calibrated with a matrix-matched standard and blank. At least three blanks and at least one Standard Reference Material (Community Bureau of Reference BCR No 357, Certified Reference Material CRM278 mussel tissue) was carried through with each batch of samples.

Freshwater challenge

Five survivors from each container were exposed to fresh water (aged and aerated tap water) for 24 h to assess the effects of exposure to copper on osmoregulation of flounder.

Statistical analysis

Statistical analyses were as for the algal and amphipod bioassays. In addition, the histology results were analysed on the basis of prevalence, using one factor ANOVA (11 levels) and each container being a replicate (4 in each treatment). If the results of ANOVA were significant, the statistically different treatments were identified using a-posteriori multiple comparison of means SNK test (Student-Neuman-Keuls test).

3.5 Risk assessment

Details of the risk assessment methodology are given in Appendix A.

4 Results and discussion

4.1 Trace metals in Macquarie Harbour waters

Total dissolved metals in Macquarie Harbour waters collected in October and December, 1995 for use in the bioassay testing program are shown in table 4.1. Dissolved copper ranged from 26 to 42 µg/L (October sampling), with the highest concentrations at stations 9, 11, 5 and 3 immediately below the outflow of the King River. Dissolved copper concentrations in water from station 9 and 14 in the December sampling were much lower (10 and 12 µg/L respectively). The dissolved copper concentrations were much higher than the ANZECC limits for total copper in seawater (5 µg/L). Concentrations of other metals including manganese, iron, aluminium and zinc were also elevated above typical seawater background concentrations. Cadmium, lead, cobalt, silver and chromium were below detection limits by ICPAES. Teasdale et al (1996) found that dissolved copper in Macquarie Harbour waters correlated highly with dissolved manganese concentrations (r^2=0.97), suggesting that dissolved copper was largely associated with colloids high in manganese (probably combined with iron) in the Harbour.

Total copper, dissolved copper, ASV-labile copper and dissolved organic carbon (DOC) in Macquarie Harbour waters are shown in table 4.2. In general there was agreement between measurements of total dissolved copper by ICPAES (CSIRO) and GFAAS (DELM). ASV-labile copper results from CSIRO were consistently higher than the ASV-labile copper determined at DELM, possibly due to less loss of copper by adsorption to the Teflon cells at CSIRO.

DOC concentrations in the Harbour were towards the upper end of the normal range for estuaries, with values of 2.2–7 mg/L in the mid-salinity waters. It is well known that dissolved organic matter can bind copper in forms that are less toxic than free metal ion (Teasdale et al 1996). The ability of DOC to bind copper is generally measured as the copper complexing capacity. None of the water samples had significant complexing capacity, in agreement with Teasdale et al (1996). Copper concentrations in all the Macquarie Harbour waters exceeded the complexing capacity of the dissolved organic matter in these waters, indicating that free copper or easily dissociable complexes were present and possibly bioavailable. ASV-labile copper was a significant component of the dissolved copper in all samples (48–65%).

Table 4.1 Total disssolved metals in Macquarie Harbour waters sampled in October and December, 1995

Station no	Cu	Mn	Fe	Zn	Cd	Pb	Co	Ni	Al	Ag	Cr
October sampling											
3	31	70	62	6	<2	<10	2	1	131	<2	<2
5	33	71	63	6	<2	<10	6	3	131	<2	<2
9	42	99	86	9	<2	<10	4	3	149	<2	<2
11	35	80	73	7	<2	<10	<1	2	135	<2	<2
14	26	52	60	5	<2	<10	<1	3	113	2	<2
28	29	62	56	5	<2	<10	<1	2	121	<2	<2
32	29	68	72	6	<2	<10	1	1	137	<2	<2
34	26	59	63	5	<2	<10	2	<1	122	<2	<2
December sampling											
9	10	17	19	21	<2	10	2	2	71	<2	<2
14	12	21	23	21	<2	10	1	2	76	<2	<2

Table 4.2 Copper and dissolved organic carbon in Macquarie Harbour waters sampled in October and December 1995

Station no	DOC (mg/L)	Total copper (µg/L)	Dissolved copper (µg/L)		ASV-labile copper (µg/L)	
			CSIRO [a]	DELM [b]	CSIRO [a]	DELM [b]
October sampling						
3	6.7	46	31	31	20	12.2
5	5.2	50	33	36	18	15.3
9	7.0	62	42	44	24	16.2
11	6.4	50	35	35	19	16.7
14	2.8	29	26	26	16	11.4
28	3.2	35	29	29	16	10.3
32	4.5	61	29	28	14	6.5
34	3.0	34	26	23	17	16.1
December sampling						
9	2.2	12	10	nd[c]	5.6	nd
14	3.1	12	12	nd	7.3	nd

a Analyses by CSIRO, CAAC, Sydney

b Analyses by DELM, Hobart

c not determined

4.2 Toxicity of ionic copper and Macquarie Harbour waters to the alga *Nitzschia closterium* (growth inhibition bioassay)

4.2.1 Toxicity of ionic copper

Because the salinities of the Macquarie Harbour waters ranged from 15 to 22‰, the growth and toxicity of copper to *Nitzschia closterium* was determined at a range of salinities (table 4.3). Growth rates of the *Nitzschia* controls decreased with decreasing salinity, from 1.2 doublings/day at 25‰ salinity to 0.79 doublings/day at 15‰.

Although growth rates in the controls decreased at lower salinity, there was no significant difference in the toxicity of ionic copper at 15 and 20‰ salinity (tables 4.4 and 4.5 and figs 4.1 and 4.2). The 72-h EC50 for ionic copper was 21 (95% confidence limits of 18–24) and 18 (15–23) µg/L at 15 and 20‰ salinity respectively. Both LOEC and NOEC values were similar at both salinities tested. Copper concentrations as low as 5 µg/L significantly reduced

Nitzschia growth rates, indicating that this species should be sensitive enough to detect copper toxicity in the Macquarie Harbour water samples if the copper was bioavailable.

Measured copper concentrations at the beginning and end of the bioassays agreed closely with the nominal copper concentrations, indicating that little copper was lost by adsorption to the flask walls throughout the assay. The pH throughout the bioassays ranged from 7.9–8.3.

Table 4.3 Toxicity of ionic copper to *Nitzschia closterium* at 20‰ salinity

Measured copper concentration[a] (µg/L)	Mean cell division rate		CV (%)
	Divisions/day	Percentage of control	
0	0.98	100	1
2.5	0.83	85	7
4.7[b]	0.73	74	6
11[b]	0.54	55	15
22[b]	0.51	52	5
43[b]	0.50	51	30
88[b]	0.15	15	41

a Copper concentration measured by ICPAES on Day 0 of the bioassay

b The mean cell division rates at these copper concentrations were significantly different to the control mean at α =0.05 by Dunnett's test. This difference corresponds to -21% of the control.

Table 4.4 Toxicity of ionic copper to *Nitzschia closterium* at 15‰ salinity

Measured copper concentration[a] (µg/L)	Mean cell division rate		CV (%)
	Divisions/day	Percentage of control	
0	0.79	100	1
3	0.70	89	5
5.6[b]	0.65	82	8
11[b]	0.58	73	2
22[b]	0.45	57	0
43[b]	0.25	32	43
87[b]	0	0	47

a Copper concentration measured by ICPAES on Day 0 of the bioassay

b The mean cell division rates at these copper concentrations were significantly different to the control mean at α =0.05 by Dunnett's test. This difference corresponds to -16% of the control.

