Assessment of the ecotoxicity of urban estuarine sediment using benthic and pelagic copepod bioassays

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Urban estuarine sediments are sinks to a range of contaminants of anthropogenic origin, and a key challenge is to characterize the risk of these compounds to receiving environments. In this study, the toxicity of urban estuarine sediments was tested using acute and chronic bioassays in the benthic harpacticoid *Quinquelaophonte sp.*, and in the planktonic calanoid *Gladioferens pectinatus*, two New Zealand copepod species. The sediment samples from the estuary tributary sites significantly impacted reproduction in *Quinquelaophonte sp*. However, results from one of the estuary sites were not significantly different to those from the tributaries sites, suggesting that chemicals other than trace metals, polycyclic aromatic hydrocarbons and ammonia may be the causative stressors. Sediment elutriate samples had significant effects on reproductive endpoints in *G. pectinatus*, and on the induction of DNA damage in cells, as shown by the comet assay. The results indicate that sediment contamination at the Ahuriri Estuary has the potential to impact biological processes of benthic and pelagic organisms. The approach used provides a standardised methodology to assess the toxicity of estuarine sediments.

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Abstract

Urban estuarine sediments are sinks to a range of contaminants of anthropogenic origin, and a key challenge is to characterize the risk of these compounds to receiving environments. In this study, the toxicity of urban estuarine sediments was tested using acute and chronic bioassays in the benthic harpacticoid *Quinquelaophonte sp.*, and in the planktonic calanoid *Gladioferens pectinatus*, two New Zealand copepod species. The sediment samples from the estuary tributary sites significantly impacted reproduction in *Quinquelaophonte sp*. However, results from one of the estuary sites were not significantly different to those from the tributaries sites, suggesting that chemicals other than trace metals, polycyclic aromatic hydrocarbons and ammonia may be the causative stressors. Sediment elutriate samples had significant effects on reproductive endpoints in *G. pectinatus*, and on the induction of DNA damage in cells, as shown by the comet assay. The results indicate that sediment contamination at the Ahuriri Estuary has the potential to impact biological processes of benthic and pelagic organisms. The approach used provides a standardised methodology to assess the toxicity of estuarine sediments.

33 **Introduction**

34 Population growth in coastal areas has resulted in the degradation of estuarine habitats and loss of

35 biodiversity (Lotze et al. 2006; Micheli et al. 2013) . Over 40% of the worldwide population lives

36 within 100 km of the coast, and in New Zealand, 75% of human settlements are within 10 km of

37 the coast (Sale et al. 2014; Statistics New Zealand 2006). The growing demand for resources and

38 services has increased the pressure for agricultural and industrial activities, and urban development

39 (Neumann et al. 2015; van Vliet et al. 2015). Estuaries have become highly vulnerable to this

40 pressure, due to the continuous input of municipal, agricultural and industrial runoff, storm water

41 discharges and accidental wastewater overflows (de los Ríos et al. 2016; Risch et al. 2018;

42 Rodrigues et al. 2017; Willis et al. 2017). These stressors, added to the increase in sedimentation

43 have made estuaries a sink for pollutants with affinity for small particles (Reichelt-Brushett et al.

44 2017; Swales et al. 2012; Vermeiren et al. 2016).

45 The potential risk of traditional pollutants and emerging contaminants has become a concern for

46 environmental regulators across New Zealand. The Ahuriri Estuary is recognized as a significant

47 conservation area under the Regional Coastal Environmental Plan for Hawke's Bay (HBRC 2014),

48 and is surrounded by 175 hectares of wetlands of ecological importance. The Purimu Stream and

49 the County Drain were created as buffering zones to reduce stormwater pollution at the Ahuriri

50 Estuary. Smith (2014) reported the presence of contaminants above threshold limits in the Ahuriri

51 Estuary. Recent studies using *in vitro* and zebrafish (*Danio rerio)* embryo tests showed that

52 sediment extracts had genotoxicity, teratogenicity and acute toxicity (Boehler et al. 2017; Heinrich

53 et al. 2017)

54 Studies on chemical extracts can identify chemicals and provide insights into the mechanisms of

55 toxicity but their ability to predict bioavailability or impact on whole organisms is limited (Burton

56 1991; Gyuricza et al. 2010). Whole sediment assessments applying *in vivo* and *in vitro* approaches

57 can complement extract studies by providing information closer to real field situations. The test

58 organisms are exposed to complex chemical mixtures that can lead to synergistic, additive, or

59 antagonistic toxicities. This is important as not considering toxicity of mixtures can result in under

60 or over-estimation of the environmental risk (Crain et al. 2008; Deruytter et al. 2017; Hasenbein

61 et al. 2015; Przeslawski et al. 2015).

62 Other endpoints can be measured to further characterise the mechanisms of toxicity like oxidative

63 DNA damage that can lead to genotoxicity (Esperanza et al. 2015; Frenzilli et al. 2009; Pellegri et

64 al. 2014). This can be measured by the comet assay, a single cell electrophoresis technique in

65 which the DNA supercouil is relaxed and exposed electrophoresis, allowing DNA with single and

66 double breaks to migrate towards a charged anode. The amount of DNA visible in the comet tail

67 under fluorescence microscopy provides an estimate of the extent of damage in a cell (Azqueta &

68 Collins 2013). DNA damage has been reported to affect enzyme functioning and impair the

69 immune system and metabolism of invertebrates, resulting in growth, development, and fitness

70 alterations (Bajpayee et al. 2017; Buschini et al. 2003). Genetic alterations can also lead to

71 mutations and cell proliferations (Mussali-Galante et al. 2014; Simonyan et al. 2016), and inherited

72 teratogenic defects, leading to decreased fitness in populations (Bachère et al. 2017; De Flora et

73 al. 1991; Osman 2014).

