonid farming industry"
2008/226	Ecological effects due to contamination of sediments with copper-based antifoulants.

As outlined in FRDC Project 2008/226, the use of antifouling paints by the Tasmanian marine salmonid industry is administered through the Australian Pesticides and Veterinary Medicines Authority (APVMA) permit to the Tasmanian Salmonid Growers Association (TSGA) which is renewable annually. The permit allows for the use of anti-fouling paints in the south-east of Tasmania which use copper oxide as their sole active ingredient whilst the industry collects the necessary information for registration of the product. The sustainable use of the paints under the permit is directed by the APVMA and Department of Primary Industries, Parks, Water and Environment (DPIPWE) with any research and monitoring being designed through an expanded working group which also includes Industry through the TSGA, various Scientific Research organisations, the CSIRO and Tasmanian Aquaculture and Fisheries Institute (TAFI), and commonwealth and state environmental regulatory authorities, Department of the Environment, Water, Heritage and the Arts (DEWHA) and the Environment Protection Authority Tasmania (EPA). The industry has been collecting data to support the Environmental requirements for registration for the product under a Research Permit for more than 10 years.

Sediments contaminated with copper-based antifouling paint were collected from in and around four fish farm leases managed by members of the Tasmanian Salmonid farming Association (TSGA) in November 2009. The sediment samples were taken in replicate from a range of sites, representing low organic carbon sites (sandy, "scouring"), high organic carbon sites (silty, "depositional"), and sites in varying stages of 'recovery' after cessation of fish stocking. Chemical analyses and whole-sediment acute toxicity tests were conducted on the sediments to provide evidence for the absence or presence of bio-available copper and these are reported in the previous study, FRDC 2008/226. The present study then employed these sediments in order to assess at what levels of paint-Cu chronic toxicity and or bio-accumulation might occur.

Consistent with previous studies (Simpson and Spadaro, 2008, FRDC 2008/218), acute lethal effects to adult amphipods or copepods were not observed in the toxicity tests. The chronic toxicity tests demonstrated that chronic effects may occur for some copper-paint contaminated sediments. The greater concentration of dissolved copper in the overlying waters was consistent with the copper being very labile for fine sediments with high copper concentrations and based on these results, the exposure to copper associated with fine particles was likely to have caused the greatest contribution to chronic toxicity to the amphipod, *M. plumulosa*. For the copepod, *N. spinipes* tests, the combination of a very high concentration of AE-Cu for the fine sediment fraction and the greatest release of copper to the overlying waters is likely to be the cause of the toxicity of the sediment to the copepod. It is possible that the much smaller copepod (generally 300-600 μm) has greater interaction with the fine (copper-contaminated) particles at the surface of the sediment than the larger amphipod (generally 6-9 mm). While the copepod is expected to feed on the algae and powdered fish food added during the tests, like the amphipod, it may also inadvertently ingest other fine particles.

The Australian assessment framework for sediments (ANZECC/ARMCANZ, 2000) incorporates a tiered assessment procedure. The first stage is to measure total particulate metal concentrations (e.g. TR-Cu) and compare these concentrations to the guideline values. It is accepted that total particulate metal concentrations are likely to overestimate metal bioavailability, but are practically the easiest fraction to measure. For any sediment samples that exceed the guideline values, it is recommended that a second assessment stage is applied. This involves the measurement of dilute acid-extractable metal fractions (e.g. AE-Cu) and then compare these concentrations to the guideline values. Compared to TRM, the AEM concentrations provide a better estimation of the bio-available fraction of metals in sediments.

The sediment quality guidelines (SQGs) are based on the effects of sediment contaminants on benthic organisms. For copper, the 'trigger value' is 65 mg/kg and the ISQG-high value is 270 mg/kg. Concerns continue to be expressed for the reliability of SQG values, however, it is important to recognise that as multiple stressors will be present in most sediments, and organisms will have the varying responses to different stressors, that the guidelines will never provide an ability to predict when ecotoxicological effects will occur. The purpose of the trigger values is to act as a guide to when effects will not occur in any sediments, and the upper values as a guide to when effects may become more likely to occur. The results also indicate that it may be feasible to derive site-specific guidelines based on AE-Cu concentrations. While the <63 μm copper concentrations also appeared to be strongly linked to copper bioavailability, it is less practical to routinely monitor the copper associated with this sediment fraction (i.e. more laborious and expensive).

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1. BACKGROUND

As stated as background to the accompanying FRDC project, 2008/226, the Tasmanian Salmonid industry is presently managed on the basis of minimising disease risk whilst optimising productivity, and operationally industry's resources (labour and capital) continue to be mainly directed towards the control of gill amoebae (AGD) which requires timely freshwater bathing precipitating significant operational bottlenecks during spring and summer. Fish health and performance is in turn reliant on a good supply of oxygenated water, and net fouling is a major obstacle to water flow through fish pens. The anti-fouling also acts as a stiffening agent and vastly improves the resistance of the net material to seal attacks, which in the past few years alone have increased to the level of being a daily occurrence in multiple pens at multiple sites or leases. The cost of maintaining barrier defence systems against seals is now counted as costing multiple millions of dollars per year. Therefore the use of antifouling paints is critical to the industry, and must be managed in such a way that it does not detract from bathing operations. Although the industry continues to trial a range of alternative anti-fouling agents only copper oxide currently provides cost effective anti-fouling sufficient also to appropriately manage operational bottlenecks. Copper oxide anti-foulings have been in use by industry for the past 8-10 years under various APVMA (formerly NRA) research permits.

Surveys of copper loadings in sediments from salmon aquaculture leases in Tasmania have indicated highly elevated copper concentrations in several locations (O'Brien, 2007). The sites with the highest concentrations of organic matter have consistently displayed the highest mean concentrations of copper in the sediments, whereas the sites with low organic matter concentrations have low sediment copper concentrations (i.e. below the sediment quality guideline trigger value of 65 mg Cu/kg). In-situ cleaning, improperly dried or coated nets and abrasive marine operations are the most likely causes for any increase in sedimentary paint or copper levels.

Research undertaken by CSIRO Land and Water (Centre for Environmental Contaminants Research, CECR) through FRDC Project 2008/226 made the following observations regarding the bioavailability of copper within paint-contaminated sediments:

- Total copper concentrations at the low total organic carbon (scouring),high TOC (depositional), and recovery sites, show considerable variability. Sites with higher TOC generally having higher copper concentrations. TRM copper exceeded the ISQG-high value of 270 mg Cu/kg at some depositional sites, but not at scouring sites.

- Analyses of total copper within a range of particle size fractions indicated that a relatively large proportion of the total copper (45-92%) was associated with particles >63 μm in size. These results indicate that much of the copper-paint contamination is likely to be present as paint particles.

- Analyses of 1-M HCl extractable copper (AE-Cu) indicated that a large proportion of the total copper is present in relative insoluble forms. The maximum AE-Cu concentrations found were <20% of total copper. These results indicate that much of the copper-paint contamination is likely to be not bio-available.

- Acute, 10-day lethality tests using the copper-sensitive amphipod, *Melita plumulosa*, found no evidence for acute toxicity due to copper, although all sediments had total copper concentrations exceeding the guideline trigger value of 65mg Cu/kg. During 10-day tests, there was negligible release of dissolved copper to the overlying seawater (Simpson and Spadaro, 2008).

In conclusion, the study recommended a number of information gaps that should be addressed to fully understand the fate and effects of the paint product in the environment. These were to identify:

(i) the form and long-term bioavailability of the paint flakes in sediments,

(ii) sub-lethal or chronic toxic effects thresholds for the paint in sediments, and

(iii) broader effects to benthic communities of the paint in sediments.

The present study is aimed at identifying those bio-availability and potential sub-lethal or chronic effects of the paint in sediments.

The project is seen as pivotal in allowing the industry to continue its use of these anti-foulings at a sustainable and ultimately well managed level, ensuring that industry can continue to expand and satisfy market demand and continue to strengthen its presence in export markets through maintaining a competitive (in export terms particularly) cost of production.

1.1 Need

The TSGA has been involved in an extended programme with DEWHA, APVMA, DPIPWE (Tas) seeking to provide the necessary information to register antifoulants for use on nets employed on fish farms. The APVMA have agreed to re-issue a trial "research" permit to allow for the use of antifouling paints that have copper oxide as their sole active ingredient and which will assess the environmental sustainability of its use on fish farms. The present allocation of paint is totally reliant on the extension of a research permit and as such provides no medium or long term assurance for the use of antifoulants, without which the industry would face collapse. All efficacy data for the registration of Cu(I)oxide paints has been provided, however the effects of the paint copper on the local ecology and ecosystem function are currently poorly understood and without evidence to the contrary, it is difficult for the industry to refute the perception that such chemicals have a detrimental effect on the environment.

The present project description (together with that of FRDC 2008/226) has been designed based on the outcomes of the meeting held to discuss the "Ecological Impacts of selected Antifoulants & Antibiotics" (February 26, 2009 - TAFI Marine research Labs, Taroona, Tasmania, as part of FRDC project 2007/246, Macleod & Eriksen). Research relating to direct measures of copper (paint) bioavailability in sediments and possible conversion into more chemically bio-available forms were included in the FRDC project 2008/226. The present project specifically addresses the need to assess whether the 'paint' copper in the sediments, is bio-available by determining if there is any potential for chronic toxicity and/or bio-accumulation to occur at the concentrations of paint copper detected under fish farm pens.

