The effects of dissolved organic carbon on copper toxicity to the development of the oyster embryo

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Introduction

The toxicity of waterborne copper to aquatic organisms is highly dependent on its chemical form (Di Toro *et al.*, 2000). The most bioavailable and subsequently toxic form is the free copper ion. However, in aquatic systems, the free copper ion has a strong tendency to form complexes with both inorganic and organic ligands, which results in a reduction in its overall toxicity. Although the inorganic copper species are believed to be bioavailable to a certain extent (MacRae *et al.*, 1999), copper complexed with organic matter is considered non-bioavailable and thus non-toxic. Consequently, factors that can influence the speciation of copper within a particular environment are likely to have significant effects on the metals toxicity to aquatic organisms.

Aims

This study investigates the effects of dissolved organic carbon (DOC) on copper speciation and its bioavailability and subsequent toxicity to the early life stages of the Pacific oyster, *Crassostrea gigas*, following a 24 h exposure.

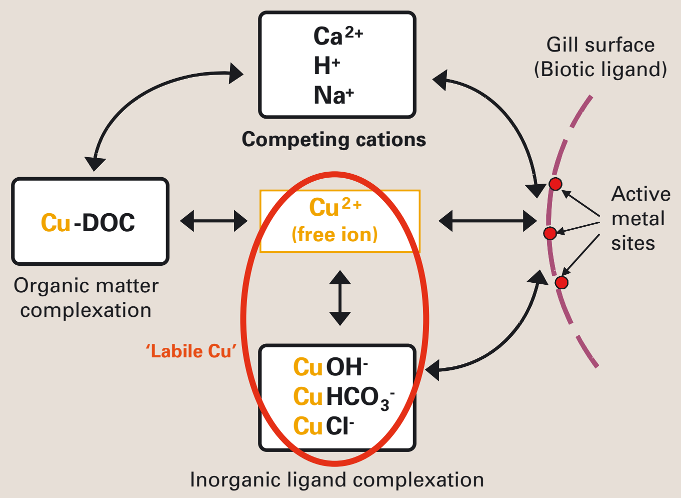

Figure 1: The Biotic Ligand Model for copper speciation

Method

Flow-through system

For copper toxicity testing, an equilibration time of at least 24 h is essential to enable the reaction kinetics of copper to stabilise prior to expose to the test organism (Ma *et al.*, 1999). A flow-through dosing system was used to expose oyster embryos to constant copper concentrations after a 32h equilibration time (see Figure 1). Copper chloride was used as the stock solution and humic acid (HA) as the source of dissolved organic carbon (DOC).