74 The aim of this study was to characterise the toxicity of urban sediment using benthic and pelagic

75 copepod species. The approach combined chemical analysis, bioassays on sediment samples and

76 their elutriates, and the comet assay to estimate genotoxicity potential.

77

78 **Materials and Methods**

79   Sampling sites and sediment preparation

80   The Ahuriri Estuary is located north of the City of Napier (population 60,000), on New Zealand's

81   North Island (39°30′S, 176°52′E) as described previously (Heinrich et al. 2017). Four sites were

82   selected: two were located 50m upstream from the Estuary at the Old Tutaekuri Riverbed, and at

83   the Humber Street that is exposed to the Tyne Street stormwater drain network. The other two

84   samples were collected downstream from the tributaries sites, at the Ahuriri Estuary (Fig 1). The

85   Waitangi estuary was used as the reference site control (based on previous chemical analysis

86   results), and samples were collected from the mouth of the Ngaruroro river. All samples were

87   collected in August and September 2017, and stored at 4°C for two days prior to use Two replicates

88   of sediment cores were collected for each site by scraping the top 0-5 cm surface sediment. Sample

89   replicates from each site were pooled, and split into subsamples for metal and organic analyses

90   and bioassays. The pH, salinity and Redox of the samples were measured using a multiparameter

91   probe (Hach HQD meter field No.58258.00).

92   Test species

93   *Quinquelaophonte sp* (M.P Charry et al. 2018, unpublished data) is a benthic harpacticoid

94   copepod, native to New Zealand coastal zones. Its geographic range expands from silty sediments

95   in the Houhora Harbour (North Island), to silty muddy sediments in Portobello Bay in the Otago

96   Harbour (South Island). *Gladioferens pectinatus (Bayly 1963)* is a pelagic species of calanoid

97   copepod commonly found in New Zealand, Australia and Tasmania (McKinnon & Arnott 1985).

98   *G. pectinatus* is highly tolerant to salinity fluctuations, allowing it to distribute from freshwater

99   lakes, estuaries and coastal areas, to open waters (Bayly 1965; Hall & Burns 2002). Both species

100   were collected, isolated and grown in monocultures in an artificial salt water reticulation system,

101 with controlled temperature (20℃), salinity (30ppt), light:dark photoperiod (12hr:12hr), light

102 intensity (10-15μmol) and dissolved oxygen (>7mg·L⁻¹), following the culturing methods

103 described in Stringer et al. (2012), Chandler and Green (1996) and ISO (2015). Dietary

104 requirements were modified for *Gladioferens pectinatus*, following Payne & Rippingale (2000)

105 study. Cultures of *Quinquelaophonte sp* and *G. pectinatus* were fed twice per week with a mixed

106 algae diet of $2x10^6$ and $5x10^5$ cells·mL⁻¹ of *Isochrysis galbana, Chaetoceros muelleri and*

107 *Dunaliella tertiolecta* respectively. The three species of microalgae were grown in F2 media at

108 Cawthron Institute.

109 Sediment toxicity test

110 Whole sediment bioassays were conducted following methods described by Chandler et al. (1996),

111 with a modification for *Quinquelaophonte sp* (Stringer et al. 2014). The assay was conducted over

112 14 days on a semi-static system, with a 14/10 light/dark photoperiod, temperature $20\pm 2℃$, water

113 salinity of 30 ppt $\pm$ 1, pH 8 $\pm$ 0.2 and DO >7 mg·L⁻¹. For each treatment there were four replicates.

114 Three replicates were used for biological analysis, and one replicate for physicochemical analyses.

115 Test chambers consisted of 50 mL borosilicate Erlenmeyers, with 1 diameter apertures in the neck

116 of the flask, covered with a 55μl nylon mesh. These openings allowed for continuous water

117 circulation. Each flask contained 10 grams of sediment, 15 adult males and 15 non-gravid females,

118 previously isolated via glass pipette. Copepods were fed every third day with $2x10^6$ cells·mL⁻¹ of

119 an algae mix of *I. galbana, C. muelleri and D. tertiolecta*. Water parameters were measured at the

120 beginning and end of tests, and endpoints were survival and potential and realized offspring.

121 Elutriate preparation and toxicity test

122   The 6 day elutriate bioassays used *G. pectinatus* neonates (<12hr old). Elutriates were prepared

123   following ASTM International (2014) guidelines. Briefly, sediments were refrigerated over 24 hr,

124   while wet and dry samples were weighed to determine wet weight/volume. Sediment was mixed

125   with artificial salt water (32g of Red Sea® Sea salt per one litre of 0.22 μm filtered dionized water,

126   pH adjusted to 8 ±0.2) to reach a 1:4 (v/v) sediment: water ratio. Samples were placed in an orbital

127   shaker at 100 rpm for 1 hour, and then centrifuged at 3000 rpm for 10 minutes at 4℃. When

128   necessary, elutriates were filtered with a 150 μm sieve, and pH was adjusted to 8 ± 0.2. All test

129   solutions were prepared in flasks previously acid washed with 10% nitric acid for 24 hr, and

130   thoroughly rinsed with deionised water.

131   The ISO/CD 16778 standard (ISO 2015) was followed to assess chronic effects on copepods

132   survival and larval development. Modifications were employed to the protocol due to the hatching

133   difference between *G. pectinatus* and the standard organism, *Acartia tonsa*. Gravid females were

134   isolated and monitored every 4 hr to ensure nauplii used were in the first larval stage (<12 hrs from

135   hatching). Neonates were isolated and exposed to each treatment in triplicates. Copepod chambers

136   were maintained at 20 ± 2℃ and under a 14:10 light dark photoperiod. Fresh elutriates were

137   prepared and exchanged in test chambers every third day, and nauplii fed a mixture of algae ($5x10^4$

138   cells·mL^{-1}) on the same day. Water samples were collected at the beginning of the test for chemical

139   analysis. At the end of the test, naupliar development and survival were assessed by calculating

140   larval development ratio (realized copepodite/ number of copepodites + number of nauplii) and

141   mortality.