The overall project is a result of extensive discussions between the TSGA, the APVMA and DEWHA and state government regulators, specifically DPIPWE/EPA. All agencies strongly believe that the work proposed in this project is vital and urgent. Both the TSGA (through Tassal and Huon Aquaculture Company) and DPIPWE are providing significant in-kind contributions.

1.2 Objectives

To provide information on the bioavailability and potential sub-lethal or chronic effects of copper-based anti-fouling paints contaminated sediments, using the following approaches.

(i) direct measures of bioavailability through measuring bio-accumulation of copper by the benthic bivalve, Tellina deltoidalis,

(ii) Sub-lethal and chronic toxicity tests using the three benthic organisms, amphipod (Melita.plumulosa), copepod (Nitocra spinipes) and bivalve (Tellina deltoidalis).

2. PROJECT SCOPE

The assessment of copper bioavailability in the sediments should consider both chemical and biological approaches. Chemical surrogate methods may be cheaper for future monitoring. Biological methods using bio-accumulation and toxic effects measures are necessary to validate the chemical approaches.

2.1 Copper bioavailability tests

The bioavailability of copper in the sediments may also be evaluated by considering copper bio-accumulation by benthic organisms. This may be achieved either by (i) sampling organisms from clean (reference) and contaminated sites and analysing accumulated metals and comparing differences, or (ii) by laboratory-based bio-accumulation tests in which organisms are exposed to copper-contaminated sediments for a predetermined period, then removed and copper accumulation is compared to controls. The second approach is recommended for this study as it allows good control of exposure conditions.

The following research was undertaken to provide information on the bioavailability of copper in sediments using biological methods:

- Bio-accumulation tests were undertaken using the benthic bivalve *Tellina deltoidalis,* involving exposure to contaminated sediment for 60 days, removal and depuration before measuring copper body concentrations. The results are compared to 'uncontaminated' controls with similar sediment properties (same exposure period and conditions) and analyses of particulate copper in sediments and dissolved copper in the pore waters and overlying waters were undertaken to aid interpretation of exposure pathways.

2.2 Sub-lethal or chronic toxicity tests

Sub-lethal or chronic effects of copper to aquatic organisms generally occur at copper concentrations that are 3- to 4-fold lower than the copper concentrations that cause acute effects (Simpson, 2006). For a range of copper-paint contaminated sediments changes in fecundity of an amphipod (*Melita plumulosa*), gravidity and development of a harpacticoid copepod (*Nitocra spinipes*), or sub-lethal growth of a benthic bivalve (*Tellina deltoidalis*) were assessed.

The toxic effects were considered in relation to natural stressors (ammonia, sulfide), and the concentration and form of copper in the sediments. In addition to the field-collected sediments, flakes taken from painted nets were used to artificially contaminant sediments in order to investigate dose-response relationships.

3. METHODS

3.1 Sediments

Fourteen sediment samples were collected by divers under the direction of Aquaculture, Management and Development Pty Ltd (AMD) during November 2009, of these seven were selected for use in the tests (Table 1). Samples from each site were collected either by divers using a shallow scoop, making sure only to collect the top 3-5 cm of sediment. Approximately 2 kg samples were transferred immediately to plastic bag. Once the samples were received at the surface they were triple bagged and placed in an Esky and dispatched within 48 h to CSIRO. The samples were supplied to CSIRO stored cold.

In general the samples sites were taken from leases for which comprehensive historical trends for paint use were available and/or they had been employed in the FRDC 2008/218 study. In addition sites at Leases 4 (S4-16,) and 2 (S2-40) were chosen as they had been very recently sampled during a TSGA/DPIPWE spatial survey of copper in and around pen sites at high and low organic sites. We therefore had recent TRM copper levels for these sites.

Table 1. Sediment samples

November 2009

Sample numbers	Organic carbon content	Expected Cu, mg/kg
Lease 4		
S4-16	Low	90
Lease 3		
S3-24	Presumed Low	100-200
Lease 2		
S2-40	High	720
Lease 1		
S1N1	High	1000
S1B	High	1100
S1C	High	700
S1-race	Presumed High	No data

3.2 General analytical procedures

Clean seawater was collected from Port Hacking, Sydney, Australia, membrane-filtered (0.45 µm), and acclimated to a room temperature of 21±1°C. Where necessary, the salinity of the filtered seawater was adjusted to the test salinity of 30‰ using Milli-Q deionised water (18 M Ω/cm; Milli-Q® Academic Water System).

All glass- and plastic-ware for dissolved metal analyses was new, and cleaned by soaking in 10% (v/v) HNO_3 (Tracepur, Merck, Darmstadt, Germany) for at least 24 h and rinsed with copious quantities of deionised water prior to use. Milli-Q deionised water was used to prepare all solutions. General methods for physical and chemical analyses of the waters and sediments are provided in Table 2.

Table 2. Methods for physical and chemical analyses

Analyte	Method
Water pH, dissolved oxygen and salinity	Measurements of pH (calibrated against National Institute of Standards and Technology certified buffers, Orion Pacific, Sydney, Australia) used a pH meter (pH 320, WTW, Weilheim, Germany) equipped with a combination pH (Sure-flow 9165BN, Thermo Orion, Beverley, MA, USA) probe. Dissolved oxygen (DO) and temperature measurements were made using a YSI 95 m (Model 95/25 Ft) and salinity measurements using a WTW meter (LF 320) with a Tetra-Con 325 probe. Salinity measurements have been reported according to the Practical Salinity Scale of 1978 (PSS 78) as dimensionless values.
Total recoverable metals (TRM)	Total recoverable metals (TRM) analyses were made by digestion of dried and ground bulk sample in hot (microwave digestion) concentrated 2:1 HCl:HNO_3 ('aqua regia'), followed by dilution and filtration (<0.45 µm). Acids were high purity (Merck Suprapur). QA/QC included sample blanks and duplicates and a certified reference sediment (PACS-2, National Research Council). The concentrations of dissolved metals in the acid extract were determined by ICP-MS and ICP-AES.
Dilute acid extractable metals (AEM, AE-Cu) (1 M HCl)	Dilute acid-extractable metals (AEM) were determined by digesting the sediment in 1 M HCl (~1 g/100 ml) for 60 min, followed by filtration (<0.45 µm). This metal fraction was designated as the simultaneously extracted metal (SEM) fraction, and the difference between the molar amounts of AVS and SEM (Cd, Cu, Ni, Pb, and Zn) was used according to AVS-SEM theory (USEPA, 2005) to predict whether these metals should be present in the sediments as metal-sulfide solid phases. The concentrations of dissolved metals in the acid extract were determined by ICP-MS and ICP-AES.
Porewater extraction	Pore water was isolated from sediment in an inert atmosphere (nitrogen) by filling a 50 mL centrifuge tube with sediment then centrifuging at 1000 g. The isolated pore water was filtered (<0.45 µm and acidified to 0.2% HNO_3 for trace-pure preservation.. The concentrations of dissolved metals were determined by ICP-AES.
Particle size fractionation	The particle size distribution of the sediment was determined by wet sieving through 63 µm nylon sieves followed by gravimetry
Acid volatile sulfide (AVS)	Acid-volatile sulfide (AVS) was analyzed according to the method described by Simpson (2001), whereby 2.5 ml of methylene blue reagent in 22 M sulfuric acid was reacted directly with 0.1 to 0.4 g of sediment in 50 ml of deoxygenated, deionized water (final H^+ concentration, ~1 N). After 1 h, the liberated sulfide was determined colorimetrically at 670 nm using a ultraviolet–visible light spectrophotometer (UVIDEC-610; Jasco), and the molar AVS concentration was calculated.
Total organic carbon (TOC)	APHA 21st ed., 5310 B.Total organic carbon (TOC) analyses in sediments were performed using a high temperature TOC analyser (Dohrmann DC-190) following removal of inorganic carbon (carbonates and bicarbonates) by acidification with 1 M HCl until effervescence was complete.
Dissolved Metals by ICP-AES	(APHA 21st ed., 3125; USEPA SW846 - 6020): The inductively-coupled plasma atomic emission spectroscopy (ICP-AES, Spectro, CIROS) using in-house methods (C-209 and C-229, respectively)..
Dissolved Metals by ICP-MS	(APHA 21st ed., 3125; USEPA SW846 - 6020): The inductively-coupled plasma mass spectroscopy (ICP-MS, in-house method C-209) technique utilizes a highly efficient argon plasma to ionize selected elements. Ions are then passed into a high vacuum mass spectrometer, which separates the analytes based on their distinct mass to charge ratios prior to their measurement by a discrete dynode ion detector.
Dissolved ammonia	Dissolved ammonia was analysed colorimetrically using a Merck Spectroquant Kit (14752) following manufacture instructions.