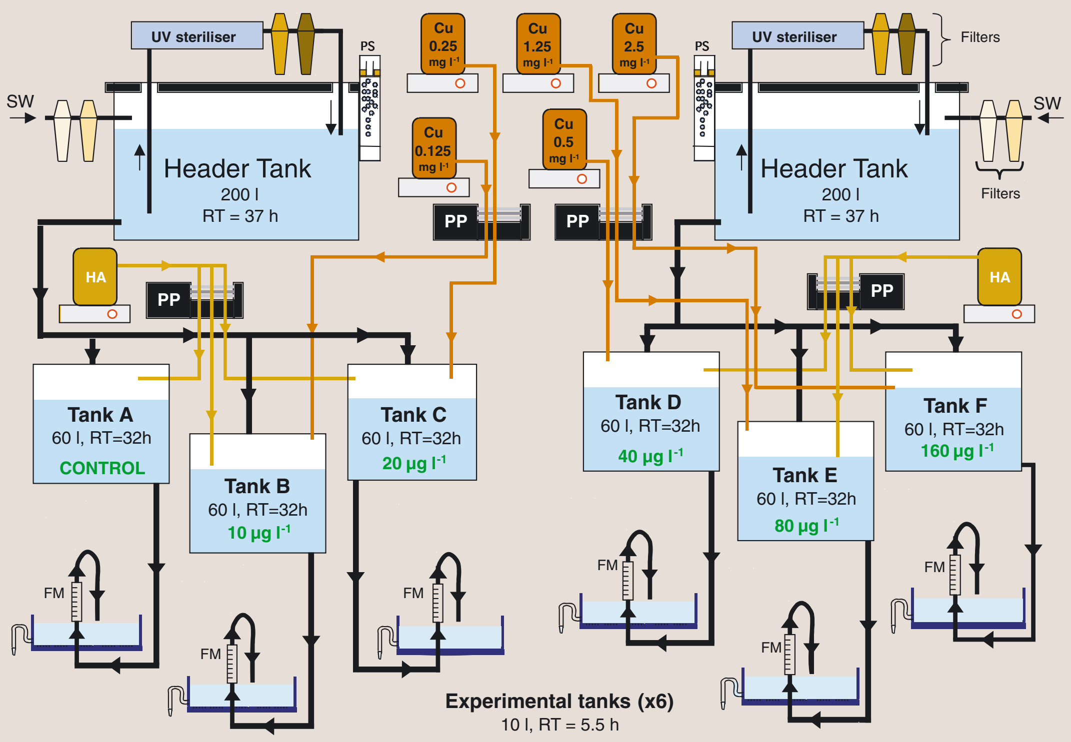

Figure 2: Schematic representation of the laboratory flow-through system for copper dosing. FM-Flow meter, HA – humic acid, PP- peristaltic pump, PS – protein skimmer, RT - residence time, SW – seawater. Copper Chloride used a copper stock and HA as DOC supply

Oyster embryo Bioassay

Conditioned oysters were obtained from Guernsey Sea farms Ltd. Male and female gametes were physically removed from the gonad and placed in filtered seawater. The sperm and egg suspensions were filtered to remove debris and combined in a ratio of 1:100. Eggs were considered appropriate for testing when greater than 80% of cells had reached the 16-32 cell stage. This stage was reached within 4h in all tests.

4ml of the embryo suspension (1000-4000 embryos/ ml) was added to each of the five holding chambers located within each experimental tank. The test was carried out at 21 ± 1°C for 24 ± 2 h in the dark. The test was terminated by fixing the embryos in the holding chambers with 20% (v/v) sodium tetraborate buffered formaldehyde solution.

The number of normal D-stage larvae within a 1 ml volume for each sample was measured using a microscope (x 400) and Sedgwick-rafter counting chamber. In all tests the proportion of normal D-larvae within the control samples was greater than the 60% quality standard.

Monitoring

At the start and end of each test the exposure media was analysed for the following:

- Total dissolved copper (TDCu) & labile copper (LCu) by Differential Pulse Anodic Stripping Voltammetry (DPASV) at a hanging mercury drop electrode (HMDE);
- DOC by high temperature catalytic oxidation (HTCO);
- Ionic concentration by ion chromatography (start of test only);
- Physicochemical readings.

Results

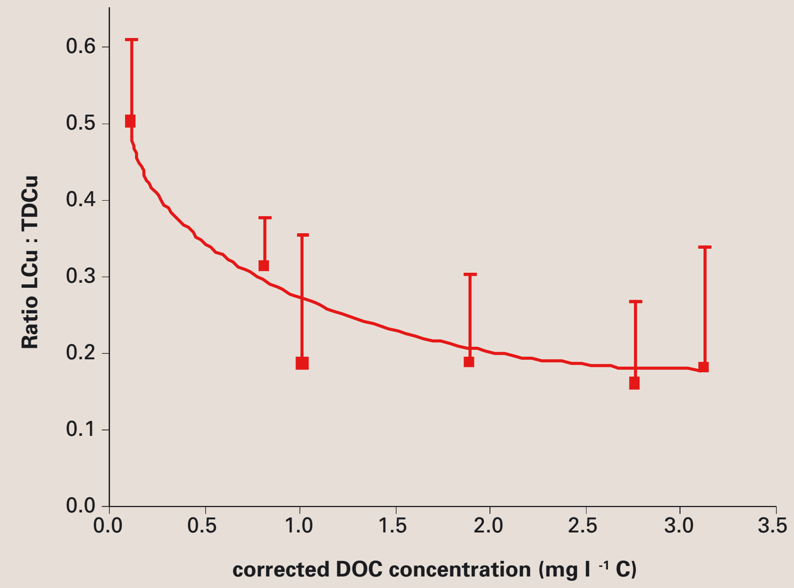

Figure 3: The Ratio of LCu to TDCu in seawater samples with increasing DOC concentration. Corrected DOC relates to the DOC concentration resulting from the addition of humic acid. (i.e. corrected for background DOC) (mean ± SD, n=5)