142

143   Comet assay

144   Genotoxicity was evaluated in *G. pectinatus* copepodites stage V. The comet assay was performed

145   following the methods described by Singh et al (1988) with modification for copepod cell

146   extractions (Pavlaki et al. 2016; Tartarotti et al. 2013). Each treatment consisted of two replicates

147   of 120 individuals. The positive control was a 15 min exposure to UV-C radiation (Gong et al.

148   2013; Han et al. 2014; Richa et al. 2015; Tartarotti et al. 2013), and the negative control used

149   filtered artificial salt water. Cell suspensions were prepared by homogenizing copepods in a Potter-

150   Elvehjem glass homogenizer with 800 µL phosphate-buffered saline (PBS). Heavy materials from

151   the homogenization were let to precipitate before transferring the cell suspension to 600 µL micro

152   tubes. Cells were centrifuged at 1.0 rcf for 8 min, the supernatant was discarded, and the cell pellet

153   was resuspended in 85 µL Low Melting Point agarose (LMP) mix (0.65% LMP in TAE 1X). Cell

154   viability was then evaluated using hematoxylin stain to identify number of living cells. Acceptable

155   samples contained around 30,000 cells·mL^{-1}. 40 µL of cell resuspension was added to the slides

156   previously coated with 1% normal melting point agarose, and cooled on ice for 10 min. A second

157   layer of 0.65% LMP was added and left to solidify before placing the slides in a lysis buffer

158   solution overnight (2.5M NaCl, 100mM Na2EDTA, 10mM TrisHCl, 10% DMSO, 1% Triton

159   x100, pH 10). Slides were then rinsed with cold deionized water and submerged in an

160   electrophoresis buffer (10M NaOH, 200mM Na2EDTA, pH >13) for 15 min to allow for DNA

161   unwinding. Electrophoresis followed for 15 min at V43 and 300mA in a horizontal gel

162   electrophoresis tank (Bio-rad Power Pac basic cat. no. 164-5050). Slides were then rinsed 3 times

163   in neutralizing buffer (0.4M Tris HCl), followed by dehydration in 100% ethanol for 20 min at

164   4℃. Slides were stained with 50 µL SafeRed (3µL/ 50 mL TAE 1X), and cell counts were done

165   with a fluorescence microscope with a 200 x magnification lens. Comet Assay VI Image analysis

166   system, PI, UK (V4.3.2) was used to determine tail DNA%, tail length and tail moment in treated

167   cells.

168   Chemical analyses

169   Elutriate samples were digested in two percent HNO_3 until analysed through Inductively Coupled

170   Plasma Mass Spectrometry (ICP-MS Agilent 7500cx) for metal analysis. Polycyclic aromatic

171   hydrocarbons (PAHs) in elutriate were analysed by liquid extraction followed by GC-MS SIM by

172   a commercial analytical laboratory (Hill Laboratories, Hamilton, NZ). All sediment samples were

173   dried for 24 hr at 35°C and sieved (<2 mm fraction). Metal samples were digested in nitric acid

174   and analysed with ICP-MS, following US EPA 200.2 protocol. PAH concentrations were analysed

175   by sonication extraction, SPE clean up and GC-MS SIM analysis (US EPA 8270C). All organic

176   compound results were normalized to 1% organic carbon to allow for direct comparison with the

177   sediment and water quality guidelines (ANZECC 2000).

178   Statistical analysis

179   Results were normalized to percent of control. Homogeneity of variance was tested using Levene's

180   test (p<0.05). One-way ANOVA with Dunnet's post hoc test was conducted to identify differences

181   between treatments and controls(ISO 2015; Stringer et al. 2014). Comet assay raw data was log

182   transformed for normality. Data meeting normality assumptions was analysed with one-way

183   ANOVA and Dunnet post hoc test. For data with variance not homogenized after log

184   transformation (Levene's test p<0.05), non-parametric Kruskal-Wallis test with a Dunn's multiple

185   comparison test were used (Pellegri et al. 2014). The R software drc, dplyr and car packages were

186   used for the analyses and graphs.

187   **Results**

188 *Sediment toxicity*

189 Only the zinc concentrations of the Old Tutaekuri riverbed and at the Humber Street drain sediment

190 samples were above the ANZECC interim sediment quality guideline (ISQG) low value (Table 1).

191 Acenaphthylene and acenaphthene were the only polycyclic aromatic hydrocarbons above the

192 ANZECC ISQG-low values at the Humber Estuary site (Table S1). Elevated levels of total

193 recoverable phosphorus were found in all samples with a much higher level at the Humber drain

194 site (Table 2). Ammonia levels were within normal limits for all sites (ANZECC 2000; Batley &

195 Simpson 2009), suggesting no masking influence on the toxicity of the samples.

196 All bioassays parameters met the acceptability criteria (adult survival > 80%, pH 8$\pm$0.2, salinity

197 30 $\pm$1ppt, DO$\geq$8, temperature 20$\pm$2°C) (ISO 14669). There was no difference between the control

198 groups except for potential offspring with the Waitangui Estuary sample in the *Quinquelaophonte*

199 *sp* tests (P<0.001; Fig 2). There were significant differences between the Humber Drain, Humber

200 Estuary and Old Tutaekuri riverbed sites and the control groups for the survival and reproduction

201 success endpoints (P<0.001; Fig 2).

202 *Water column toxicity*

203 Many of the sediment elutriate samples had dissolved trace metals at levels above ANZECC water

204 quality guidelines except for cadmium (Table 3). All sites, including the reference site (Waitangi

205 Estuary), had zinc levels above ANZECC trigger values for a level of protection of 95%. Copper

206 level at the Waitangi site was also above the ANZECC water quality guideline.