Table 4.5 Toxicity of ionic copper to *Nitzschia closterium* at varying salinities

Salinity (‰)	Control cell division rate (doublings/day)	Copper toxicity (µg/L)		
		72-h EC50	LOEC	NOEC
15	0.79	21 (18–24)	5.6	3
17.5	0.95	–	–	–
20	0.98	18 (15–23)	4.7	2.5
25	1.2	–	–	–

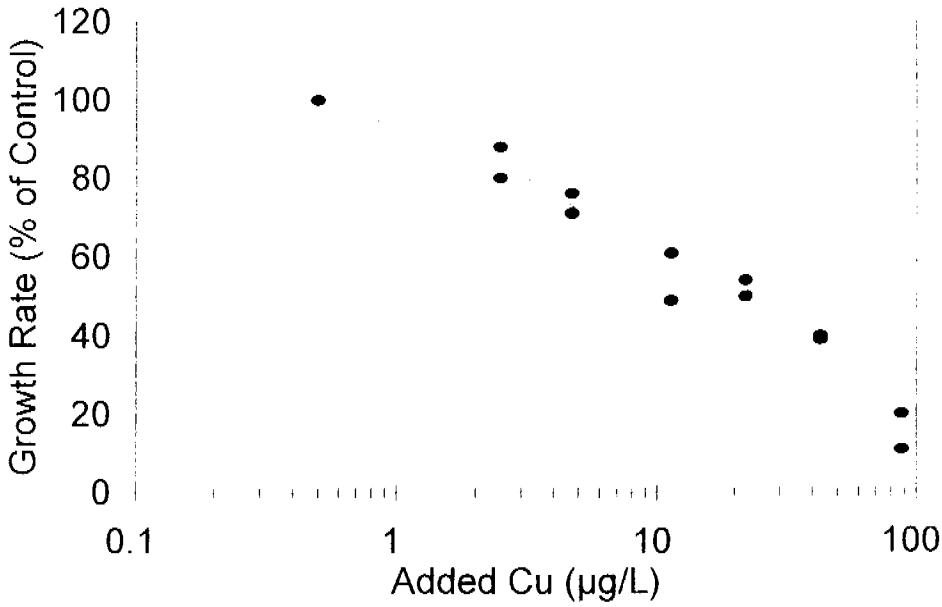

Figure 4.1 Inhibition of growth of *Nitzschia closterium* by ionic copper at 20‰ salinity

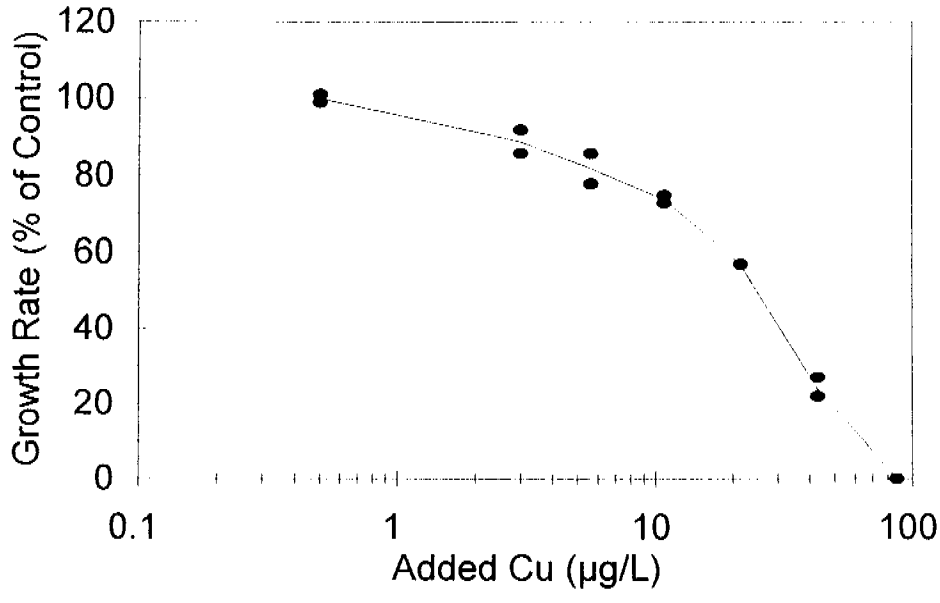

Figure 4.2 Inhibition of growth of *Nitzschia closterium* by ionic copper at 15‰ salinity

Table 4.6 Growth of *Nitzschia closterium* in filtered Macquarie Harbour water (station 11)[a] at 15‰ salinity

Concentration of Macquarie Harbour waters (%)	Mean cell division rate		CV (%)
	Divisions/day	Percentage of control	
0	0.69	100	8
6.25	0.76	110[b]	7
12.5	0.80	116[b]	1
25	0.88	128[b]	6
50	0.79	114[b]	4
100	0.76	110[b]	2

a Station 11 water contained 35µg Cu/L.

b Growth enhancement was not significant at $\alpha = 0.05$ by Dunnett's test.

4.2.2 Toxicity of Macquarie Harbour waters (October sampling)

Filtered waters

The growth of *Nitzschia closterium* in various dilutions of filtered station 11 water is given in table 4.6. Although full-strength station 11 water (100%) contained 35 µg Cu/L, of which 18 µg Cu/L was ASV-labile, there was no significant decrease in *Nitzschia* growth over the 72-h exposure. Because the *Nitzschia* growth bioassay is sensitive enough to detect copper concentrations as low as 5 µg Cu/L at 15‰ salinity, it appears that <5 µg/L of the copper in the station 11 water was bioavailable. Although algal growth appeared to be enhanced in the Macquarie Harbour water, this was not significant at the alpha=0.05 level by Dunnett's test.

Table 4.7 shows the growth of *Nitzschia* in the other full-strength filtered Macquarie Harbour waters sampled in October, 1995 (all at 18‰ salinity). None of the waters were toxic to *Nitzschia*, with growth rates 88–100% of the controls in clean seawater at 18‰ salinity. Although these waters contained from 26–42 µg Cu/L, less than 5 µg Cu/L was bioavailable otherwise significant decreases in algal growth would have been observed.

Table 4.7 Growth of *Nitzschia closterium* in filtered Macquarie Harbour waters at 18‰ salinity

Station no	Dissolved Cu (µg/L)	Mean cell division rate		CV (%)
		Divisions/day	Percentage of control	
Control		1.0	100	3
3	31	0.88	88	16
5	33	0.98	98	7
9	42	1.0	100	3
14	26	1.0	100	2
28	29	1.1	110	6
32	29	1.0	100	4
34	26	1.0	100	4

Unfiltered waters

To determine whether toxicity of the Macquarie Harbour waters may be associated with particulate material, the toxicity of six of the unfiltered waters from the October sampling was investigated (table 4.8). Two concentrations of each sample (100% and 50%, diluted with 18‰ salinity clean seawater) were tested in triplicate. There was no significant decrease in algal growth in any of the water samples tested, despite total copper concentrations ranging from 29–62 µg Cu/L. No significant growth enhancement was observed.

4.2.3 Toxicity of Macquarie Harbour waters (December sampling)

Growth of *Nitzschia* in filtered and unfiltered Macquarie Harbour water collected from station 9 in December 1995 (21‰ salinity) is shown in table 4.9. A small but significant decrease in algal growth rate in full-strength (100%) filtered and unfiltered water was observed, with rates 88% and 81% of the controls respectively. However, this small decrease in algal growth was far less than that predicted by ASV-labile copper determinations. There was no effect on algal growth at concentrations of station 9 water of 75% or less ie the NOEC was 75%.

Similar results were found for filtered and unfiltered station 14 water (table 4.10). A small but significant decrease in algal growth rate in full-strength (100%) filtered and 100% and 75% unfiltered water was observed, with rates 88%, 92% and 92% of the controls respectively. The LOEC for the filtered water was 100%, with an NOEC of 75%. The unfiltered water showed similar toxicity with an LOEC of 75% and an NOEC of 50%.

Copper concentrations in each dilution of filtered and unfiltered Macquarie Harbour water were measured on Day 0 and Day 3 of the bioassays (table 4.11). There was excellent agreement between nominal and measured copper concentrations at each dilution. Copper concentrations at the end of the bioassay were the same as at the beginning of the bioassay confirming that copper did not adsorb to the walls of the silanised bioassay flasks. Thus the lack of toxicity of the Macquarie Harbour water samples was not due to losses of copper to the test containers.