3.3 Bio-accumulation and toxicity tests

3.3.1 Bivalve bio-accumulation tests

The bioavailability of copper to the benthic bivalve *Tellina deltoidalis* was assessed using 8-week exposures to undiluted test sediments. This test was carried out according to the general procedures described in Simpson et al. (2005) using the test conditions summarised in Appendix 3. Sediments were homogenised immediately prior to being added to test beakers (220 g sediment per 1 L beaker, 3 replicates per sediment). Filtered seawater (30 ‰) was added, and each beaker was incubated at 21°C with aeration overnight to allow sediments to settle. The following day, overlying water was replaced and 13 bivalves of an unknown size (20-160 mm^2) were randomly assigned to each beaker. Bivalves used in the tests were collected from a local estuary in NSW and allowed to acclimate to laboratory conditions. Overlying water concentrations of metals and ammonia, along with physio-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured throughout the 8 week test. The number of surviving bivalves in each beaker was recorded and their size after 8 weeks measured using a flat-bed scanner and Scion® imaging software. Results are expressed as a percentage of the survival in the control sediment. Treatments were fed at a rate of 1 mg Sera micron® fish food/bivalve per week.

At the termination of the tests, surviving organisms were counted and allowed to depurate overnight in clean seawater. Organisms were then scanned to determine their size and calculate their growth over the 8 weeks. Their shells were then removed using a Teflon-coated razor blade and their soft tissue mass collected using plastic tweezers. Tissue masses from the same replicate were placed in a pre-weighed 30 mL polycarbonate vial and left overnight in a domestic freezer at -20 °C. They were then freeze-dried (Christ Alpha 1-2 LD Plus Freeze Dryer) until completely dry tissues were obtained (approximately 5 h), and the tissue dry weight (DW) was determined. Dried tissues were combined with 2 mL of ultrapure HNO_3 (Tracepur, Merck, Darmstadt, Germany), allowed to stand at room temperature for 24 h, then microwave heated (1100 W domestic microwave) for 7 minutes at 30% power, 10 minutes at 20% power and 30 minutes at 10% power. This program was performed twice. Vials were uncapped and let to cool down and degas in a fume hood between each heating. Cooled digests were combined with 2 mL of H_2O_2 (30%, GR for analysis ISO, Merck, Kilsyth, Australia), and were allowed to stand for 18 h in a fume hood. Digests were reheated in the microwave for 20 minutes on 10 % power and then diluted with Milli-Q water to a final volume of 25 mL. Samples were shaken, left to settle overnight, and then diluted with Milli-Q water. Copper and zinc concentrations were measured by inductively coupled plasma-mass spectrometry (ICPMS, Agilent 7500ce) calibrated with matrix-matched standards. For quality control purposes, one blank (Milli-Q water) and one reference sample (DORM-3, Fish Protein Certified Reference Material for Trace Metals, National Research Council Canada) were analysed for every 7 samples.

3.3.2 Amphipod survival and reproduction tests

This test measures the survival and number of amphipod (*Melita plumulosa*) embryos and <1 day old juveniles in the second brood following exposure to undiluted test sediments over a 10 day period (Mann et al., 2009). The test conditions are summarised in Appendix 3. Sediments were homogenised immediately prior to being added to test vials (40 g sediment per 250 ml vial, 4-5 replicates per sediment). Filtered seawater (30 ‰) was added, and each beaker was incubated at 21°C with aeration overnight to allow sediments to settle. The following day, overlying water was replaced, five gravid females (gravid for <24 h) and 7 males were randomly assigned to each beaker. Amphipods used in the tests were isolated from laboratory cultures. The sediments were renewed after 5 days by gently sieving away the adults and placing them into the same fresh sediment that had been equilibrated overnight. This allows for the removal of juveniles from the first brood which is typically unaffected by contaminates in the test sediment.

Overlying water concentrations of metals and ammonia, along with physio-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured at 1 h, before and after the 5 day sediment renewal and on day 10. The number of embryos per female was counted by microscopy and expressed as a percentage of the control. The sediment was also checked (by sieving the sediment through 180 μm mesh) for juvenile amphipods that had escaped the marsupium (pouch where the young are held securely) during the latter stages of the test. Treatments were fed at a rate of 1 mg sera micron® fish food/amphipod twice a week.

3.3.3 Copepod life-cycle (reproduction and development) tests

This test measures the reproductive output of the copepod *Nitocra spinipes* following exposure to undiluted test sediments over a 10-day period (Perez and Simpson, unpublished method). The test conditions are summarised in Appendix 3. Sediments were homogenised immediately prior to being added to test vials (0.5 g sediment per 10 ml vial, 5-6 replicates per sediment). Filtered seawater (30 ‰) was added, and each vial was incubated at 21°C overnight to allow sediments to settle. The following day, overlying water was replaced and five gravid females (3-5 weeks old) were randomly assigned to each vial. Copepods used in the tests were isolated from laboratory cultures. Overlying water concentrations of metals and ammonia, along with physio-chemical parameters (temperature, pH, salinity and dissolved oxygen) were measured twice during the 10-day test. The number of nauplii (first juvenile lifestage of the copepod) and copepodites (second juvenile lifestage) in each vial was recorded by microscopy. Results were expressed as a percentage of the survival in the control sediment. Treatments were fed a diet of 1×10^4 cell per mL of both *Isochrysis* sp. and *Tetraselmis* sp. as well as 0.3 mg Sera micron® fish food (<63 μm) per test vial twice a week.

4. RESULTS

4.1 Sediments

The concentrations of TRM measured in the sediments are shown in Table 3. Total copper concentrations ranged from 64-110 (S4/S3 sites), 310 (S2 sites) and 400-810 (S1 sites) mg/kg, respectively. These concentrations can be compared to the sediment quality guideline trigger value of 65 mg/kg and the ISQG-high value of 270 mg/kg, respectively (ANZECC/ARMCANZ, 2000).

Table 3. . Total recoverable metal concentrations in the bulk sediment.

	Total recoverable metals (TRM), mg/kg								
Sample Name	**As**	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**V**	**Zn**
Lease 4									
S4-16	6	0.6	18	64	33	6	5	23	120
Lease 3									
S3-24	6	0.4	15	110	22	6	21	10	148
Lease 1									
S1-N1	22	0.2	51	810	55	22	21	82	310
S1-B	10	0.3	25	720	32	7	7	59	170
S1-C	21	0.6	47	790	52	23	20	70	230
S1-race	18	0.6	56	400	70	28	20	83	150
Lease 2									
S2-40	23	0.8	54	**310**	59	26	22	89	200
Trigger value	20	1.5	80	65	NV	21	50	NV	200
ISQG-high	**70**	**10**	**370**	**270**	**NV**	**53**	**220**	**NV**	**410**

NV = no guideline value available.

4.2 Bioavailability of copper to benthic bivalves

For samples S1-N1, S1-C and S2-40, 8-week bio-accumulation tests were undertaken using the benthic deposit-feeding bivalve, *Tellina deltoidalis*, and the differences in growth are shown in Table 4. The control sediment comprised a 2:1 mixture (~67% <63 μm) of Bonnet Bay sediment and Sydney sand, where the Bonnet Bay sediment comprised entirely silt. The growth of *T. deltoidalis* was significantly lower in the S1-

N1 and S1-C sediments which had the highest total copper concentrations, compared to bivalve growth in sediment S2-40. However, since bivalve growth in all three sediments was greater than that in the control sediment which had negligible copper (<15 mg/kg), there were likely to have been factors other than copper affecting bivalve growth. Copper concentrations in the bivalve tissues at the completion of the tests are compared to the sediment copper concentrations in sediment and overlying water (Table 5, Figure 1).

Table 4. Growth of the bivalve T. deltoidalis in test sediments over 8 weeks.

Sediment	Total copper in sediment	Growth of bivalve (*Tellina deltoidalis*)			
	mg/kg	% growth	SE	% control	SE
Control	<15	16	4.8	100	31
S1-N1	810	24	3.9	156	25
S1-C	790	21	2.6	137	17
S2-40	310	39	1.7	253	33

SE = standard error

Table 5. Copper concentrations in the bivalve T. deltoidalis after 8-week growth tests compared with copper concentrations in the sediments.