207 The tests met the ISO 16778 (2016) quality assurance standards. Nauplii survival in samples from

208 all sites was significantly different from the control, especially at the Old Turaekuri Riverbed site

209 and the Humber Estuary (Fig 3A). There were no differences in the larval development between

210   the control, the Waitangi Estuary site and the Old Turaekuri Estuary elutriates (P>0.05; Fig 3B).

211   The Old Tutaekuri Riverbed elutriates was the most toxic to *G. pectinatus* larval development

212   (P<0.01; Fig 3B).

213   *Comet assay*

214   The comet assay was not done on the Waitangi Estuary as it was not sampled at the same time as

215   the other sites. Test acceptability was based on the statistically significant difference between a

216   negative and a positive control (P<0.001). A significant difference was identified between the

217   DNA damage from the negative and the positive controls (Dunn's, p<0.05) on the three parameters

218   measured (tail moment, intensity and length). There were no differences in tail moment and tail

219   intensity between the negative control and the estuary sites. The Old Tutaekuri Riverbed samples

220   induced DNA damage as indicated by the tail moment and tail intensity (DNA%) (Fig 4). There

221   was no significant DNA damage in samples from Humber Drain based on tail intensity endpoint,

222   however results from tail moment suggest a marginal significance (p = 0.06).

223   **Discussion**

224   Between 79 and 87% of runoff loads have been attributed to urban runoff (residential galvanized

225   roofs and urban roads), with 86% of the load containing zinc, especially after wet events (Davis &

226   Birch 2010; Li et al. 2015). Metal concentrations observed in this study were comparable to earlier

227   reports at the Ahuriri Estuary (Smith 2014), and studies in urbanized New Zealand cities (Charters

228   et al. 2016; Mills 2016) and overseas (Ács et al. 2016). The results of metals in the systems studied

229   suggest that stormwater runoff is a major source of metals in the estuary, specially at the Old

230   Tutaekury Riverbed, and Humber Drain samples.

231   The high mortality and low reproduction of *Quinquelaophonte sp* in the Humber Estuary sample

232   could not be fully explained by the individual metal or PAH content (except for acenaphthylene

233   and acenaphthene which were above the ISQG-low values). This suggests the presence of multiple

234   stressors affecting the toxicity in copepods. In this study, the Humber Estuary site had the highest

235   sum of low molecular weight PAHs (Table S1). Recent studies have shown that copepods can

236   significantly bioaccumulate PAHs, even when these are below threshold values (Cailleaud et al.

237   2009). However, low weight PAHs can be eliminated more efficiently than high molecular PAHs

238   due to their lower hydrophobicity nature (Almeda et al. 2013). This could have partially

239   contributed to the high mortality of copepods (91%) exposed to the Humber Estuary sample, and

240   the unsuccess in egg hatching. Our study aligns with those conducted by Boehler et al. (2017) and

241   Heinrich (2017) on extracts from sediment samples collected near the Ahuriri Estuary. They

242   showed multiple toxicity responses including androgenic and glucocorticoid activities in cell lines,

243   and moderate teratogenicity in zebrafish embryos exposed to PAHs and musk extracts mixtures at

244   concentrations as low as 1.6 mg SEQ. mL-1.

245   The total mortality of copepods in the tributary sites can be partially explained by the concentration

246   of zinc. Stringer (2014) calculated a zinc LC_{50} of 196 mg. Kg-1 and a mobility inhibition EC_{50} of

247   137 mg.Kg -1 for *Quinquelaophonte sp*. The results from the drain and riverbed samples were well

248   above that value (310 and 280 mg. Kg-1 respectively), suggesting that zinc had an effect on the

249   mobility and mortality of *Quinquelaophonte sp* individuals during the 14-day exposure.

250   Physicochemical results showed that the Humber Estuary samples had higher redox potential,

251   which can trigger sulphides and trace metal sulphides oxidation increasing metal bioavailability in

252   pore water (Chapman et al. 1998; Kalnejais et al. 2015; Lu et al. 2016). In addition, the presence

253   of mucilage (predominantly from diatom) was also observed to cover the sediment samples from

254   the Humber Estuary site at the end of the tests. Mucilage has been reported to affect copepod's

255   grazing ability (Malej & Harris 1993; Pančić & Kiørboe 2018), and cause teratogenic effects in

256   the gonadal tissue, affecting reproductive success (Ianora et al. 2011; Wolfram et al. 2014). Further

257   studies are needed to confirm the contribution of these stressors to the toxicity.

258   Bioassays using *G. pectinatus* have been confirmed as good indicators for the toxicity of water

259   dissolved compounds through contact or ingestion. Despite of the reduction of metal

260   concentrations from the tributaries to the estuary sites (zinc and lead dropping by 2.27X and 2.5X

261   respectively), survival and larval development were significantly affected. The toxicity of the

262   sediment samples to *G. pectinatus* is unlikely to be solely due to independent metals, as the levels

263   from the estuary sites were similar to those measured in the Waitangi reference and yet, there were

264   differences between the larval development ratio. The presence of other compounds not addressed

265   in this paper (e.i. organochlorine compounds and emerging contaminants) may have further

266   influenced the toxicity in *G. pectinatus,* as reported in Boehler (2017). This may be due to the

267   higher exposure to pollutants emerging from the Napier city, while the Waitangi estuary is

268   distanced from the urban stormwater and waste water runoff. Extensive literature has reported the

269   effect of additive toxicity of chemicals mixtures in marine invertebrates reproductive success and

270   mortality (Cooper et al. 2009; Hagopian-Schlekat et al. 2001; Picone et al. 2018). However, these

271   effects highly depend on the type of interaction (metal-metal, metal-organic, organic- organic) and

272   on the concentration of each compounds in the mixture, which can either trigger synergistic,

273   antagonistic, or non-interactive toxicities (Gauthier et al. 2014; Nys et al. 2016; Traudt et al. 2017).