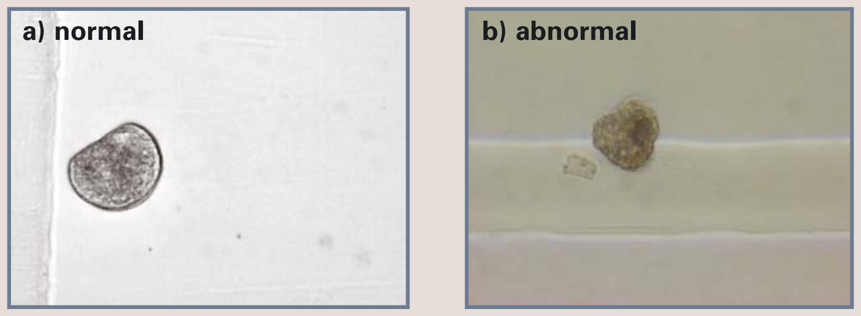

Figure 4: Examples of normal (a) and abnormal (b) development of the D-shaped larvae of the Pacific oyster, Crassostrea gigas

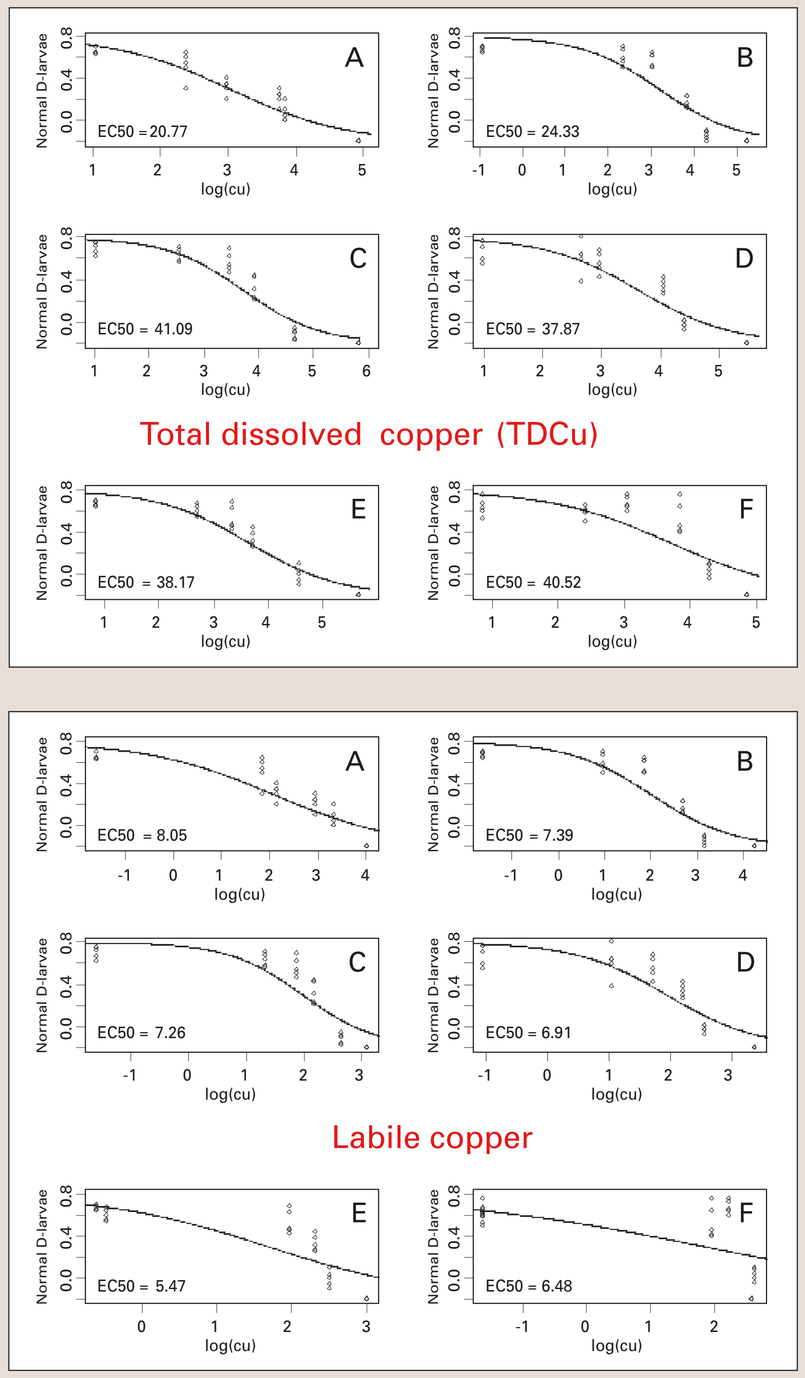

Figure 5: The effects of total dissolved copper (TDCu) and labile copper (LCu) on oyster D-larvae development. Logistical models formulated from the proportion of normal D-larvae with respect to the natural log of the copper concentration. A) Control test, 0.12 mg l^{-1} corrected DOC, B) 0.82 mg l^{-1} corrected DOC, C) 1.02 mg l^{-1} corrected DOC, D) 1.9 mg l^{-1} corrected DOC, E) 2.77 mg l^{-1} corrected DOC, F) 3.13 mg l^{-1} corrected DOC. The estimated EC50 value is shown on each plot.

Table 1: Physicochemical and ionic readings of the seawater within the experimental tanks during the separate oyster embryo bioassays. Values represent means and standard deviations of readings taken at the start and end of each test from all experimental tanks (i.e. control test n=12, all other tests n=14)