	Copper in bivalve tissues, mg/kg		Total copper, mg/kg		1-M HCl extractable copper, mg/kg		Dissolved copper, µg/L	
Sediment	Mean	SE	Bulk	<63 µm	Bulk	<63 µm	mean	SD
Control	93	2	<15	15	<10	10	1.2	2.0
S1-N1	192	13	810	430	203	162	3.8	2.6
S1-C	118	28	790	120	86	58	1.9	3.0
S2-40	88	32	310	47	31	22	0.6	0.8

SE = standard error. SD = standard deviation

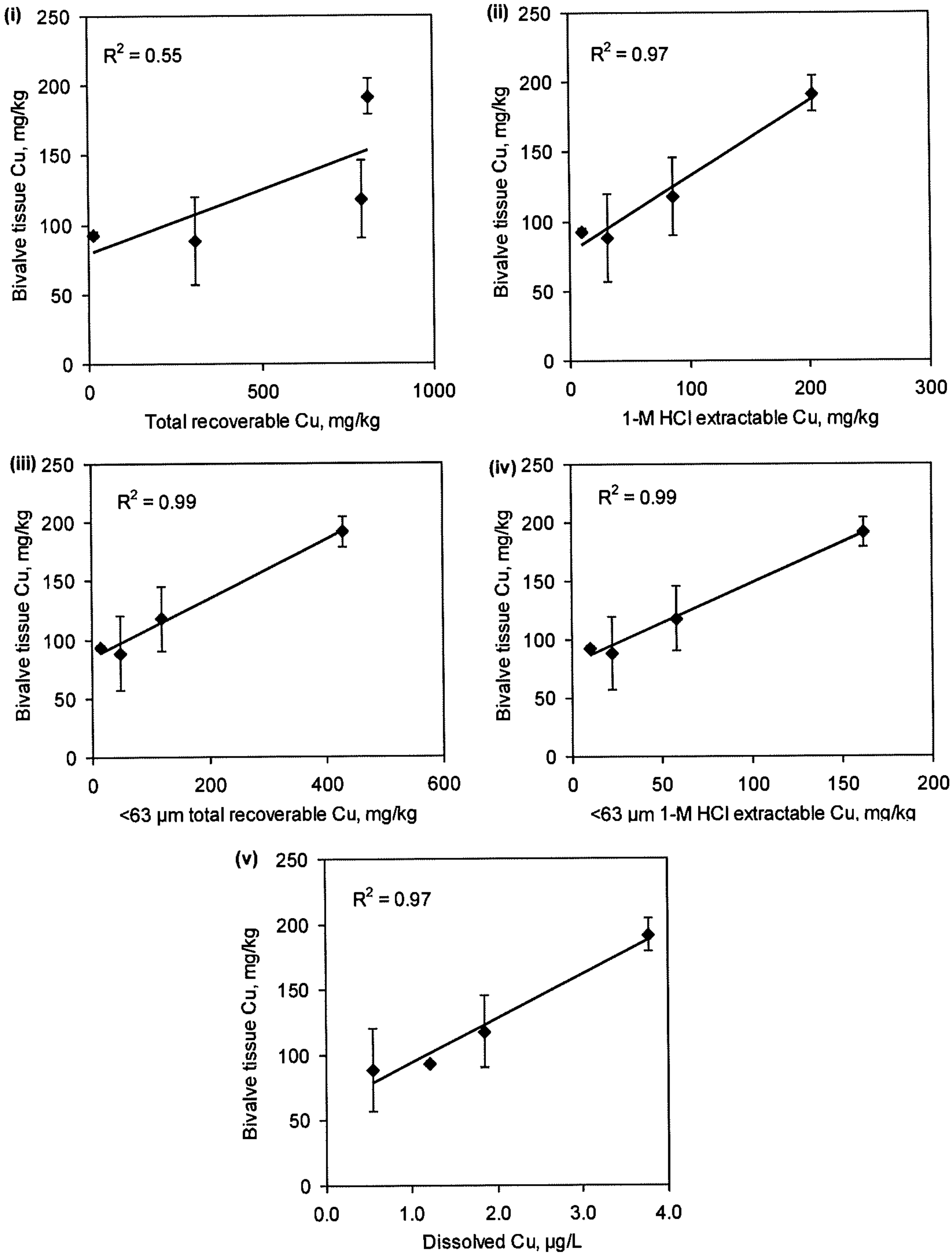

Figure 1. Comparison of copper accumulation by the bivalve, T. deltoidalis, with (i) total recoverable copper, (ii) 1-M HCl extractable copper, (iii) TRM for <63 µm sediment fraction, (iv) AEM for <63 µm sediment fraction, and (v) the average dissolved copper concentration in the overlying water during the 8-week tests.

The relationship between total copper and the copper bio-accumulation by the bivalves was weak (Figure 1 (i)). However relationships were previously shown (O'Brien et al, FRDC 2008/226), between the copper bio-accumulation by the bivalves, the AE- Cu concentration of the bulk sediment and the copper concentration of the <63 µm sediment fraction (for TRM or AEM) were significant ($p<0.001$) and very strong (r^2 - 0.97).

Although the dissolved copper concentrations were quite low during the tests (Table 5), they also correlated strongly with measured copper bio-accumulation (Figure 1 (v)). As the porewater copper concentrations were not monitored during these tests it is not possible to determine if low porewater copper concentrations also contributed to the copper bio-accumulation. However, it is important to note that for these sediments in the field, the dissolved copper concentrations in the overlying water would be much lower due to dilution with surrounding seawater. As a consequence, the rate of bio-accumulation by organisms in the field is also expected to be lower

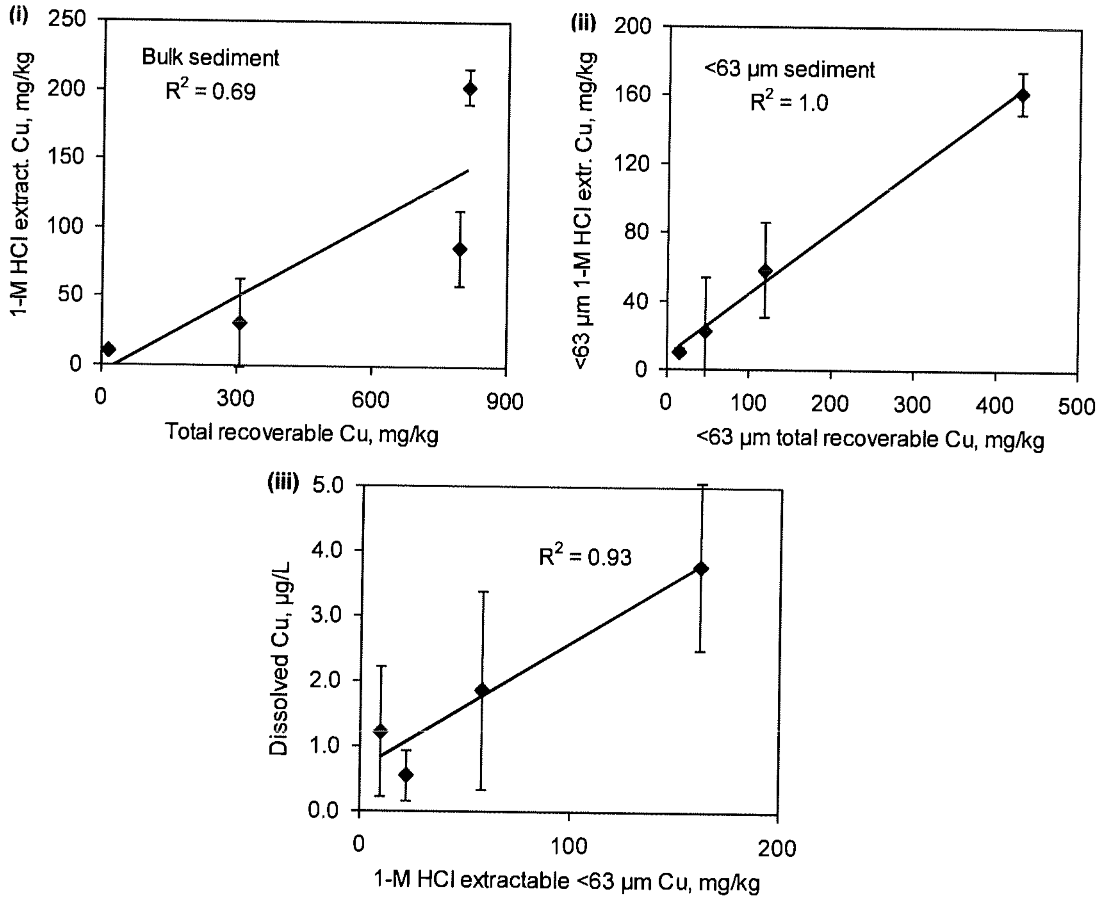

Figure 2. Relationships between copper in the forms of AEM vs TRM for (i) bulk sediment and (ii) <63 µm sediment fraction, and (iii) between <63 µm AE- Cu and dissolved copper in the overlying waters (sampled daily during the tests).

As noted earlier, there was a very strong relationship between TRM and AEM for the <63 µm sediment fraction. There were also a strong relationship between the copper concentration of the <63 µm sediment fraction and the dissolved copper released to the overlying water during the tests (Figure 2 (iii)).

While these experiments cannot be used to determine whether increasing AE-Cu, increasing portions of <63 µm copper, or whether the dissolved copper concentration in the overlying water contributed more significantly to increased copper accumulation by the bivalves, it is likely that the bio-accumulation was due to a combination of these copper exposure sources and routes (Simpson and Batley, 2007). The observation of copper in each of these phases indicates a greater degree of bioavailability than copper present as total particulate phase. *T. deltoidalis* is a deposit feeding bivalve, actively ingesting fine particles, and both dissolved and particulate exposure routes have been demonstrated to contribute to copper bio-accumulation by this species (King et al., 2005).

4.3 Chronic whole-sediment toxicity test results

4.3.1 Amphipod survival and reproduction

Consistent with past observations (Simpson and Spadaro, 2008), the sediments exhibited no acute toxicity to the amphipods. The toxic effects to the reproductive output of the amphipod *Melita plumulosa* was assessed for the six sediments (Table 6). The number of embryos produced by the female amphipods in the controls (either clean silty-sand or mixtures of less-contaminated, field-collected sediments from Tasmania) in the three tests, were within test acceptability limits of 13-18 embryos per female. Five out of the six sediments tested were considered non-toxic, only S1-N1 was toxic to the reproduction of the amphipod.