274   A recently published framework for ecological risk assessment (Nys et al. 2018) was developed to

275   better characterise the bioavailability of metal mixtures, based on a sensitive species distribution

276   (SSD) model. This approach could be further addressed when assessing community impacts at the

277   Ahuriri estuary.

278

279   The comet assay is a well recognised method to measure DNA damage in individual cells, and an

280   accepted tool for environmental monitoring (Colin et al. 2016; de Lapuente et al. 2015; Martins &

281   Costa 2014). The present study confirms the genotoxicity in the Old Tutaekuri samples, and the

282   potential genotoxicity at the Humber drain, previously characterised with sediment extracts of

283   organic compounds from the Ahuriri estuary (Boehler et al. 2017; Heinrich et al. 2017). The

284   Humber Drain and Old Tutaekuri Riverbed sites had zinc and lead concentrations above guidelines

285   values. Zinc has been reported to induce low levels of DNA damage in zooplankton copepods

286   (Goswami et al. 2014), the marine clam *Ruditapes philippinarum,* rainbow trout *Oncorhynchus*

287   *mykiss*, and grasshopper *Chorthippus brunneus* (Augustyniak M 2006; Marisa et al. 2016).

288   However, while lead genotoxicity has not been sufficiently studied in copepods, it has been

289   reported to be significant in amphipod haemocytes and spermatozoa at 25 µg. L^{-1} (Di Donato et

290   al. 2016), in freshwater mussel at 120µg.L^{-1} (Black et al. 1996; Sohail et al. 2017) and in

291   polychaetes at 100 µg. L-1 after 3 days (Singh et al. 2017).

292   In addition, multiple co-genotoxic mechanisms between chemical compounds have been reported

293   on aquatic organisms (Gauthier et al. 2014). These mechanisms include the inhibitory effect of

294   metals on the cytochrome P450, essential for PAHs detoxification; the enhanced production of

295   reactive oxygenated species (ROS) which translates into cellular oxidative stress, and the effects

296   of PAH on membrane integrity, which enhances the permeability to metals. This indicates that

297   mixtures of organic and inorganic chemicals can modulate synergistic, additive or antagonistic

298   toxicities at the cellular and genetic level (Di Poi et al. 2018; Kousar & Javed 2015; Warne &

299   Hawker 1995).

300   The initial protective mechanism of copepods to minimize cellular and DNA damage is to

301   metabolize and eliminate toxicants (Han et al. 2017). However, in the presence of toxicants with

302   pharmacological modes of action (non-specific disruption or overloading of enzyme activities),

303   the enzymes in charge of metabolising compounds and lipid storage regulators may be inhibited.

304   The presence of compounds with these modes of action were identified at the Humber drain and

305   the Old Tutaekuri riverbed in previous studies at 0.012 and 0.0034 mg. Kg-1 respectively (Boehler

306   et al. 2017). Pharmacological effects have been reported in the pelagic copepod *Calanus*

307   *finmarchicus* exposed to naphthalene, pyrene, crude oil (Han et al. 2015; Hansen et al. 2008),

308   *Acartia tonsa* exposed to four synthetic musk substances (Wollenberger et al. 2003), *Tigriopus*

309   *japonicus* exposed to polybrominated diphenyl ethers (Han et al. 2015), and *Nitocra spinipes*

310   exposed to galaxolide (Breitholtz et al. 2003). This may suggest that the chemicals present at the

311   impacted estuary sites may have modes of action affecting the precursor of larval molding and

312   deoxidation (P450 enzymes such as CYP330A1 or CYP305A1) as shown on the bioassays, as

313   opposed to directly fractioning DNA brands (Hansen et al. 2008). Further, DNA repairing

314   mechanisms present in copepods remains to be confirmed.

315   It has been suggested that risk assessment frameworks and decision support systems consider the

316   genotoxicity of chemical mixtures as part of a weight of evidence approach. This has been

317   demonstrated by the method integrating nine lines of evidence in contaminated estuaries in

318   Portugal (Caeiro et al. 2017), the policy frameworks and risk assessment tools at contaminated

319   sites in the Netherlands (Dagnino et al. 2008), and the acceptance of genotoxic tools as one of the

320   standard monitoring strategies within the European Union Water and Marine Strategy Framework

321   Directives (Allan et al. 2006; Martins & Costa 2015).

322   **Conclusion**

323   The sediment and elutriate samples from the urban Ahuriri Estuary tributaries were genotoxic and

324   impacted reproduction in the two copepod bioassays. The toxicity from the estuaries sites was

325 lower than that of the tributaries, but genotoxicity and reproduction inhibition were evidenced.

326 This confirms the bioavailability of biologically active chemicals in the sediment samples. The

327 approach using bioassays in pelagic and benthic copepod species was successful to characterise

328 the toxicity of urban sediment. The results provides useful effects-based information to inform

329 environmental managers.

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334

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Figure 1

The Ahuriri Estuary and Waitangi Estuary sampling sites.

(A) Old Tutaekuri Estuary site. **(B)** Old Tutaekuri Riverbed site. **(C)** Humber Estuary site. **(D)** Humber Drain site. **(E)** Waitangi Estuary site. The Old Tutaekuri Riverbed receives most of the inflow from a rural and urban catchment, and the Humber Drain receives stormwater inflow from the Pandora industrial area and residential suburbs.