Test	Temp (°C)		pH		Salinity (‰)		DO (%)		Na (mM)		K (mM)		Mg (mM)		Ca (mM)		Cl (mM)		SO_4 (mM)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Control	20.21	0.17	8.17	0.01	33.25	0.27	94.42	1.66	463.0	5.3	9.9	0.2	52.4	1.4	9.9	0.2	564.3	4.6	27.1	1.1
4HA	20.74	0.12	8.08	0.02	32.04	0.6	95.54	1.05	461.5	7.3	10.1	0.2	54.2	1.1	9.8	0.2	544.5	13.8	27.6	0.8
8HA	20.81	0.21	8.12	0.01	32.62	0.58	94.31	0.99	460.9	11.5	10.1	0.3	54.0	1.2	9.8	0.2	545.3	9.5	27.5	0.8
12HA	20.49	0.20	8.04	0.02	32.42	0.67	95.64	1.89	457.1	11.4	9.9	0.3	53.1	1.3	9.5	0.4	546.5	13.9	27.4	0.8
16HA	20.56	0.24	8.04	0.02	32.66	0.46	91.71	0.91	467.1	4.3	10.0	0.2	52.2	0.6	10.1	0.3	556.5	10.8	27.0	1.2
20HA	20.64	0.16	8.08	0.01	33.01	0.57	91.71	1.07	457.8	11.4	9.8	0.3	51.3	1.3	9.7	0.5	562.2	13.2	27.3	0.7