Table 4.8 Growth of *Nitzschia closterium* in unfiltered Macquarie Harbour waters at 18‰ salinity

Station no	Concentration (%)	Mean cell division rate		CV (%)
		Divisions/day	Percentage of control	
Control		1.0	100	2
3	100[a]	1.2	120	22
	50[b]	1.0	100	2
5	100	0.94	94	3
	50	1.0	100	3
9	100	1.0	100	3
	50	1.1	110	3
11	100	1.1	110	18
	50	1.1	110	6
14	100	1.0	100	2
	50	1.0	100	4
28	100	1.1	110	6
	50	1.1	110	8

a 100% is full strength Macquarie Harbour water

b 50% is full strength (100%) Macquarie Harbour water diluted 1:2 with 18‰ salinity seawater

Table 4.9 Growth of *Nitzschia closterium* in filtered and unfiltered Macquarie Harbour water, station 9 (21‰, December 1995 sampling)

Concentration of station 9 water (%)	Measured copper concentration (µg/L)	Mean cell division rate		CV (%)
		Divisions/day	Percentage of control	
Unfiltered				
0	-[b]	1.2	100	4
50	-	1.2	100	6
75	-	1.1	92	5
100[a]	-	0.97	81	7
Filtered				
0	<1	1.1	100	2
12.5	2	1.1	100	1
25	3	1.1	100	2
50	6	1.0	91	2
75	7	1.1	100	7
100[a]	10	0.97	88	2

a the mean cell division rates at full strength Macquarie Harbour water were significantly lower than the controls at $\alpha = 0.05$

b not determined

Table 4.10 Growth of *Nitzschia closterium* in filtered and unfiltered Macquarie Harbour water station 14 (20‰, December 1995 sampling)

Concentration of station 14 water (%)	Measured copper concentration (µg/L)	Mean cell division rate		CV (%)
		Divisions/day	Percentage of control	
Unfiltered				
0	-[b]	1.2	100	4
50	-	1.2	100	6
75[a]	-	1.1	92	3
100[a]	-	1.1	92	4
Filtered				
0	<1	1.1	100	4
12.5	2	1.1	100	3
25	3	1.1	100	6
50	5	1.1	100	2
75	9	1.2	109	1
100[a]	11	0.96	88	3

a the mean cell division rates for these concentrations of Macquarie Harbour waters were significantly lower than the controls at $\alpha = 0.05$ by Dunnett's test

b not determined

Table 4.11 Nominal and measured copper concentrations in Macquarie Harbour waters (stations 9 and 14, December 1995 sampling) at the beginning and end of the *Nitzschia closterium* bioassay

Sample station	Concentration (%)	Nominal copper concentration (µg/L)	Measured copper concentration (µg/L)	
			Day 0	Day 3
9	100	10	10	10
	75	7.5	7	7
	50	5	6	5
	25	2.5	3	3
	12.5	1.3	2	3
14	100	12	11	10
	75	9	9	8
	50	6	5	6
	25	3	3	4
	12.5	1.5	2	2

4.2.4 Copper complexing capacity of Macquarie Harbour waters

The copper complexing capacity of filtered station 9 and unfiltered station 14 water (collected in December, 1995) was determined by spiking full-strength water with various amounts of ionic copper (5–80 µg/L) and measuring the decrease in algal growth rate over 72 h.

Copper additions as high as 20 µg/L (total dissolved copper=32 µg/L) to the filtered station 9 water had no effect on algal growth (table 4.12). The first observable effect on algal growth rate was at 52 µg Cu/L, an order of magnitude higher than copper concentrations required to

first affect algal growth in clean seawater at the same salinity. The 72-h EC50 was approximately 90 µg Cu/L, with broad 95% confidence limits of 60–138 µg Cu/L. This is also much higher than the 72-h EC50 of 18 µg Cu/L in clean seawater at 20‰ salinity. The 72-h EC15 value (a measure of the copper complexing capacity of the water) was approximately 20 µg/L. Although the station 9 water contained 10 µg Cu/L initially, of which 5.6 µg Cu/L was ASV-labile, further copper additions to this water were complexed or bound and not available for uptake into the algal cells to cause toxicity until copper concentrations exceeded 20 µg/L.

Table 4.12 Growth of *Nitzschia closterium* in copper-spiked, filtered Macquarie Harbour water, station 9 (21‰ salinity)

Added copper (µg/L)	Total copper[b] (µg/L)	Mean cell division rate		CV (%)
		Divisions/day	% control	
0	10	0.92	100	11
5	15	0.86	93	7
10	21	0.93	101	2
20	32	0.89	97	9
40[a]	52	0.70	76	8
80[a]	95	0.47	51	4

a The mean cell division rates at these added copper concentrations were significantly less than the control mean at a = 0.05 by Dunnett's test. This corresponds to -14% at the control.

b Total copper (copper in station 9 water plus copper added) was measured by ICPAES on Day 0.

Similar results were found for unfiltered station 14 water spiked with ionic copper (table 4.13). Total dissolved copper concentrations up to 22 µg/L had no effect on algal growth rate, whereas copper concentrations exceeding 42 µg/L significantly decreased algal growth. The 72-h EC50 was 117 µg Cu/L (95% confidence limits of 100–144 µg/L) and the copper complexing capacity of the sample (EC15) was 42 µg/L (34–50 µg /L). Again, further copper additions to this water were not bioavailable until copper concentrations exceeded the complexing capacity of the sample (42 µg Cu/L).

Table 4.13 Growth of *Nitzschia closterium* in copper-spiked, unfiltered Macquarie Harbour water station 14 (21‰ salinity, December 1995 sampling)

Added copper (µg/L)	Total copper[b] (µg/L)	Mean cell division rate		CV (%)
		Divisions/day	% control	
0	12	0.84	100	6
10	22	0.83	99	2
30[a]	42	0.75	89	5
60a	72	0.68	81	5
90[a]	102	0.52	62	2
120[a]	132	0.37	44	14

a The mean cell division rates for these added copper concentrations were significantly less than the control mean at $\alpha = 0.05$ by Dunnett's test. This corresponds to -9% of the control.

b Total copper (copper in station 14 water plus copper added) was measured by ICPAES on Day 0.

These results were surprising because chemical complexing capacity experiments using ASV had shown that these waters had no copper complexing capacity and indeed contained 'free' copper. This ASV-labile copper represents ionic copper, inorganic copper complexes and organic copper complexes which can dissociate at the ASV electrode. It is generally (and simplistically) assumed that this ASV-labile copper is similar to the bioavailable fraction. However, the *Nitzschia* growth inhibition bioassays showed that none of this copper was able to penetrate

the algal cell membrane where it could exert a toxic effect on cell division. Moreover, additional copper could be added to the waters and a decrease in algal growth rate was not observed until copper concentrations exceeded 20 and 42 µg Cu/L in filtered station 9 and unfiltered station 14 waters respectively. This apparent discrepancy between chemically labile and bioavailable copper was investigated further in a series of additional experiments outlined below.

4.2.5 Prevention of copper toxicity to *Nitzschia* growth: Inorganic versus organic binding

Once dissolved copper in the Macquarie Harbour waters exceeds the complexing capacity of the dissolved organic matter (such as humic and fulvic acids), the free copper would be detected as ASV-labile copper and potentially bioavailable. The lack of toxicity of the water samples to algae suggests that this ASV-labile copper is not bioavailable, at least to algae. It is possible that this copper is present as organic complexes which are directly reduced at the ASV electrode (rather than first dissociating to free copper which then diffuses to the mercury electrode and is reduced) so it is detected as ASV-labile copper, whereas the organic copper complex itself does not dissociate at the algal cell membrane and therefore cannot penetrate the cell to cause a toxic effect on algal growth. If this were the case, UV irradiation of the water sample should break down the organic copper complexes so that more copper is available to inhibit algal growth.

In two experiments, filtered Macquarie Harbour water from station 9 (October sampling) containing 42 µg Cu/L and station 14 (December sampling) containing 12 µg Cu/L, were irradiated under UV light for 4 h. The toxicity of the UV irradiated water was then compared with the toxicity of the non-irradiated water over 72 h in the *Nitzschia* growth inhibition test. In both waters the toxicity of the irradiated and non-irradiated water was the same. This suggests that either copper was still bound in a non-bioavailable form or that copper losses during UV irradiation occurred. It appears unlikely that copper was solely bound as organic complexes otherwise a reduction in algal growth after copper release from the organic complexes would have been detected.

It is possible that copper in the Macquarie Harbour waters is adsorbed to humic coated colloidal inorganic compounds, such as manganese, iron or aluminium oxyhydroxides (Teasdale et al 1996). A portion of these colloids may dissociate at the ASV electrode (and are therefore measured as ASV-labile copper) and adsorb to, but do not penetrate, the algal cell membrane. Previous work in our laboratory has shown that copper adsorbs to manganese, iron, cobalt and aluminium hydroxides on the *Nitzschia* cell surface and prevents copper from penetrating into the cell to cause an inhibitory effect on cell division (Stauber & Florence 1985a, b, 1987). Concentrations of manganese, cobalt, aluminium and iron as low as 4, 2, 110 and 550 µg/L respectively could reduce the toxic effect of copper by 2.5 to 5 times. Copper concentrations of over 50 µg/L were required to reduce algal growth rates by 50%. Manganese and cobalt were the most effective in reducing copper toxicity because they could also catalytically scavenge free radicals.