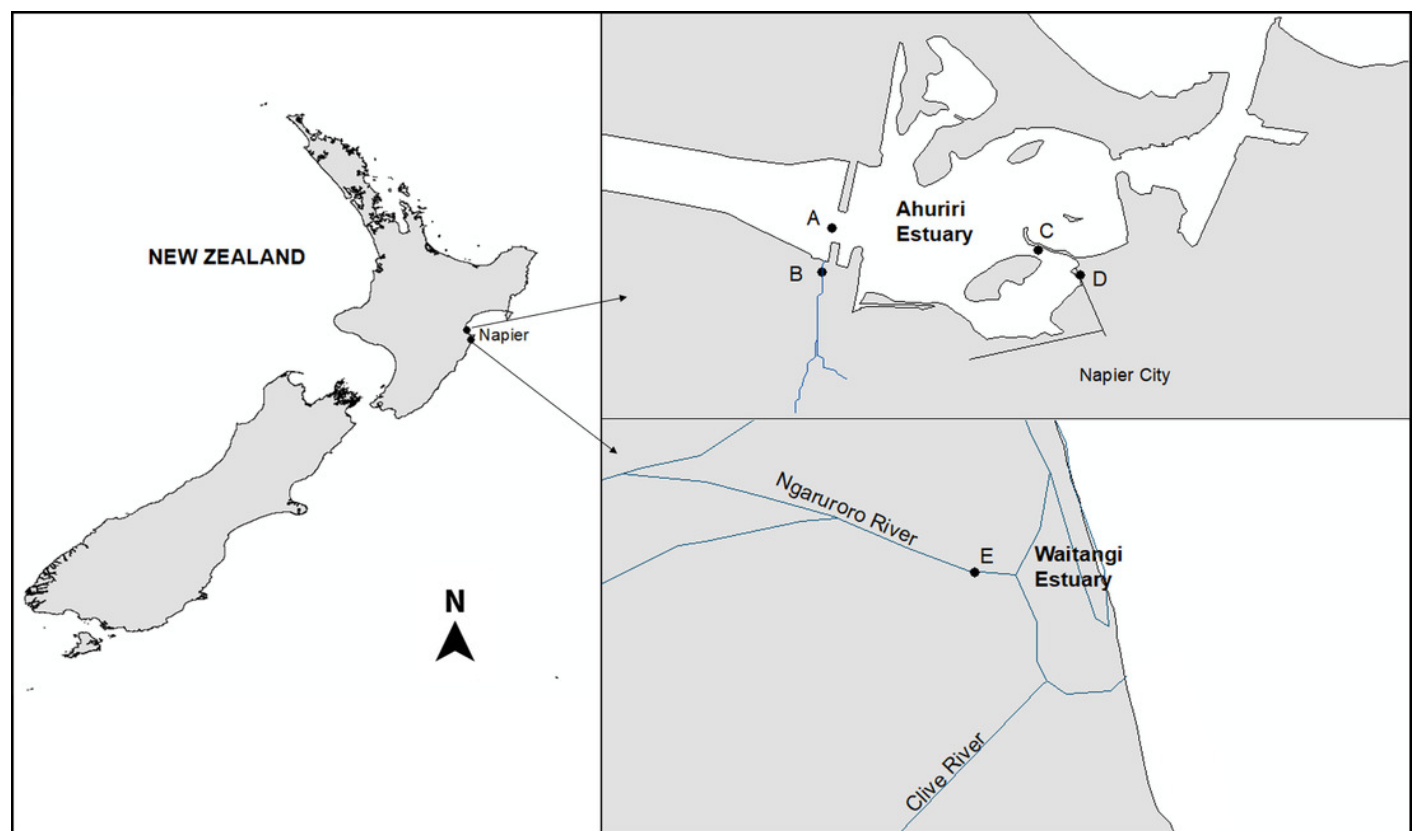



Figure 2

Results of the 14-day chronic test using the benthic copepod *Quinquelaophonte sp* exposed to sediments.

Three endpoints were assessed for the toxicity of sediments from the Ahuriri Estuary and reference site, Waitangi estuary. **(A)** Survival. **(B)** Total offspring hatched during the test. **(C)** Potential offspring (eggs in female pouch). Site codes: Waitangi Estuary (Wa), Old Tutaekuri Estuary (OT Est), Humber Estuary (Hum Est), Old Tutaekuri Riverbed (OT River), Humber Drain (Hum Dr). Diagonal lined bars: control site (Waitangi), white bar: laboratory control sediment, black bars: tested sites. Significance levels: '*' $p < 0.05$, '**'$p < 0.001$ Dunnett's multiple comparison against the control.