Table 6. Amphipod reproduction tests

Sediment	10-day survival (% control)	Reproduction: Juvenile per female	Reproduction: % control	Toxic/ Not Toxic
S4-16 (Control)	100 ± 2	17 ± 1	100 ± 8	Not Toxic
S1-B	100 ± 2	16 ± 2	91 ± 9	Not Toxic
S3-24	86 ± 5	13 ± 2	76 ± 13	Slightly Toxic
S4-16:BB (Control)	100 ± 6	16 ± 2	100 ± 15	Not Toxic
S2-40	100 ± 8	13 ± 2	83 ± 12	Not Toxic
S1-race	108 ± 4	15 ± 1	91 ± 7	Not Toxic
2BB:1SS (Control)	100 ± 3	17 ± 1	100 ± 8	Not Toxic
S1-N1	98 ± 4	10 ± 2	56 ± 10 [b]	Toxic
S1-C	102 ± 2	16 ± 1	92 ± 7	Not Toxic

[a] Mean ± Standard Error, [b] Significantly less than silty sand control.
Controls are matched with respect to general sediment properties

Based on the dissolved copper and ammonia concentrations measured in the overlying water during the tests, it appears unlikely that these dissolved constituents contributed to the toxicity observed in the S1-N1 sediment (Table 7). The S1-N1 sediment had the highest concentrations of TR-Cu (Table 3) and AE-Cu (FRDC Report 2008/226, Table 8). Of the three sediments used for bivalve bio-accumulation tests, S1-N1 resulted in the greatest copper bio-accumulation, i.e. indicating copper bioavailability. Based on the knowledge that *M. plumulosa* ingests fine sediments to obtain nutrition and the relatively high portion of fine sediments that were contaminated with copper (FRDC Report 2008/226, Table 8), i.e., 429 mg Cu/kg for the <63 μm sediment fraction, we believe this is the likely cause of the toxicity exhibited by the S1-N1 sediment.

The highest concentrations of dissolved copper in the overlying water were measured in the test of the non-toxic S1-B sediment and slightly toxic S3-24 sediment (Table 7). As the porewater copper concentrations were negligible, the dissolved copper in the overlying water is believed to originate from copper desorbing from particles at the sediment-water interface of the surface sediments. For these sediments in the field, the dissolved copper concentrations in the overlying water would be much lower due to dilution with surrounding seawater.

Table 7. Dissolved copper and ammonia concentrations during the 10-day amphipod toxicity test

Sediment	Pore water, μg Cu/L	Overlying water, μg Cu/L				Overlying water, mg total NH_4/L				
	Initial	Day 0	Day 5i	Day 5ii	Day 10	pH	Day 0	Day 5i	Day 5ii	Day 10
Silty Sand	<3	<3	<3	<3	<3	7.9	0.7	3.5	0.9	2.3
S4-16:Silt	<3	<3	<3	<3	<3	NA	NA	NA	NA	NA
S4-16	<3	<3	<3	<3	<3	7.9	0.8	6.9	2.4	7.3
S1-B	<3	<3	28	<3	28	8.0	0.9	6.3	1.9	6.6
S3-24	<3	<3	15	<3	14	8.0	0.8	6.9	1.7	7.3
S1-N1	<3	<3	<3	<3	<3	8.0	0.9	4.2	0.7	2.6
S1-C	<3	<3	<3	<3	<3	8.0	0.7	2.7	0.5	1.4
S2-40	<3	<3	<3	<3	<3	8.0	0.8	3.3	0.7	2.2
S1-race	<3	<3	<3	<3	<3	8.0	0.5	4.8	1.4	5.7

On day 5, measurements were made before (i) and after (ii) sediment and overlying water renewals. NA = not analysed

4.3.2 Copepod reproduction and development

The toxic effects to the reproductive output of the copepod *Nitocra spinipes* was assessed for six of the sediments (Table 8). The numbers of juveniles produced by the female copepods in the controls were within test acceptability limits of 20-54 juveniles per female. The S1-B sediment was considered toxic to the reproductive output of the copepod (48±4 and 69±3 % control for the repeated tests on this sediment, respectively). The S1-N1, S1-C and S1-race sediments may also indicate low levels of toxicity (79-81% control, and statistically different to controls), but as this is a relatively new test procedure we do not have adequate information to determine if this is environmentally significant.

Total dissolved ammonia concentrations in the overlying water were below the 'no effects' concentration of 10 mg total NH_3/L (Table 9; Perez, Simpson and Spadaro, unpublished results). The sediments S3-24 and S2-40 had some of the higher dissolved ammonia concentrations and showed no reproductive toxicity to the copepods. Ammonia was not considered to be causing toxic effects in any of the tests.

Table 8. Copepod reproduction and development tests

Sediment	Nauplii		Total Juvenile Production		Toxic/ Not Toxic
	per female	% control	per female	% control	
Silty Sand	28 ± 3	100 ± 12	34 ± 3	100 ± 14	Not Toxic
S4-16 – Control	34 ± 2	100 ± 5	40 ± 2	100 ± 5	Not Toxic
S1-B	16 ± 1	48 ± 4	17 ± 2	43 ± 4 [b]	Toxic
S1-B (repeated)	30 ± 1	69 ± 3	30 ± 1	69 ± 3	Toxic
S3-24	30 ± 2	89 ± 4	36 ± 2	90 ± 6	Not Toxic
S4-16:Silt (Control)	23 ± 2	100 ± 8	25 ± 1	100 ± 6	Not Toxic
S1-N1	17 ± 1	75 ± 5	20 ± 2	80 ± 7	Slightly Toxic
S1-C	17 ± 1	73 ± 3	20 ± 1	79 ± 3	Slightly Toxic
S2-40	21 ± 2	93 ± 7	23 ± 1	93 ± 5	Not Toxic
S1-race	18 ± 1	80 ± 2	21 ± 1	81 ± 4	Slightly Toxic

[a] Mean ± Standard Error, [b] Significantly less than silty sand control.

Table 9. Dissolved copper concentrations during the 10-day copepod toxicity test

Sediment	Pore water, µg Cu/L	Overlying water, µg Cu/L		Overlying water, mg total NH_3/L		
	Initial	Day 6	Day 10	pH	Day 6	Day 10
Silty Sand	<3	<3	<3	7.7	4.2	6.5
S4-16:Silt	<3	<3	<3	-	-	-
S4-16	<3	<3	<3	7.9	3.8	4.3
S1-B	<3	25	38	7.9	2.3	3.7
S3-24	<3	4.5	6.5	7.9	3.9	5.0
S1-N1	<3	<3	<3	7.9	0.8	0.8
S1-C	<3	<3	<3	7.9	0.8	0.9
S2-40	<3	<3	<3	7.9	1.2	1.7
S1-race	<3	<3	<3	8.2	1.7	2.0

The S1-B sediment that exhibited toxicity has the greatest release of copper to the overlying waters (Table 9). As the porewater copper concentrations were negligible, this result indicated that the HB sediment contained 'labile' forms of copper that may be bio-available to organisms inhabiting this surface sediment layer. A 10-day water-only copper EC50 (the concentration that causes a 50% reduction in copepod reproductive output) has not been accurately established for this test, however, the 7-day copper EC50 (which would underestimate the sensitivity to copper over ten days) is 95 µg Cu/L, with a no-observable effect concentration (NOEC) of ~40 µg Cu/L. As the dissolved copper concentration in the S1-B test was below the 7-day NOEC it is likely that exposure to other forms of bio-available copper, was also likely to be contributing to the toxic effects. The feeding behaviour of this copepod species has not been thoroughly studied, and it is not known whether copper associated with fine particles is a significant route of metal exposure.

5. DISCUSSION

5.1 Bio-accumulation and bio-availability

The accumulation of copper by the bivalves confirmed the presence of bio-available copper in the copper-paint contaminated sediments.

Of the three sediments used for bivalve bio-accumulation tests, S1-N1 resulted in the greatest copper bio-accumulation. The copper bio-accumulation was poorly correlated with TR-Cu (bulk sediment), but strong relationships were observed with TR-Cu for the <63 μm fraction, AE-Cu and the dissolved copper released to the overlying water during the tests. While it was not possible to accurately proportion the copper bio-accumulation to the different copper sources, based on the strength of the relationships and the chemical measurements, it is likely that the AE-Cu associated with the fine sediments is the major source of the labile copper resulting in the dissolved exposure and also the major contributor to the dietary exposure route. Additional insight into which copper exposure route is the most important might be gained if bio-accumulation tests were also made with sediment S1-B. Compared with the tested sediment S1-C which had 50% <63 μm particles, low AE-Cu, and low dissolved copper release, the S1-B sediment comprised only 8% <63 μm particles, but the AE-Cu was very high for this sediment fraction and the dissolved copper release was high.

5.2 Toxicity

Consistent with previous studies (Simpson and Spadaro, 2008), acute lethal effects to adult amphipods or copepods were not observed in the toxicity tests. The chronic toxicity tests demonstrated that chronic effects (in the copepod and amphipod) may occur for some copper-paint contaminated sediments, but not necessarily for the same sediments. Copper released to the overlying water during the toxicity tests indicated that labile forms of copper exist in the surface sediments.