Concentrations of dissolved manganese (17–99 µg/L) and aluminium (71–149 µg/L) in the Macquarie Harbour waters, if present as colloidal oxides/hydroxides, were well in excess of the concentrations required to protect *Nitzschia* from copper toxicity. Iron (19–86 µg/L) and cobalt (<1–6 µg/L) in these waters may also protect to a lesser extent. To test this hypothesis, we added 20 µg Mn/L (as manganese chloride) and/or 20 µg Fe/L (as iron(III) chloride) to clean seawater at 20‰ salinity containing *Nitzschia* cells and allowed the flasks to stand for three days, prior to the addition of copper (10–100 µg/L). Bioassays were then run as usual and the inhibitory effect of copper in the presence of manganese or iron compared with

alone. There were no differences in the toxicity of copper in the presence or absence of iron and manganese. It is possible that three days was not long enough for oxidation of the added Mn(II) and Fe (III) at the cell surface, to form hydroxides and prevent copper penetration into the cell. It is also possible that colloidal aluminium, iron (including humic coated iron oxide), manganese and cobalt are together contributing to prevent copper uptake by this or another as yet unknown mechanism.

Removal of colloidal manganese and/or adsorbed copper from the Macquarie Harbour waters (station 9 and 14) was also attempted by ultrafiltration through an Amicon Centricon 10 (10,000 molecular weight cut-off). It was hoped that by determining the metal concentrations and toxicity of the waters before and after removal of this fraction, it may help indicate to what the copper was bound. However, all the manganese and 62–100% of the copper passed through the membrane indicating that most of these metals were present in dissolved forms with less than 10,000 molecular weight. Further work is required to understand the speciation of copper in Macquarie Harbour waters and its impact on algae.

4.3 Toxicity of ionic copper and Macquarie Harbour waters to the alga *Dunaliella tertiolecta* (enzyme inhibition bioassay)

4.3.1 Toxicity of ionic copper

The effect of ionic copper on the activity of the enzyme ß-D-galactosidase in the marine green alga *Dunaliella tertiolecta* at 17‰ salinity is shown in table 4.14 and fig 4.3. This bioassay was extremely sensitive to copper, with concentrations of copper as low as 2.5 µg/L significantly decreasing algal enzyme activity (86% of controls). The 72-h EC50 was 18 µg Cu/L, with 95% confidence limits of 15–22 µg/L. Thus this enzyme inhibition bioassay showed similar sensitivity to the *Nitzschia* growth inhibition test, and should be able to detect toxicity in the Macquarie Harbour waters if the copper is present in a bioavailable form.

4.3.2 Toxicity of Macquarie Harbour waters

Filtered Macquarie Harbour waters from the October and December samplings were assayed for toxicity using the *Dunaliella* enzyme inhibition bioassay. Due to the bioassay procedure, 90% was the maximum concentration of sample that could be assayed. Controls were matched to the same salinity as each of the water samples.

Inhibition of ß-D-galactosidase activity in *Dunaliella* in each of the water samples is given in table 4.15. Significant inhibition of enzyme activity (46–72%) was observed in all the Macquarie Harbour water samples tested, with station 5 being the most toxic. Enzyme inhibition was weakly correlated with dissolved copper concentrations in the samples (r^2=0.65).

Table 4.14 Effect of ionic copper on β-galactosidase activity in *Dunaliella tertiolecta* at 17‰ salinity

Added copper (µg/L)	β-Galactosidase activity (% of control)	CV (%)
0	100	4
2.5[a]	86	3
5[a]	78	3
10[a]	60	5
20[a]	46	4
40[a]	35	9

a β-galactosidase activity at these copper concentrations was significantly lower than the control at $\alpha = 0.05$ by Dunnett's test. This corresponds to -10% of the control.

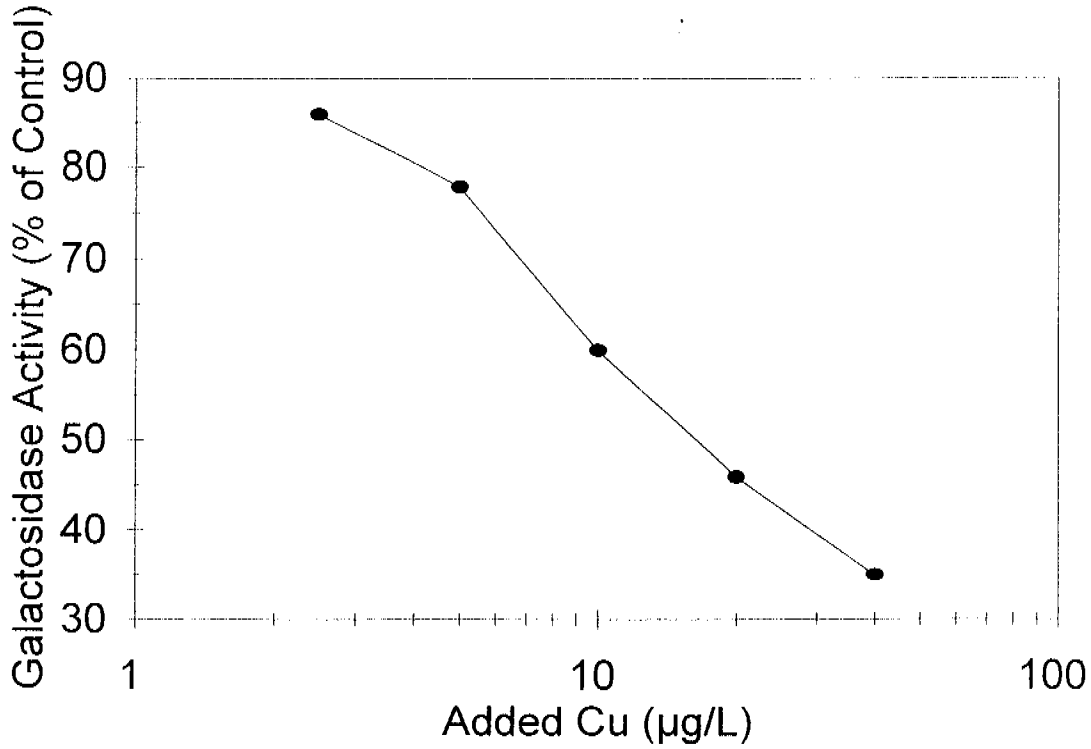

Figure 4.3 The effect of ionic copper on the activity of β-D-galactosidase in *Dunaliella tertiolecta* at 17‰ salinity

Because the enzyme bioassay is carried out 44.5°C, it was important to determine if toxicity of the samples was likely at realistic water temperatures or was due simply to breakdown of the copper complexes at this high temperature. A portion of the station 14 water was autoclaved at 121°C for 20 min, and the toxicity of the autoclaved and non-autoclaved water to ß-D-galactosidase activity in *Dunaliella* assessed using the standard enzyme bioassay. It was assumed that if high temperatures caused dissociation of the copper complexes, more copper would be bioavailable leading to greater enzyme inhibition in the assay with the autoclaved sample. Enzyme inhibition in both the autoclaved and non-autoclaved sample was the same (82% of the control). It appears that copper complexes present in the sample do not dissociate at high temperatures and suggests that copper bioavailability and toxicity in these samples is not influenced by the incubation temperature of 44.5°C in the enzyme inhibition bioassay.

Using the ß-D-galactosidase inhibition versus ionic copper calibration graph, the inhibition of ß-D-galactosidase activity in the Macquarie Harbour water samples was converted to an estimate of bioavailable copper. Bioavailable copper (determined in this way) and ASV-labile copper (determined electrochemically) for each of the Macquarie Harbour waters are shown in table 4.16 and fig 4.4. There was a weak correlation between the two measurements (r^2= 0.51).

Though there was reasonable agreement between ASV-labile copper and bioavailable copper estimated from the galactosidase inhibition bioassay, this was not the case for bioavailable copper determined in the growth inhibition test. It is possible that the enzyme ß-D-galactosidase is located on the cell membrane and that copper, adsorbed to iron, manganese and aluminium hydroxides at the cell surface, can inhibit the enzyme on the cell membrane. Alternatively, the enzyme may compete with carrier proteins (which transport copper into the cell) leading to copper inhibition of the enzyme at the cell surface. However, because copper is bound to the metal hydroxides at the cell surface, less copper penetrates the cell and therefore an effect on cell division is not observed.