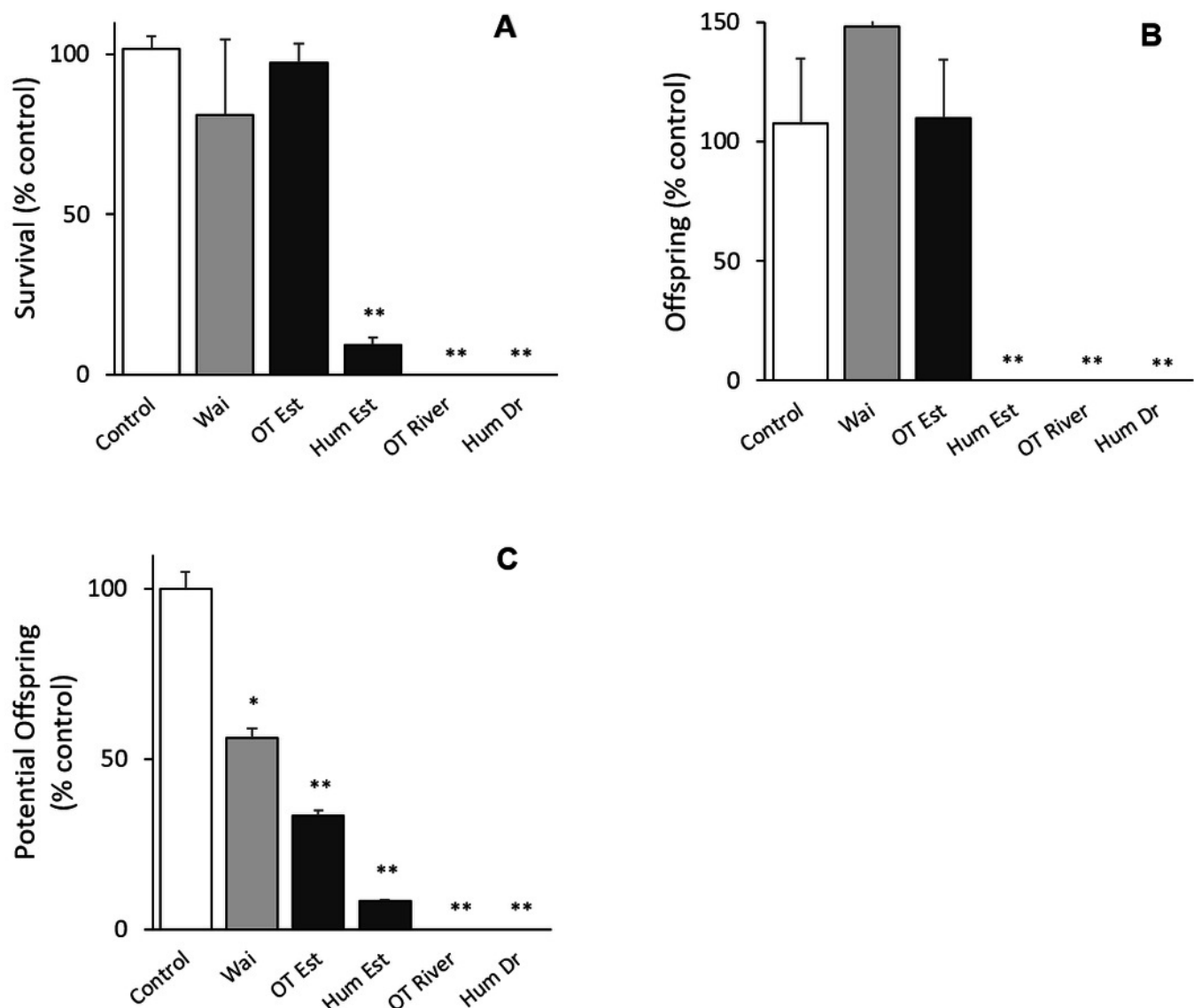



Figure 3

Elutriates acute and chronic results.

(A) Survival and (B) larval development ratio of *Gladioferens pectinatus* after exposure to elutriates from selected sites at the Ahuriri Estuary, and Waitangi Estuary. Significance levels: '*'p < 0.05, '**'p< 0.001, Dunnett's multiple comparison against the control.

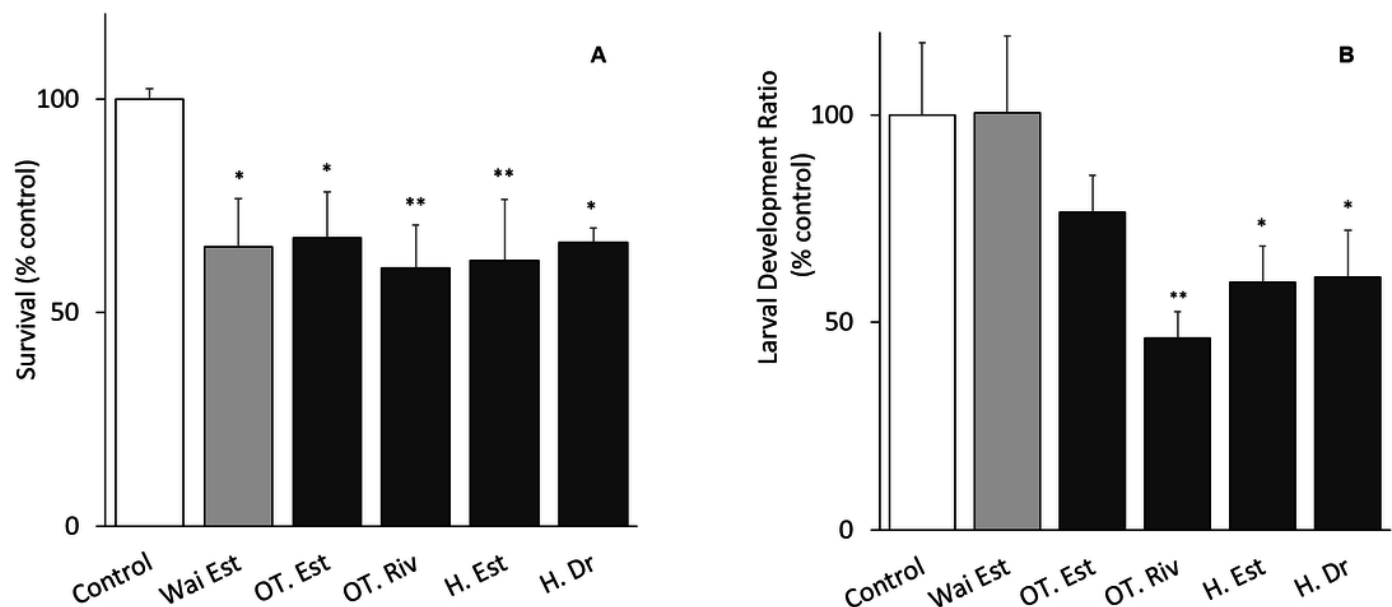
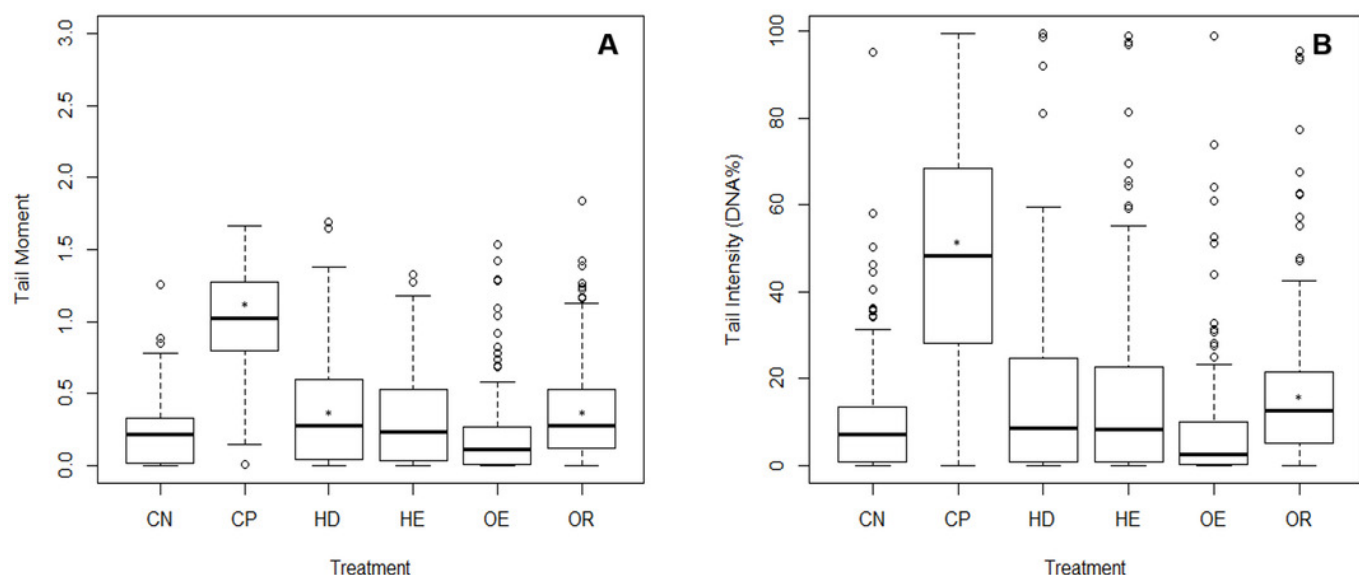