Chronic effects occurred to *M. plumulosa* in a sediment with ~800 mg Cu/kg (S1-N1) and to *N. spinipes* in a sediment with 290-720 mg Cu/kg (S1-B – TR-Cu concentrations differed for the two sub-samples analysed). However, the S1-C sediment which had 320-790 mg Cu/kg was not toxic to *M. plumulosa* and only slightly toxic to *N. spinipes* (79±3, % control).

For the *M. plumulosa* tests, the S1-N1 sediment (~800 mg Cu/kg) caused chronic toxicity but the sediments S1-B, S1-C and S1-race with TR-Cu concentrations of 290-790 mg Cu/kg did not cause chronic toxicity. The greater toxicity of the S1-N1 sediment was attributed to its higher 'load' of <63 μm particles that were high in copper. The <63 μm sediment fraction of the S1-N1 sediment was 65% of the total and had 429 mg Cu/kg. In contrast, the <63 μm sediment fraction comprised just 8% of the S1-B sediment although the TR-Cu concentration was 1420 mg/kg for this fraction.

The S1-C and S1-race sediments has 50 and 79% <63 µm particles with TR-Cu concentrations of 119 and 332 mg/kg, respectively.

The exposure of *M. plumulosa* to copper in the fine sediment fraction was therefore likely to be greater for the S1-N1 sediment. The greater concentration of dissolved copper in the overlying waters during the S1-B sediment tests compared to the S1-N1 sediment tests was consistent with the copper being very labile for fine sediments with high copper concentrations, i.e., <63 µm S1-B was 913 mg AE-Cu/kg; <63 µm S1-N1 was 162 mg AeE-Cu/kg (FRDC Report 2008/226, Table 12). However, for the *M. plumulosa* tests, the dissolved copper concentration remained below or similar to the predicted no-observable effect concentration. Based on these results, the exposure to copper associated with fine particles was likely to have caused the greatest contribution to chronic toxicity to *M. plumulosa*.

For the *N. spinipes* tests, the S1-B sediment caused chronic toxicity (48±4 % control). The combination of a very high concentration of AE-Cu for the fine sediment fraction at 1420 mg/kg (FRDC Report 2008/226, Table 8) and the greatest release of copper to the overlying waters is likely to be the cause of the toxicity of the S1-B sediment to the copepod. This conclusion is drawn despite the fact that the <63 µm sediment fraction comprised only 8% of the S1-B sediment. It is possible that the much smaller copepod (generally 300-600 µm) has greater interaction with the fine (copper-contaminated) particles at the surface of the sediment than the larger amphipod (generally 6-9 mm). While the copepod is expected to feed on the algae and powdered fish food added during the tests, like the amphipod, it may also inadvertently ingest other fine particles.

Based on dilute-acid extractable metal concentrations (AE-Cu), chronic effects occurred to *M. plumulosa* at 200 mg AE-Cu/kg (S1-N1) and to *N. spinipes* at 140 mg AE-Cu/kg (S1-B). However, the S1-race sediment which had 170 mg AE-Cu/kg was not toxic to *M. plumulosa* and only slightly toxic to *N. spinipes* (81±4, % control). The S1-C sediment had 80 mg AE-Cu/kg.

Note that it is difficult to determine the threshold for chronic effects of dissolved metals (including copper) to *M. plumulosa* (Mann et al., 2009) during whole-sediment toxicity tests. Based on thresholds for effects from copper to *M. plumulosa* during 10-day acute water-only tests (Spadaro et al., 2008), and to *N. spinipes* during 13-day life-cycle water-only tests (unpublished results), we believe that the copepod *N. spinipes* and *M. plumulosa* have a similar sensitivity to dissolved copper.

5.3 Effects thresholds and measures of copper bio-availability in paint contaminated sediments

The Australian assessment framework for sediments (ANZECC/ARMCANZ, 2000) incorporates a tiered assessment procedure. The first stage is to measure total particulate metal concentrations (e.g. TR-Cu) and compare these concentrations to the guideline values. It is accepted that total particulate metal concentrations are likely to overestimate metal bio-availability, but are practically the easiest fraction to measure. For any sediment samples that exceed the guideline values, it is recommended that a second assessment stage is applied. This involves the measurement of dilute acid-extractable metal fractions (e.g. AE-Cu) and then compare these concentrations to the guideline values. Compared to TRM, the AEM concentrations provide a better estimation of the bio-available fraction of metals in sediments.

The sediment quality guidelines (SQGs) are based on the effects of sediment contaminants on benthic organisms. The sediment quality guideline trigger value (or ISQG-low) is based on the lower 10^{th} percentile of an effects database. This is used as a screening value that if exceeded, additional investigations are made to determine whether there is indeed a risk posed by the guideline exceedance. A second upper guideline value, the ISQG-high, is also derived from the median of the effects data. It has no particular significance except as being indicative of a value above which there is a high probability of toxicity to benthic organisms. For copper, the 'trigger value' is 65 mg/kg and the ISQG-high value is 270 mg/kg.

Concerns continue to be expressed for the reliability of SQG values, however, it is important to recognise that as multiple stressors will be present in most sediments, and organisms will have the varying responses to different stressors, that the guidelines will never provide an ability to predict when ecotoxicological effects will occur. The purpose of the TVs is to act as a guide to when effects will not occur in any sediments, and the upper values as a guide to when effects may become more likely to occur. In this context, we know that because the empirical guidelines were derived from a ranking of toxicity and other effects data, and because contaminants typically co-occur (e.g. metals and organics), then any toxicity was equally attributed to all components of the mixture. For example, toxicity may be due to high concentrations of PAHs in a sediment, but this is equally ascribed to copper which may be present but not causing effects. The copper guideline then becomes overly conservative (Batley and Simpson, 2008).

For the present study, chronic effects occurred to *M. plumulosa* in a sediment with ~800 mg TR-Cu/kg (S1-N1) and to *N. spinipes* in a sediment with 290-720 mg TR-Cu/kg (S1-B). Based on AE-Cu, chronic effects occurred to *M. plumulosa* at 203 mg AE-Cu/kg (S1-N1) and to *N. spinipes* at 139 mg AE-Cu/kg (S1-B). However, the S1-race sediment which had 173 mg AE-Cu/kg was not toxic to *M. plumulosa* and only slightly toxic to *N. spinipes* (81±4, % control). The S1-C sediment which had 320-790 TR-Cu/kg and had 80 mg AE-Cu/kg did not cause effects to either species. Overall, these results are consistent with the upper guideline value of 270 mg TR-Cu/kg providing a conservative guideline for managing the risks associated with the copper contamination in sediments associated with the aquaculture leases. The results also indicate that it may be feasible to derive site-specific guidelines based on AE-Cu concentrations. While the <63 μm copper concentrations also appeared to be strongly linked to copper bioavailability, it is less practical to routinely monitor the copper associated with this sediment fraction (i.e. more laborious and expensive).

6. PLANNED OUTCOMES/ BENEFITS AND ADOPTION

The present project provides the first data to support a quantitative basis for understanding the long term bio-availability of the 'paint'-derived copper, and, helps to ensure that any decision on the regulatory limits applicable to the practice of using anti-fouling paints for aquaculture nets can provide for long term protection against chronic effects as well as acute short term effects. Further it provides confidence that the present Sedimentary Guideline limits used to regulate the industry's use of the paint are conservative, and are therefore effective at providing protection and ensuring sustainability, albeit that in future we may be able to provide more accurate 'bio-available based' guideline limits.

Together with project FRDC 2008/226 the present study therefore provides information that will now be used by regulators to define limits on the deposition of paint derived copper and has aided in the design of a monitoring system for TRM and bio-available copper in sediments. This information will also provide the basis for the assessment for the full registration of copper-based anti-foulings for fish farm nets and provide for a well-defined set of guidelines for its sustainable use, allowing the industry to more reliably plan and manage its continued expansion.

Results for the present study, in combination with the 2010 monitoring survey are currently being adopted into the management strategies of the fish farm companies employing copper based paints. The focus at the farm level is for:

- the minimisation of fish net manipulations through the use of soft (non-antifouled) nets for fish crowding, this eliminates the need for bending and folding anti-fouled nets during fish transfers.
- The increased use of monofilament and plastic nets especially at high organic (depositional) sites, and,
- The general shift in the use of anti-foulings to low organic (scouring) sites.

The data from the present study together with those provided in FRDC project 2008/226, are being incorporated into the Risk Assessment process employed by the APVMA in order to determine the limits associated with the environmentally sustainable use of Cu(I)O anti-fouling paint on fish farm nets as part of the requirements for its' registration, and/or continuance of the Research permit in the interim. A modified monitoring regime is now underway for 2010, that takes into consideration the information provided in the present study, including, the possibility for assessing a more accurate method for assessing the bio-availability of the copper in those sediments.

7. FURTHER DEVELOPMENT

Specific recommendations for further development are as follows.

As the copper contamination in sediments has arisen through the use of the copper-based antifouling paints and has been demonstrated to be bioavailable and toxic to benthic invertebrates, improved management practices are required to assess and monitor the risks associated with the copper contamination.