Table 4.15 Inhibition of β-galactosidase activity in *Dunaliella tertiolecta* in filtered Macquarie Harbour waters (90%)[a]

Station no	Salinity (‰)	Dissolved copper concentration (µg/L)	β-galactosidase activity (% of control)	CV (%)
October Sampling				
3	17	31	55	4
5	17	33	46	1
9	12	42	60	1
11	14	35	55	3
14	17	26	62	2
28	17	29	50	2
32	17	28	63	2
34	17	23	56	2
December Sampling				
9	21	10	72	3
14	20	12	66	6

a Full strength (100%) Macquarie Harbour waters could not be tested, due to additional reagents added in the enzyme assay

4.4 Toxicity of ionic copper and Macquarie Harbour waters to the amphipod *Allorchestes compressa*

4.4.1 Toxicity of ionic copper

The effect of ionic copper (3–16 µg/L) on the growth and survival of *Allorchestes compressa* over 27 days is shown in table 4.17. Throughout the experiment, the temperature ranged from 18 to 19°C, pH from 8.0 to 8.3, salinity from 21 to 23‰ and dissolved oxygen from 5.3 to 6.9 mg/L. Measured copper concentrations in each tank were relatively constant over the 27-day experiment, but were generally lower than nominal copper concentrations, possibly due to absorption of copper onto the glass tank walls. Results from the bioassays are therefore expressed in terms of measured copper concentrations.

Survival in the controls over the 27 days ranged from 67 to 87% (mean 77%), and was lower than the 90–100% expected. Similar survival (53–87%) was found at all copper concentrations tested. Only the mean amphipod survival at 4 µg Cu/L was significantly lower than the controls at alpha = 0.05 by Dunnett's test.

Amphipod growth was a more sensitive and reliable endpoint for copper toxicity than amphipod survival. Growth of the amphipods over 27 days was significantly lower than the controls at copper concentrations of 4–16 µg/L. The LOEC was 4 µg Cu/L and the NOEC was 3 µg Cu/L. The 27-day EC50 was 15 µg Cu/L, with 95% confidence limits of 13-17 µg Cu/L, using trimmed Spearman Karber analysis. Because dissolved copper concentrations in the Macquarie Harbour waters ranged from 10 to 42 µg Cu/L, the 27-day amphipod growth bioassay should be sensitive enough to detect copper toxicity in these waters if the copper is present in a bioavailable form.

4.4.2 Toxicity of Macquarie Harbour waters

The growth and survival of *Allorchestes compressa* in Macquarie Harbour station 9 water over 27 days is shown in table 4.18. Throughout the experiment, the temperature ranged from 18 to 19°C, the pH ranged from 7.8 to 8.2, salinity from 21 to 22‰ and dissolved oxygen from 5.3 to 6.9 mg/L. The measured copper concentrations throughout the bioassay agreed well with the expected copper concentrations in the water samples, except for 100% station 9 water which contained 6 µg Cu/L instead of 10 µg Cu/L.

Table 4.16 Bioavailable copper (from the β-galactosidase inhibition bioassay) compared with ASV-labile copper in Macquarie Harbour waters

Station no	Date sampled	Bioavailable copper[a] (µg/L)	ASV-labile copper[b] (µg/L)
3	Oct 1995	13.5	20
5	Oct 1995	20	18
9	Oct 1995	10	24
9	Dec 1995	6.5	5.6
11	Oct 1995	13.5	19
14	Oct 1995	9.5	16
14	Dec 1995	8	7.3
28	Oct 1995	17	16
32	Oct 1995	9	14
34	Oct 1995	12	17

a Bioavailable copper was determined from the inhibition of β-galactosidase activity in *D. tertiolecta* (as a % of the control) using the ionic copper calibration graph

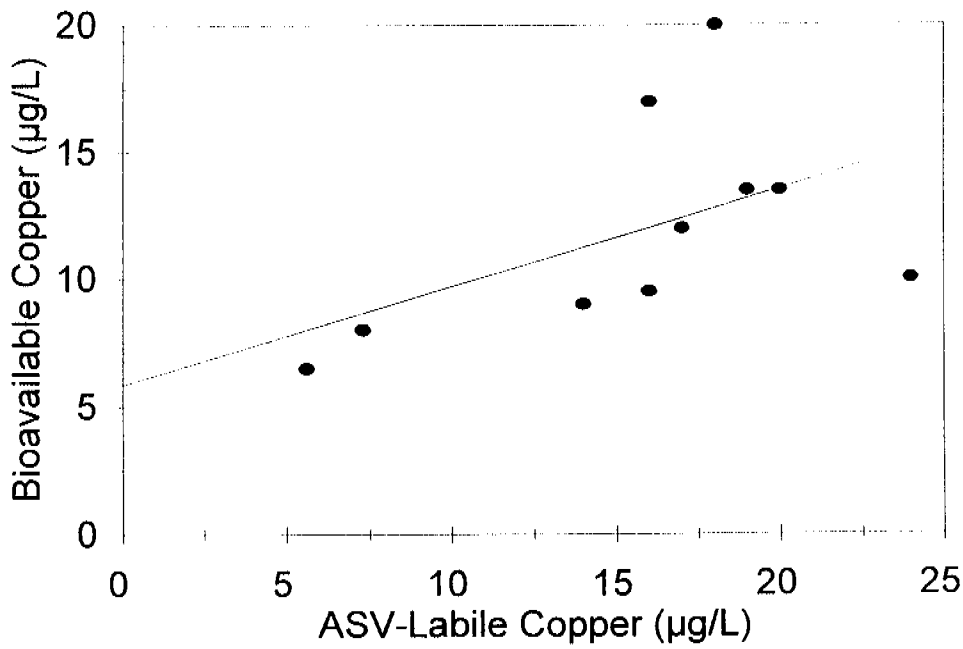

Figure 4.4 Bioavailable copper (determined from β-D-galactosidase bioassay) versus ASV-labile copper (determined electrochemically)

Table 4.17 Toxicity of ionic copper to *Allorchestes compressa* after a 27-day exposure

Measured copper concentration (µg/L)	Mean amphipod survival		Amphipod growth	
	(% recovery)	CV (%)	Mass per amphipod (mg)	CV (%)
0	77	11	0.24	9
3	87	7	0.25	8
4	53[a]	40	0.16[b]	26
8	83	7	0.18[b]	10
13	76	7	0.14[b]	14
16	78	9	0.11[b]	8

a The mean % recovery at this copper concentration was significantly lower than the control at $\alpha = 0.05$ by Dunnett's test.

b The mean mass per amphipod was significantly lower than the control at $\alpha = 0.05$ by Dunnett's test.

Table 4.18 Growth and survival of *Allorchestes compressa* in Macquarie Harbour, station 9 water

Concentration of station 9 water (%)	Measured Cu concentration (µg/L)	Mean amphipod survival		Amphipod growth	
		% recovery	CV (%)	Mean mass per amphipod (mg)	CV (%)
Control	1	104	3	0.28	8
12.5	2	98	3	0.26	9
25	2	99	4	0.25	5
50	4	101	3	0.26	11
100	6	100	7	0.27	5

Mean amphipod survival (% recovery) in the controls was 104%, possibly because more than 30 amphipods were added initially. Amphipod survival ranged from 98 to 104% and was not reduced at any concentration of station 9 water tested. Similarly, amphipod growth (0.25–0.28 mg per amphipod) was not affected at any concentration tested. The highest measured concentration of copper in the station 9 water (100%) was 6 µg Cu/L and it appears that <4 µg/L of this copper was available to cause toxicity to the amphipod.

The growth and survival of *Allorchestes compressa* in Macquarie Harbour station 14 water over 27 days is shown in table 4.19. Throughout the experiment, the temperature was maintained at 18°C, the pH ranged from 7.9 to 8.2, salinity from 19 to 22‰ and dissolved oxygen from 4.9 to 6.9 mg/L.

Concentrations of station 14 water (25–100%) containing 5–10 µg Cu/L, had no effect on amphipod survival, which ranged from 86-99%. There was a small but statistically significant effect on amphipod survival at the lowest concentration tested (12.5%). This result is unlikely to be biologically meaningful, because higher concentrations of station 14 water had no effect.

Growth of the amphipods over 27 days was significantly reduced in all concentrations of station 14 water. The LOEC was 12.5%, corresponding to a copper concentration of 3 µg Cu/L. Within the error of the copper determination by ICPAES (3 ± 1 µg/L), this is similar to the LOEC of 4 µg Cu/L for ionic copper alone. It appears that some of the copper in the station 14 water was bioavailable, and that this contributed to the toxicity of the station 14 water to amphipod growth.

In summary, the Macquarie Harbour waters had no effect on amphipod survival as predicted from the ionic copper experiments. However, station 14 water did reduce amphipod growth and this growth inhibition was possibly due to copper in the sample. No effect of station 9 water on amphipod growth was observed.