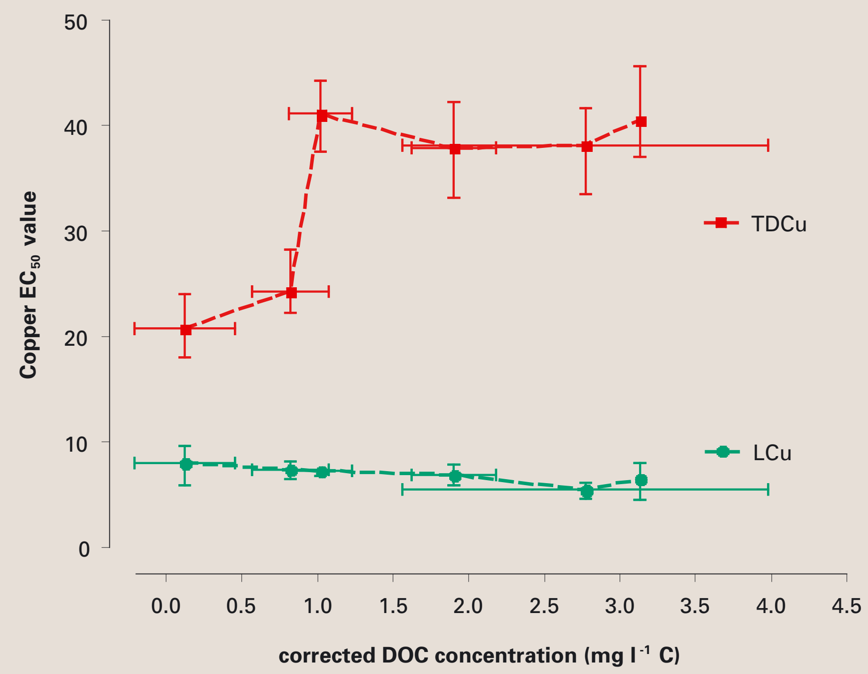

Figure 6: Comparison of the effects of corrected DOC concentration on the EC_{50} of TDCu and LCu for the developing oyster embryo. Data points denote a calculated value using the binomial Generalised Linear Model with logistic link function. The confidence limits for the EC_{50s} were calculated by simulation. One hundred sets of simulated values were obtained using binomial random variables and the EC_{50} estimate was calculated each time. The 3rd and 98th largest EC_{50} estimates were used to obtain the displayed 95% confidence limits for the EC_{50s}

Table 2: Summary table of ecotoxicity test data. NOEC-No observable effect concentration, LOEC – Lowest observable effect concentration

Corrected DOC	NOEC		LOEC		EC_{10}		EC_{50}	
(mg l^{-1})	TDCu	LCu	TDCu	LCu	TDCu	LCu	TDCu	LCu
0.12	10.89	6.36	19.7	8.5	3.24	0.45	20.77	8.05
0.82	10.42	2.61	20.58	6.35	3.38	1.08	24.33	7.39
1.02	12.83	3.84	31.81	6.58	8.90	1.76	41.09	7.26
1.9	19.53	5.53	57.69	9	6.86	1.37	37.87	6.91
2.77	28.19	7.23	40.86	10.2	6.27	0.48	38.17	5.47
3.13	47.13	7.02	72.73	13.8	5.48	0.07	40.52	6.48

Summary

1 The proportion of Labile Copper (LCu) to Total dissolved copper (TDCu) was affected by the concentration of DOC in the seawater samples. The proportion of LCu was approximately 50% of the TDCu concentration at the lowest levels of DOC (0.12 mg l^{-1} corrected DOC). The proportion of LCu was reduced to approximately 20% of TDCu at a corrected DOC concentration of 1 mg l^{-1} and above.

2 TDCu and LCu EC_{50s} were 20.77 µg l^{-1} and 8.05 µg l^{-1} respectively when DOC was not added to the exposure medium.

3 Addition of 1 mg l^{-1} DOC (corrected, as humic acid) reduced TDCu toxicity, resulting in an increase in the EC_{50} to approximately 40 µg l^{-1} TDCu.

4 The addition of DOC had no significant effect on the EC_{50} of LCu to the OEB.

5 This study has shown that DOC provided protection for the development of *C. gigas* against high levels of waterborne copper, by reducing the concentration of the more bioavailable forms. Overall the LCu concentration (Cu^{2+} and inorganic copper), not TDCu, was responsible for the observed copper toxicity to the OEB.

Acknowledgments

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References

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