Figure 4

DNA damage on cells isolated from copepods exposed to sediment elutriates.

(A) Tail Moment. **(B)** Tail Intensity. **(CN)** Negative control, **(CP)** Positive control, and elutriates from the **(HD)** Humber Drain, **(HE)** Humber Estuary, **(OE)** Old Tutaekuri Estuary and **(OR)** Old Tutaekuri Riverbed sediments. * Indicates significance difference from control (p<0.05). Humber drain (p<0.01) and Old Tutaekuri Riverbed (p<0.001).

Mean concentrations (mg·kg $^{-1}$ dry weight) of trace metals in sediment samples.

Analytes from the four sites of the Ahuriri Estuary, and from the reference site, Waitangi Estuary. The ANZEEC Interim Sediment Quality Guidelines (ISQG) represent levels above which there is a low probability of biological effects (ISQG-Low) and high probability of biological effect (ISQG High). Analytes above thresholds values are indicated as * for ISQG-low.

	Arsenic	Cadmium	Copper	Lead	Zinc
ANZECC ISQG-Low	20	1.5	65	50	200
ANZECC ISQG-High	70	10	270	220	410
Humber Drain	4.7	0.32	16.7	32	310*
Humber Estuary	3.7	0.018	4.7	9.9	57
Old Tutaekuri Riverbed	4.7	0.127	13.9	16.1	280*
Old Tutaekuri Estuary	3	0.024	4.4	7.3	47
Waitangi Estuary	6.4	0.079	11.9	15.3	61

Sediment physicochemical properties from the five studysites.

TOC= Total Organic Carbon; TRP= Total Recoverable Phosphorus; TN= Total Nitrogen; Sal = Salinity. Values for pH and redox are the average between results from beginning and end of test.

	pH	Redox (mV)	Ammonia (mg·kg⁻¹ dry weight)	TOC	TRP (mg·kg⁻¹ dry weight)	TN (g·100g⁻¹ dry weight)	Sal (ppt)
Humber Drain	7.87	145	2.9	2.50	1160	0.12	29.6
Humber Estuary	7.84	228.5	4.4	0.36	360	0.05	29.6
Old Tutaekuri Riverbed	7.77	109.0	22.0	0.15	340	<0.05	29.6
Old Tutaekuri Estuary	7.87	171.4	9.0	1.06	390	0.11	29.6
Waitangi Estuary	7.99	126.0	5.7	1.45	650	0.21	29.6

Dissolved concentrations (μg·L^{-1}) of trace metals from sediment elutriates.

Analytes obtained from the four study sites at the Ahuriri Estuary, and from the reference site, the Waitangi Estuary. ID= Insufficient data; ND= Not detected. Analytes above the ANZECC guidelines thresholds values are indicated with * for a level of protection of 95% and above.

	Arsenic (μg·L⁻¹)	Cadmium (μg·L⁻¹)	Copper (μg·L⁻¹)	Lead (μg·L⁻¹)	Zinc (μg·L⁻¹)
ANZECC Guidelines 99% [a]	ID	0.7	0.3	2.2	7
ANZECC Guidelines 95% [a]	ID	5.5	1.3	4.4	15
Humber Drain	<4	<0.2	<1	4.1*	75*
Humber Estuary	ND	0.2	1	1.6	33*
Old Tutaekuri Riverbed	<4	<0.2	<1	5.3*	59*
Old Tutaekuri Estuary	ND	0.2	2.2*	2.2	44*
Waitangi Estuary	<4	0.5	8.3*	4	33*

2   [a] ANZECC 2000 water quality guidelines trigger values for 99 and 95% of protection levels for

3   species in marine waters.