Ahsanullah and Williams (1991) investigated the sub-lethal effects of copper (and zinc, cadmium and chromium) on survival, average weight and biomass (average weight x survival proportion) of *Allorchestes compressa* at 19°C and 31‰ salinity. Copper concentrations producing minimum detectable decreases in mean survival, weight and biomass (equivalent to LOEC values) were 24, 5.2 and 3.7 µg Cu/L respectively. The LOEC for mean weight (5.2 µg Cu/L) reported by Ahsanullah and Williams (1991) was similar to the LOEC found in the present study (4 µg Cu/L) despite the different salinities used.

Table 4.19 Growth and survival of *Allorchestes compressa* in Macquarie Harbour, station 14 water

Concentration of station 14 water (%)	Measured Cu concentration (µg/L)	Mean amphipod survival		Amphipod growth	
		% recovery	CV (%)	Mean mass per amphipod (mg)	CV (%)
Control	2	99	7	0.32	11
12.5	3	82[a]	18	0.23[b]	22
25	5	95	7	0.24[b]	12
50	6	90	3	0.20[b]	9
100	10	86	12	0.20[b]	4

a The mean survival at this concentration was significantly less than the control at $\alpha = 0.05$ by Dunnett's test.

b The mean amphipod mass at these concentrations was significantly less than the control at $\alpha = 0.05$ by Dunnett's test.

4.5 Toxicity of ionic copper and Macquarie Harbour waters to juvenile flounder *Rhombosolea tapirina*

4.5.1 Survival

Survival of juvenile flounder after a 14-day exposure to ionic copper and Macquarie Harbour water (station 9 and 14) is shown in table 4.20. In general, measured copper concentrations were close to nominal concentrations throughout the 14-day bioassay.

There was no significant effect on survival in any of the Macquarie Harbour waters tested (at alpha = 0.05 by Dunnett's test). Copper concentrations of 0–80 µg/L also had no significant effect on flounder survival, with a NOEC at 80 µg Cu/L. Survival of flounder was only reduced at the highest copper concentration tested (160 µg/L). Most mortalities occurred within the first 24 hours of exposure and the last fish died on day 8 of the test.

The 14-day EC50 was 138 µg Cu/L, with 95% confidence limits of 126–152 µg Cu/L (based on nominal concentrations). This was well above expected concentrations of dissolved copper in mid-depth Macquarie Harbour waters at 21‰ salinity. Flounder survival, like amphipod survival, was not a sensitive test endpoint for copper.

Juvenile flounder were more sensitive to copper at 20‰ salinity over 14 days than juvenile glass perch and juvenile scaled mullet in 4-day tests at 20‰ salinity (Denton & Burdon-Jones 1986). They reported 96-h LC50 values of 6 and 2.65 mg Cu/L for glass perch and mullet respectively.

4.5.2 Copper content of fish

Residues of copper in juvenile flounder after a 14-day exposure to ionic copper were significantly higher at the 0.05 level than control fish at all copper concentrations tested (table 4.21), with the highest copper content in fish exposed to 160 µg Cu/L. There was high variability in the copper contents and in some treatments there were significant differences between containers, particularly in the treatments which resulted in greater accumulation of copper in the fish.

There was no significant difference (at the 0.05 level) in the copper content of fish exposed to Macquarie Harbour water (both concentrations of station 9 and 14 water) compared with controls. Even though dissolved copper concentrations in these waters were similar to the dissolved copper concentrations in the ionic copper treatments (7 and 11 µg Cu/L) in which there were significant increases, there was no significant accumulation of copper from Macquarie Harbour waters. This suggests that the copper present in the Macquarie Harbour waters was not available for uptake into fish tissue over 14 days.

Table 4.20 Survival of juvenile flounder after a 14-day exposure to ionic copper or Macquarie Harbour waters (stations 9 and 14)

Treatment	Measured copper concentration (µg/L)	Mean survival (%)	CV (%)
Control	2	100	0
5 µg Cu/L	7	97	7
10 µg Cu/L	11	98	4
20 µg Cu/L	23	97	4
40 µg Cu/L	52	100	0
80 µg Cu/L	76	96	4
160 µg Cu/L[a]	170	38	32
100% station 9	13	100	0
50% station 9	7	100	0
100% station 14	13	100	0
50% station 14	6	100	0

a The mean survival at this copper concentration was significantly less than the control at $\alpha = 0.05$ by Dunnett's test.

The accumulation of copper in the body of the fish was relatively low. This may be due to the short exposure time, as it was shown that there were no significant differences in whole body copper levels in rainbow trout (*Oncorhynchus mykiss*) exposed to 0–194 µg Cu/L after 14 days exposure (Dixon & Sprague 1981). These authors, however, did report significant increases in copper accumulation after 21 days.

4.5.3 Histopathological changes

Table 4.22 shows the prevalence of the most common histopathological changes in juvenile flounder after a 14-day exposure to copper and Macquarie Harbour waters. The main organs affected by exposure to copper were gills and liver, in agreement with Baker (1969). The flounder which were exposed to copper showed epithelial necrosis (death of individual cells), epithelial lifting (lifting of respiratory epithelium covering the lamellae, which increases the respiratory diffusion distance and thus can affect physiological processes such as gas exchange), and proliferation of chloride cells in their gills (fig 4.5). The prevalence and degree of these changes seemed to be related to the concentration to which the fish were exposed and generally increased with increasing copper concentration (with the exception of the fish exposed to 5 µg Cu/L).

Table 4.21 Residues of copper in juvenile flounder after a 14-day exposure to ionic copper or Macquarie Harbour water (stations 9 and 14)

Treatment	Measured copper concentration (µg/L)	Mean copper content (mg/kg dry weight)	CV (%)
Control	2	2.01	13
5 µg Cu/L[a]	7	3.99	23
10 µg Cu/L[a]	11	2.89	14
20 µg Cu/L[a]	23	3.08	13
40 µg Cu/L[a]	52	4.46	38
80 µg Cu/L[a]	76	3.92	21
160 µg Cu/L[a]	170	11.55	79
100% station 9	13	1.97	29
50% station 9	7	2.31	19
100% station 14	13	2.60	36
50% station 14	6	2.28	18

a Copper contents of flounder at these concentrations were significantly greater than the control at $\alpha = 0.05$ by Dunnett's test (after transformation)

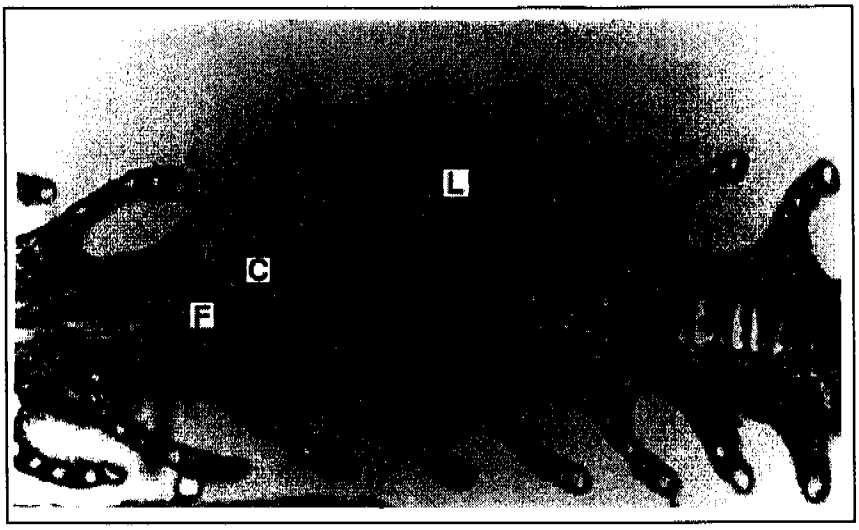

Figure 4.5a Gill filament from a control fish (F – filament, L – lamellae, C – chloride cell)