To improve the management of sediment contamination arising from the fish aquaculture in Tasmania, the following recommendations are made:

(i) Site-specific guidelines should be developed for copper contamination arising from fish aquaculture practices. A guideline that considers the bioavailability of copper in the sediments, e.g. using AE-Cu, would provide a more predictive method for managing the risks associated with the copper-paint contaminated sediments;

(ii) Tests are required to better understand the changes in the bioavailability of paint-associated copper on nets. These tests should be undertaken on the same net materials after known periods of field-deployment. Specific attention should be given to the effects of UV-light exposure on nets that are brought ashore, but the redeployed. The influence of *in situ* cleaning on abrasion of paint particles from the nets should be assessed;

(iii) Changes in the bioavailability of copper within the sediments that occur over time need to be assessed. Particular attention should be given to the natural remediation that may occur at sites after fallowing and should include measurements of deposition rates, analyses of phases such AVS that will reduce the mobility of copper in anoxic sediments, and both particulate and porewater copper measurements through sediment depth profiles.

To derive site-specific guidelines for both low- and high-organic sediment types, the following tests and experiments are recommended. Copper paint removed from used nets (e.g. following 6-months deployment) should be used to artificially contaminate sediments with a range of total copper concentrations, e.g. 0, 50, 100, 200, 400, 800 mg/kg total copper. Chronic toxicity tests should be undertaken on these 'spiked' sediments to create concentration-response curves based on the copper concentrations. Both the amphipod, *M. plumulosa*, and the copepod, *N. spinipes*, should be used, but may also need to be supported by other test species. The tests should be supplemented by chemical analyses that allow effects thresholds to be derived based on TR-Cu and AE-Cu concentrations, but also consider the attributes of others measurements that may provide useful information on copper bioavailability. Additional toxicity tests should also be undertaken on contaminated sediments collected from fish aquaculture leases to strengthen the concentration-response relationships.

In addition, the results from the present study have been reviewed in the 2nd FRDC Workshop on the research objectives for the use of Cu(I)O anti-fouling paints by the fish farming industry in Tasmania. The workshop was comprised of all major stakeholders (comprised of Federal & State Regulatory Authorities, the Industry and Research Organisations). The Outcomes and Action items from this workshop are provided in detail in Appendix 4. Previous to the workshop a meeting of all stakeholders had already considered the implications of the results from the two projects and requested that Dr Stuart Simpson (CECR) provide a brief outline of the further research that might be undertaken to address continued concerns of the group, for consideration at the 2nd workshop.

In brief the recommendations from the workshop and meeting identified that there were still a number of knowledge gaps in terms of research required to assist the Registration process and general continued use of the Cu-paints, principle amongst these were:

- To develop effective concentration response relationships based on AE Cu.
- To provide information on the potential for natural remediation through assessment of form and fate of copper.

A project that reflects these priorities and also takes into consideration the broad additional recommendations of the workshop is currently being drafted by the CECR in conjunction with the Tasmanian Aquaculture and Fisheries Institute.

8. CONCLUSION

The present study together with FRDC project 2008/226 now form the major part of the scientific basis for both the continued sustainably managed use of Cu(I)O anti-foulings in the Tasmanian salmon industry, and, their registration.

In detail; this project has demonstrated that:

- The accumulation of copper by the bivalves confirmed the presence of bio-available copper in the copper-paint contaminated sediments. The copper bio-accumulation was poorly correlated with TR-Cu (bulk sediment), but strong relationships were observed with TR-Cu for the <63 µm fraction, AE-Cu and the dissolved copper released to the overlying water during the tests.

- Consistent with previous studies (Simpson and Spadaro, 2008), acute lethal effects to adult amphipods or copepods were not observed in the toxicity tests.

- The chronic toxicity tests demonstrated that chronic effects (in the copepod and amphipod) may occur for some copper-paint contaminated sediments, but not necessarily for the same sediments.

- Copper released to the overlying water during the toxicity tests indicated that labile forms of copper exist in the surface sediments.

- Overall, the results provided are consistent with the upper guideline value of 270 mg TR-Cu/kg providing a conservative guideline for managing the risks associated with the copper contamination in sediments associated with the aquaculture leases.

- The results also indicate that it may be feasible to derive site-specific guidelines based on AE-Cu concentrations. While the <63 µm copper concentrations also appeared to be strongly linked to copper bioavailability, it is less practical to routinely monitor the copper associated with this sediment fraction (i.e. more laborious and expensive).

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10. APPENDICES

Appendix 1- Intellectual Property

There is no IP associated with the current project.

Valuable information arising from the research includes bioavailability and toxicology data that is to be included in the Risk Assessment for the use of antifouling paints by the fish farming industry in Tasmania. In addition this information together with the data on the nature of the paint in the sediments is being employed in the design of the monitoring programme for the paints and as a means for defining the limits of the sustainable management of their use.

Appendix 2 - Staff engaged on the project

As part of the Centre for Environmental Contaminants Research (CECR), CSIRO Land and Water:
Stuart Simpson, David Spadaro, Graeme Batley, Anthony Chariton, Ian Hamilton and Chad Jarolimek (all of CSIRO Land and Water)
Joel Davis of ANSTO (SEM and x-ray microanalysis of the paint)

Through the Tasmanian Salmonid Growers Association (TSGA):
Cameron Dalgleish and Craig Selkirk, of Tassal Ltd.,
Dom O'Brien, of Aquaculture Management and Development P/L and also on behalf of Huon Aquaculture Company P/L.
Pheroze Jungalwalla, Executive Officer of the TSGA.
DiveWorks P/L provided a team of divers for sampling.
Numerous boat skippers from both Tassal Ltd and Huon Aquaculture Company P/L.

From the Department of Primary Industry, Parks, Water and Environment, Hobart, as part of the sampling team and with input to sampling methodology:
Graham Woods, Eric Brain and Marc Santo

Appendix 3. Toxicity test conditions

Table 3.1. Summary of bivalve growth toxicity test conditions

Test type	Static renewal (renewal twice weekly)
Test duration	60 day
Temperature /Salinity	21 ± 1°C / 30 ± 1 ‰
Light intensity	3.5 µmol photons/s/m^2
Photoperiod	12 h light, 12 h dark
Test chamber	1L glass beakers
Sediment weight	200 mL
Overlying water volume	~750 mL
Total test volume	950 mL
Age/size of test organisms	16-100 mm^2
No. test organisms/ test chamber	10-15
No. replicate beakers / sample	3
Feeding regime	1 mg Sera micron® fish per tellina twice a week.
Test chamber aeration	1 outlet with slow bubbling to maintain ≥ 85 % dissolved oxygen throughout the test
Control sediment	Uncontaminated sediment with similar physico-chemical parameters (grain size, porewater salinity) to the test sediment
Overlying water	Fresh uncontaminated seawater (Port Hacking), NSW, 0.45 µm filtered and diluted with Milli-Q to salinity of 30±1 ‰
Endpoint	Percentage growth (total area)
Test acceptability criteria	Physico-chemical parameters (dissolved oxygen, pH, salinity and temperature) within acceptable limits throughout the test

Table 3.2. Summary of amphipod reproduction toxicity test conditions

Test type	Static non-renewal
Test duration	10 day
Temperature /Salinity	21 ± 1°C / 30 ± 1 ‰
Light intensity	3.5 μmol photons/s/m^2
Photoperiod	12 h light, 12 h dark
Test chamber	250 ml glass beakers
Sediment weight	40 g
Overlying water volume	~220 mL
Total test volume	280 mL
Age/size of test organisms	2-4 month old
No. test organisms/ test chamber	5 females and 7 males
No. replicate beakers / sample	4-5
Feeding regime	1 mg Sera micron® fish per amphipod twice a week.
Test chamber aeration	1 outlet with slow bubbling to maintain ≥ 85 % dissolved oxygen throughout the test
Control sediment	Uncontaminated sediment with similar physico-chemical parameters (grain size, porewater salinity) to the test sediment
Overlying water	Fresh uncontaminated seawater (Port Hacking), NSW, 0.45 μm filtered and diluted with Milli-Q to salinity of 30±1 ‰
Endpoint	Reproductive output (total embryo/juvenile numbers)
Test acceptability criteria	13-18 embryos/juveniles per female, physico-chemical parameters (dissolved oxygen, pH, salinity and temperature) within acceptable limits throughout the test

Table 3.3. Summary of copepod reproduction toxicity test conditions

Test type	Static non-renewal
Test duration	10 day
Temperature /Salinity	21 ± 1°C / 30 ± 1 ‰
Light intensity	3.5 µmol photons/s/m^2
Photoperiod	12 h light, 12 h dark
Test chamber	10 ml polycarbonate vial
Sediment weight	0.5 g
Overlying water volume	~9 mL
Total test volume	10 mL
Age/size of test organisms	3-5 week gravid females
No. test organisms/ test chamber	5
No. replicate beakers / sample	5-6
Feeding regime	1×10^4 cell per mL of both alga *Isochysis* sp. and *Tetraselmis* sp. + 0.3 mg Sera micron® fish food (<63 µm) per test vial twice a week.
Test chamber aeration	Algae photosynthesis maintaining ≥ 85 % dissolved oxygen throughout the test
Control sediment	Uncontaminated sediment with similar physico-chemical parameters (grain size, porewater salinity) to the test sediment
Overlying water	Fresh uncontaminated seawater (Port Hacking), NSW, 0.45 µm filtered and diluted with Milli-Q to salinity of 30±1 ‰
Endpoint	Primary – juvenile production (nauplii + copepodite). Secondary – Copepodite development
Test acceptability criteria	20-54 juveniles per female, physico-chemical parameters (dissolved oxygen, pH, salinity and temperature) within acceptable limits throughout the test

Appendix 4. Workshop Notes, Priorities and Knowledge Gaps

2nd FRDC Workshop on the research objectives for the use of Cu(I)O anti-fouling paints by the fish farming industry in Tasmania

Brief Notes from the meeting including Discussion and Action items

Meeting Date: 2 December, 2010

Location: TAFI Conference Room, Taroona

Subject: Antifoulant Meeting

Attendees.