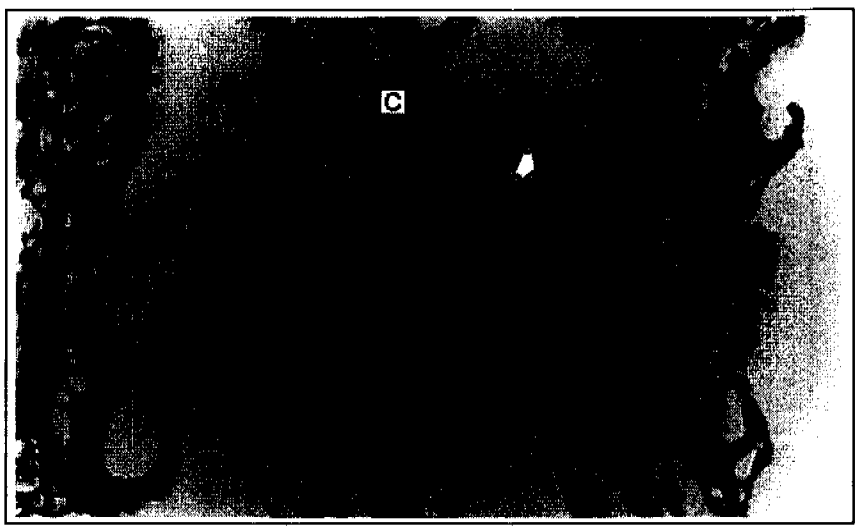

Figure 4.5b, c Gill filament from an exposed fish (N: necrosis, arrow: lifted epithelium, open arrow: lamellae fusion)

Epithelial lifting and necrosis

Significant epithelial lifting and necrosis occurred at copper concentrations of 40–160 µg/L, with an LOEC of 40 µg Cu/L and no effect (NOEC) at 20 µg Cu/L. The 14-day EC50 for epithelial lifting was 22 µg Cu/L, with 95% confidence limits of 19–26 µg Cu/L. Epithelial necrosis was slightly less sensitive as an endpoint with a 14-day EC50 of 35 µg Cu/L (30–39 µg Cu/L). Although dissolved copper concentrations in the Macquarie Harbour waters were lower than the copper concentrations which would cause a significant effect, significant epithelial lifting and necrosis was observed at all concentrations of Macquarie Harbour water tested (both station 9 and 14). This suggests that copper was not solely responsible for the toxic effects of Macquarie Harbour water and that some other metal, combination of metals or other compounds in Macquarie Harbour water contributed to epithelial lifting and necrosis in juvenile flounder.

Chloride and mucous cell proliferation

There was no significant increase in mucous cell numbers in juvenile flounder exposed to either ionic copper or Macquarie Harbour waters. This test endpoint was not sensitive enough to detect copper toxicity in these waters.

There was a significant increase in the number of chloride cells in the highest copper concentration tested (160 µg Cu/L) compared with controls at alpha = 0.05 by Dunnett's test. There was also a significant increase in chloride cells in both concentrations of station 9 water tested, with no effect in station 14 water. Again, dissolved copper concentrations in station 9 water were not high enough to explain the histopathological changes observed.

Cloudy swelling in liver

Large, swollen, faintly granular cells were present in livers of fish exposed to 50% and 100% Macquarie Harbour water from station 9 and 100% Macquarie Harbour water from station 14, as well as in the flounder exposed to 5, 20 and 40 µg Cu/L (fig 4.6). The prevalence of this change was significantly greater in the fish exposed to 100% Macquarie Harbour water (both station 9 and 14) compared with controls.

The content of these cells was PAS negative excluding the possibility of glycogen storage which can have similar appearance and would be normal. Because these swollen cells were not present in control fish and the material inside was not glycogen, they were presumed to be degenerated cells, possibly due to copper interference with normal cell functions. It is possible that it was due to failure of the sodium pump mechanism at the cell membrane, leading to accumulation of intracellular fluid. This type of cellular damage is called 'cloudy swelling'.

Other effects

The fish exposed to 80 and 160 µg Cu/L and some fish exposed to 100% Macquarie Harbour water from station 14 also showed necrotic changes in their liver and kidney (fig 4.7).

On the basis of the prevalence of histopathological changes in juvenile flounder, the fish exposed to 100% Macquarie Harbour water from both sites were affected as much as the fish exposed to 160 µg Cu/L. This suggests that copper is not solely responsible for the effects of Macquarie Harbour water on juvenile flounder.

Similar histopathological changes after copper exposure have been found in other studies with rainbow trout and winter flounder. Respiratory epithelium lifting has been reported in the gills of rainbow trout (*Oncorhynchus mykiss*) exposed to 64 µg Cu/L for 48 h (Bilinski & Jonas 1973). Increases in the number of chloride cells in gills as well as necrotic changes in liver, kidney and haematopoietic tissues were shown in winter flounder (*Pseudopleuronectes americanus*) exposed to 320–560 µgCu/L for 29 days (Baker 1969).

Figure 4.6 Cloudy swelling in liver of an exposed fish

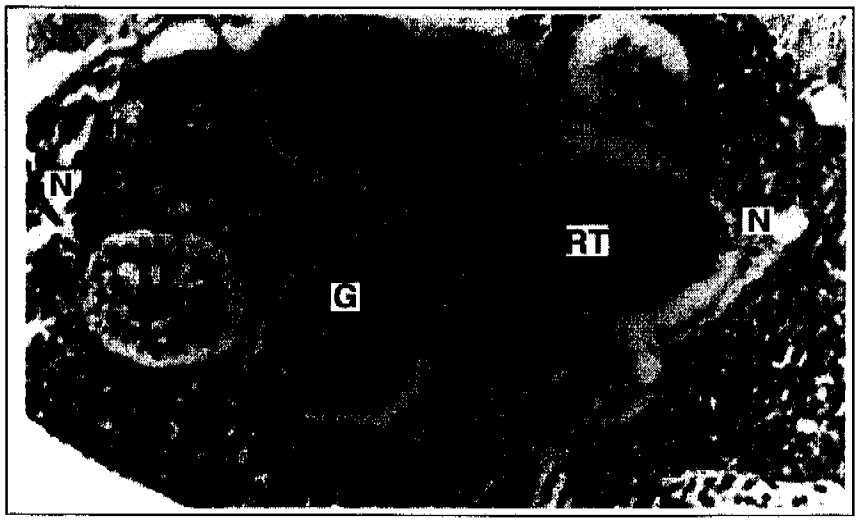

Figure 4.7 Kidney of an exposed fish (N – necrosis, G – glomerulus, RT – renal tubule)

4.5.4 Freshwater challenge

There were no mortalities or any behavioural effects of freshwater challenge, indicating that 14-days exposure to Macquarie Harbour water from station 9 and 14 or to 5, 10, 20, 40 and 80 µg Cu/L does not affect osmoregulation in juvenile flounder.

4.5.5 Summary

Survival, osmoregulation and copper contents of juvenile flounder were not affected in 14-day exposures to Macquarie Harbour waters. Significant histopathological changes were observed, however, these were not attributable solely to copper. Other compounds or metals in the Harbour waters may be responsible for these effects.

Considering that increased salinity protects fish from copper poisoning, the greenback flounder seems to be sensitive to copper, relative to other species (Sorensen 1991). This may be due to the small size of the fish tested and their age or the species-specific differences in sensitivity to toxicants.

Table 4.22 Prevalence of the most common histopathological changes in juvenile flounder after a 14-day exposure to ionic copper or unfiltered Macquarie Harbour water (stations 9 and 14)

Treatment	Endpoint							
	Epithelial lifting		Epithelial necrosis		Increase in chloride cells		Increase in mucous cells	
	Prevalence (%)	CV (%)	Prevalence (%)	CV (%)	Prevalence (%)	CV (%)	Prevalence (%)	CV (%)
Control	12.5	116	0	0	0	0	0	0
5 µg Cu/L	38	39	31	77	6	13	0	0
10 µg Cu/L	12.5	116	6	200	0	0	0	0
20 µg Cu/L	38	39	13	116	0	0	6	13
40 µg Cu/L	94[a]	13	75[a]	27	19	15	6	13
80 µg Cu/L	81[a]	30	69[a]	35	14	20	6	13
160 µg Cu/L	100[a]	0	100[a]	0	50[a]	71	0	0
Macquarie Harbour								
Stn 9 (100%)	81[a]	30	88[a]	116	38[a]	52	6	13
Stn 9 (50%)	69[a]	35	50[a]	41	38[a]	40	19	30
Macquarie Harbour								
Stn 14 (100%)	94[a]	13	75[a]	82	19	30	12	17
Stn 14 (50%)	69[a]	46	44[a]	43	6	13	0	0

a The means for these groups were significantly greater than the control mean at $\alpha = 0.05$ by Dunnett's test

4.6 Risk assessment

Table 4.23 summarises the NOEC values for copper in Macquarie Harbour waters compared with clean seawater (20‰ salinity) for each of the bioassays used in the current study. Amphipod growth and algal enzyme inhibition were the most sensitive endpoints in Macquarie Harbour bioassays with NOEC values of <10 µg Cu/L. Algal growth was much less sensitive to copper in Macquarie Harbour waters than in clean seawater, due to adsorption of copper by organic and inorganic colloids in the harbour waters.

Table 4.23 No effect values for copper in clean seawater (20‰ salinity) compared with Macquarie Harbour water

Bioassay	NOEC (µg Cu/L)	
	Seawater	Macquarie Harbour water
Algal growth	2.5	>42
Algal enzyme	<2.5	<10
Amphipod survival	>16	>12
Amphipod growth	3	2, 6
Flounder survival	80	>12
Flounder histopathology	20	<10

A preliminary risk assessment of copper in Macquarie Harbour waters is given in Appendix A. This risk assessment compared recent monitoring data of ASV-labile, dissolved and total copper in mid-salinity waters of Macquarie Harbour with literature data on copper toxicity using lethal and sub-lethal end-points for a wide range of species in brackish, estuarine and marine waters. This assessment showed that all of the measured total copper concentrations (dissolved + filterable) were above the ANZECC marine water quality guideline value of 5 µg/L. There was a probability greater than 0.90 of the occurrence of ASV-labile copper at concentrations harmful to 5% of all species. For total dissolved copper the probability was higher than 0.98. It was concluded that dissolved copper in Macquarie Harbour would have to be reduced by a factor of 30 to provide sub-lethal protection for 95% of species for 90% of the time.

This preliminary risk assessment based on literature data for copper toxicity was necessarily conservative, largely because bioavailable copper was overestimated. Using the toxicity data from the algal and fish bioassays carried out in actual Macquarie Harbour waters, the probability of copper concentrations in the mid-salinity harbour waters exceeding no effect concentrations was reduced. However, it was not possible to estimate a single copper value which would protect against adverse effects in the Macquarie Harbour from the limited bioassay data in this study. It was possible to conclude that:

- Based on the algal growth bioassays in Macquarie Harbour waters, there was a probability of only 0.13 and 0.28 of the occurrence of ASV-labile and dissolved copper respectively occurring at concentrations above these values in Macquarie Harbour waters. The preliminary risk assessment showed that the copper concentration likely to be hazardous to 5% of algae was 15 µg/L, requiring a four-fold reduction in dissolved copper and a two- fold reduction in ASV-labile copper in Macquarie Harbour waters to protect 95% of the algal species 90% of the time. The algal growth bioassays however, showed no effect at copper concentrations as high as 42 µg Cu/L.

- Amphipod growth was more sensitive, with a probability of 1.0 that dissolved copper concentrations in Macquarie Harbour would exceed the NOEC of 3 µg/L. To protect this species of amphipod 90% of the time, dissolved copper concentrations would have to be reduced by a factor of 20. However, amphipod growth inhibition was only observed in one Macquarie Harbour sample tested (station 14) and poor recovery of controls was obtained in the copper calibration experiment. Further bioassays with this species would be necessary.
- Bioassays with flounder showed that there were no adverse effects on flounder survival, osmoregulation or copper accumulation at concentrations of copper found in Macquarie Harbour. Histopathological effects were observed in Macquarie Harbour bioassays but these effects were not solely due to copper.

Clearly, more toxicity data from a larger number of Macquarie Harbour samples are required to confidently assess the environmental impact of copper in these waters. Based on the risk assessment and data from the Macquarie Harbour toxicity tests, dissolved copper concentrations in mid-salinity Macquarie Harbour waters should be reduced at least four-fold. Dissolved copper concentrations of below 20 µg/L should have no adverse effect on algal growth, amphipod survival, or osmoregulation, survival and histopathology of flounder. Without the use of additional safety factors, we estimate that the maximum acceptable concentration for copper in Macquarie Harbour mid-depth waters lies between 10 to 20 µg/L. Effects on amphipod growth and algal enzyme levels were observed at lower copper concentrations, however the ecological significance of these endpoints is uncertain.

5 Conclusions

Chemical measurements of ASV-labile copper and copper complexing capacity of ten Macquarie Harbour mid-salinity waters (sampled in October and December 1995) have shown that copper concentrations in these waters exceed the complexing capacity of the dissolved organic matter in Macquarie Harbour. Similarly, a preliminary risk assessment comparing dissolved copper levels in Macquarie Harbour waters with concentrations of copper reported in the literature to have significant impacts on aquatic organisms, has shown that dissolved copper concentrations in Macquarie Harbour would need to be reduced. However, sensitive toxicity tests with algae, invertebrates and fish suggest that much of the dissolved copper in the Macquarie Harbour waters is not bioavailable.

Growth inhibition tests with the marine alga *Nitzschia closterium,* which can detect bioavailable copper at concentrations as low as 5 µg/L, showed that Macquarie Harbour waters containing up to 42 µg Cu/L were not toxic. Similarly, no significant increases in copper accumulation in juvenile flounder were observed after a 14-day exposure to Macquarie Harbour waters containing up to 12 µg Cu/L, even though significant increases in copper contents in fish exposed to ionic copper at similar concentrations were found.

No effect of Macquarie Harbour waters on amphipod mortality or juvenile flounder survival or osmoregulation were observed, although these bioassay endpoints were not sensitive enough to detect effects at copper levels environmentally relevant to Macquarie Harbour.

Small but significant effects of Macquarie Harbour waters on algal enzyme activity, amphipod growth (station 14 only) and juvenile flounder histopathology were found. However, histopathological changes in flounder could not be solely attributed to copper in the Harbour waters, because similar effects did not occur at equivalent low ionic copper concentrations.

Despite the limited sampling on two occasions only, it appears that much of the copper present in the Macquarie Harbour waters is not bioavailable. It is likely that colloidal metal oxyhydroxides in the waters reduce copper bioavailability, because electrochemical measurements showed that the waters (with up to 24 µg/L of copper ASV-labile) had no complexing capacity. Colloidal aluminium, manganese and iron oxides/hydroxides were present in sufficient concentrations to bind copper and reduce its uptake into organisms. Part of this fraction of copper may be detected as labile, but not able to dissociate at the cell membrane.

This work highlights the importance of using toxicity tests, not just chemical monitoring, to assess the impacts of pollutants in aquatic systems. Use of chemical analyses and literature data alone in this environmental risk assessment would have greatly overestimated the toxicity of copper in mid-salinity waters of Macquarie Harbour. Use of appropriate and sensitive toxicity tests has shown that only a small fraction of the measured copper is available to cause toxicity. A four-fold reduction in dissolved copper would be required to achieve acceptable copper concentrations of 10–20 µg/L in Macquarie Harbour mid-depth waters. More extensive sampling of Macquarie Harbour waters covering a broader range of salinities over different seasons would be required to ensure copper in these waters causes minimal impact on biota.

6 Recommendations for future work

Toxicity of only a limited number of water samples on two sampling occasions was determined in this study. Temporal and spatial variability in bioavailability and toxicity of copper in Macquarie Harbour waters is required with a more extensive sampling program.

This study focused only on the toxicity of mid-depth waters (20‰ salinity) to marine organisms. Further testing of surface, low-salinity waters which contain higher concentrations of copper should be carried out. To do this, suitable bioassays with estuarine species able to tolerate salinities as low as 5‰ should be developed.

The physico-chemical speciation of copper is critical to its toxicity in water bodies. More research is required to find the analytical speciation methods that correlate best with bioassays (eg algal assays) of copper-containing waters. At present, regulatory bodies base their criteria on *total* copper concentration, but many experts expect this to change in the future if reliable speciation techniques are developed and accepted. There is a similar need for correlation between copper speciation and toxicity in aquatic sediments.

There are very limited data on the toxicity of copper to marine fish. This is principally because marine fish are more difficult than freshwater fish to keep in culture. A survey of the chronic toxicity of copper to a range of early life stages and juvenile Australian marine fish would provide a data bank that would be useful to both industry and regulatory bodies in Australia.

Algal assays are particularly useful for assessing copper toxicity in waters, because they are relevant, sensitive and rapid. Unfortunately, much of the literature data on the toxicity of copper to various algal species are of limited value because the assays were carried out in culture media containing chemicals which complex copper and reduce its toxicity. More toxicity data need to be collected using natural waters as growth media. Also, it is evident that the susceptibility of a particular species of alga to copper can be quite different in an assemblage of algal species than if tested alone. More research should be carried out on the effects of copper on natural assemblages of algae. In addition, the toxicity of copper to algae is highly dependent on other metals present, and further work on the toxicity of metal mixtures to algae is required.