Graham Woods (DPIPWE)	Catriona MacLeod (TAFI)
Marc Santo (DPIPWE)	Ruth Eriksen (TAFI)
Alastair Morton (DPIPWE)	Greg Dowson (EPA)
Dom O'Brien (Huon Aquaculture)	Craig Selkirk (Tassal)
David Cahill (Huon Aquaculture)	Stuart Simpson (CSIRO)
Ken Young (APVMA)	

Apologies:

Cameron Dalgleish (Tassal)	Pheroze Jungalwalla (TSGA)
Sarah Richards (EPA)	Phil Sinclair (DEH)

Background

The workshop forms part of the requirements of both: FRDC project 2009/218, entitled, Ecological effects due to contamination of sediments with copper-based antifoulants (Part 2), and the need to review the progress of the research aimed at providing the Environmental data in support of full registration of Cu(I)Oxide paint for the salmonid farming industry in Tasmania under APVMA Research permit #10924. The workshop aimed to identify the progress against agreed research priorities identified at the previous workshop (February, 2009, as part of FRDC project 2007/246), to report on progress to date with respect to the level of understanding of the toxicity and bio-availability of the Cu(I)O paint, and, to assess the need for further research on;

1. the fate of the paint copper in the environment, and

2. any further information that may be required for the registration and or continuation of further permits for the use of the paint

Workshop Agenda

Discussion and Action Items only, and where relevant to the Research programme.

Discussion of the progress on any outcomes since the 1st Workshop

Industry Operational overview/update

Overview/update on Research since 1st workshop

Overview/update on CFOC Project to Assess In-situ cleaning

Actions

1. Industry to appoint a Post-Doc researcher to undertake the design and implementation of the workload in the project as a priority.
2. Industry to establish and coordinate a project steering committee specific to in-situ cleaning research.

2010 Regulatory Monitoring Survey

Discussion

- Development of regulatory monitoring program - DPIPWE will progress the development of a regulatory monitoring program specific to targeting exceedance of Cu levels relative to licence conditions as opposed to permit specific research projects.
- Discussion on proposed active remediation project (proposal by Tassal). DPIPWE likely to provide support on some form of pilot remediation work on the condition that appropriate investigation of risks associated with active remobilisation of Cu at relevant cages sites and compliance sites are conducted.
- Notwithstanding assessment of Cu remobilisation risk in the active remediation project, passive remediation at representative sites still needs to be investigated (refer Stu Simpson's part 3 project brief)

APVMA noted that they will need to be kept informed on any relevant work being done – given concerns over the "what if's" should there be a major disturbance of the sediments.

Actions

1. Industry to include remediation investigation in stage/part 3 research plan across various sites and, where relevant, include site specific investigations for any active remediation research activities. Industry to resolve how this work is

undertaken from research funding perspective and therefore whether active remediation component is covered under part 3 investigation.

2. Industry to meet with Marine Farming Branch to work through overall sediment residue data set - this will include working through site specific issues such as cage site classifications and duration of stocking/fallowing.

Plan and process for moving forward with research

- Need to formalise research going forward
- Address two important knowledge gaps
    1. Develop effective concentration response relationships based on AE Cu.
    2. Provide information on the potential for natural remediation through assessment of form and fate of copper.

APVMA noted (1) above is not necessary for registration, but (2) must be addressed.

Discussion on Research Proposal prepared by Stu Simpson (CECR)

- APVMA acknowledged that the concentration response can be used in the registration process and that it is necessary to confirm whether Cu(I)O 'contamination' is acceptable beyond 65 mg Cu/kg (i.e. to or beyond 270 mg Cu/kg current used by the EPA).
- Need to understand the science behind any modification to the guideline value
- Concentration response work to be assessed and if possible to include local species (to improve the level of investigation from a trophic and behavioural response perspective). Suggested local species of Brittle Star may be appropriate.
- Assess likelihood of levels above 65 mg Cu/kg outside lease?

Flow on discussion looked at need to determine risk of ecological effects (APVMA); potential for Stu Simpson to include a few trophic levels to add value to registration. All parties agree on point (1) including the accepted sampling methodology of using divers to collect samples if required.

Actions

1. Industry is to progress a Part 3 research application to the FRDC by Christmas

   To include investigation into toxicity responses, sediment remediation and further investigation/confirmation of leaching data.
2. Refer revised knowledge gap table.

Update of knowledge gaps and priorities - antifoulants

- Tables and work plan diagrams (1^{st} workshop work plan) updated. Changes made are to be reflected in registration-specific versions of the priority table circulated to regulators following the last workshop.
- Flow on discussion included a need to understand relationship between age of paint and leaching rates, and what happens when the nets are not re-dipped?

Actions

1. Part 3 research application to be provided to the FRDC by Christmas as per above.
2. Further work into leaching rates is still a priority that needs resolution (refer to updated knowledge gap table).

Knowledge gap - Antifoulants	Comments at meeting
Environmental Fate	
1. What are the mechanisms for fluctuations in sediment copper levels? Are reductions in measured levels due to: a) High spatial variability? Work completed b) Recovery processes resulting in sediments being reworked and buried or redistributed within the sediments? c) Dispersal or removal of metals from the sediments by oxidative release, sediment transport or other mechanisms? d) Trophic transfer and accumulation processes?	H b) through c) are short term priorities Note: That d) is not considered a high priority
2. a) What proportion of metals measured in sediments is due to paint flakes and what is due to soluble metals bound to sediments? Basic analysis completed. b) Does copper/zinc complex to dissolved organic matter, and become less bio-available? (PREVIOUSLY POINT 4 IN TABLE 34) Done but not quantified Notes: • Temporal analysis should be considered • Use of gravity separation should be considered to distinguish between organic matter and paint flakes	M Note: This question is only a priority if the proportion of the paint in the sediment is significant.

Knowledge gap - Antifoulants	Comments at meeting
3. How significant is the release of copper/zinc from nets in dissolved form into the pelagic environment? Work completed	Work completed Note: A desktop study may be sufficient to meet this data need
4. Develop standardised sampling and assessment protocols. Protocols completed, may need modifications into the future	Work completed
5. How are metals in sediment partitioned between the solid and porewater phases and what are the implications for toxicity? Some work already undertaken through toxicity studies, further assessment to be made during recovery study(ies). Notes: • Dependent on outcome of 2 • As such, it is not possible to determine if this is a requirement for registration	H This point is dependent on the outcomes of 2.
Management/Operational	
6. Are net coating and drying protocols adequate to ensure effective minimal release rates upon immersion? Ongoing	L Note: It is assumed that protocols have already been implemented & evaluated

Knowledge gap - Antifoulants	Comments at meeting
7. a) What is the concentration of copper in water after freshly painted nets are immersed? Work completed for RA. b) How does this change with exposure time and with the degree of fouling? c) Does in-situ net washing contribute unacceptable levels of soluble and particulate copper and zinc to the environment? To be undertaken under the CFOC project d) How does this compare to use of the net washing facility? Note: 7. is connected to 3.	M Note: c) – H A desktop study may be sufficient to meet this data need
Biological Effects Research questions and can seek alternative funding	
8. Is there an appropriate measure of bioavailability that corresponds to levels of toxicity observed in key test species? Covered partially in Overall Research Project Part 2 (FRDC 2009/218) and planned to continue in Part 3.	H
9. What is the sediment concentration above which a statistically significant biological effect is expected? Covered partially in Part 2 (FRDC 2009/218) and planned to continue in Part 3. Note: Depends on outcomes of 8, 9, & 10 as are interconnected	H
10. How are benthic community structure & function and rate of response affected by increase in metal levels in sediment Partially being covered through Ecotox work. Note:	H Note: a. Aggregate value revised to H but to be re-assessed depending on results of 8

Knowledge gap - Antifoulants	Comments at meeting
• Depends on outcome of 8, 9, &11 as are interconnected • Recommended that 8 and 9 are undertaken, followed by 10 and 11	b. Lab-based experimental work may be sufficient to meet this data need
11. How are sediment recovery processes affected by increased metals levels? Includes microbial ecology Note: • Depends on outcome of 8, 9, &10 as are interconnected.	M Note: a. Aggregate value revised to M but to be re-assessed depending on results of 10
Mass Balance Budget / combination	
12. What is the contribution of metals to the sediments from uneaten food and additives and faeces/metabolic waste?	L
13. a) Is there significant uptake, bioaccumulation or toxicity in organisms that reside or spend part of their reproductive cycle in the water column? E.g., algae, fouling organisms. b) Or sediment?	L
14. What is the relative importance of each of these sources and pathways? Note: This is an output and not a priority	L