

Paper Summary:

**Toxicity of Ozonated Seawater to Marine Organisms. II:
Comparative Toxicity of Natural Waters**

This paper was written for submission to Environmental Toxicology and Chemistry by members of Nutech O3, Inc.'s scientific team. The purpose of this study was to determine if differing levels of salinity, dissolved organic carbon, and total dissolved nitrogen had any effect on total residual oxidant (TRO) formation. TRO is generated in ballast water by treating the water with ozone. The ozone then reacts with bromide ion in seawater to form mainly bromine and some bromate, both of which are toxic to marine organisms. Waters from Cape Fear River Estuary, North Carolina; Shannon Point Marine Center, Washington; Vallejo, California; Yaquina Bay Oregon; and artificial seawater were used in this experiment.

This paper supports Nutech O3, Inc.'s claim that TRO is an accurate measurement of the effectiveness of an oxidant based ballast water treatment system. There exist direct correlations between TRO and species mortality and these correlations are not affected by the origin of the water being treated.

The water samples were populated with juvenile mysid shrimp, sheepshead minnows, and topsmelt and then dosed with ozone. Ozonation of all waters caused a steady increase in TRO concentration with the Vallejo, CA sample with higher impurities increasing more slowly. TRO increased during the first 3 hours of treatment with saturation occurring after 6 hours of treatment. Mortality rates were measured after 24 and 48 hours with higher mortality observed after 48 hours than at 24 hours. These mortality rates were not greatly affected by the type of water used during testing, leading to the conclusion that the effectiveness of ozone in seawater will likely be the same regardless of the site-specific ozone demand properties of individual seawater samples. The rate of TRO formation, however, is directly correlated with impurities so the dosing time to reach a lethal TRO level will be longer in waters with higher organic and nitrogen concentrations..

In addition to the toxicity experiments, similar tests were conducted in which each ozonated water sample was treated with sodium thiosulfate before organisms were added. TRO measurements were taken after this treatment to confirm that oxidants were reduced. Results showed that this treatment was effective in both reducing toxicity and preventing mortality from occurring in any species in this study.

APPENDIX 3

Running head: Toxicity of Ozonated Seawater to Marine Organisms

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TOXICITY OF OZONATED SEAWATER TO MARINE ORGANISMS II:
COMPARATIVE TOXICITY OF NATURAL WATERS

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1 **Abstract** - Ballast water transport of non-indigenous species (NIS) has been recognized
2 as a significant contributor to biological invasions and a threat to coastal ecosystems.
3 Recently, the use of ozone gas as an oxidant to eliminate NIS from ballast while ships are
4 in transit has been considered. We measured total residual oxidant (TRO, as Br₂)
5 formation with ozone treatment over time in artificial seawater and four site-specific
6 seawaters. Toxicity tests with each of the waters over 48 hours were conducted using
7 juvenile mysid shrimp (*Americamysis bahia*). Studies were also conducted using selected
8 waters for topsmelt (*Atherinops affinis*), and sheepshead minnows (*Cyprinodon*
9 *variegatus*). There appeared to be a correlation between water salinity and the rate of
10 TRO formation during ozonation, but dissolved organic carbon and total dissolved
11 nitrogen did not affect TRO concentrations. Sheepshead minnows were most sensitive to
12 TRO concentrations, while topsmelt were less sensitive than sheepsheads but more
13 sensitive than mysids. Toxicity tests run concurrently in which sodium thiosulfate
14 (Na₂S₂O₃) was added before test initiation resulted in TRO elimination and survival of all
15 organisms. The results of this study should be considered in designing ozone treatment
16 in ballast tanks. 188 words

17 **Keywords:** ozone, non-indigenous species, marine invertebrates, total residual
18 oxidant, toxicity

19 INTRODUCTION

20

21 The transport of non-indigenous species (NIS) via ballast water on ocean-going
22 vessels has been recognized as a serious threat to the ecology of coastal ecosystems
23 worldwide (e.g. Carlton and Geller 1993; Ruiz et al. 1997, Carlton 1999; Bax et al. 2001)
24 In analyses of ballast water samples from over 150 cargo ships, Carlton and Geller (1993)
25 identified at least 367 taxa and found representatives of all marine trophic groups.
26 Invasions that could be attributed to ballast water transport in marine and freshwater
27 include the introduction of the ctenophore *Mnemiopsis leidyi* to the Black Sea
28 (Vinogradov et al. 1989), the Asian shore crab *Hemigrapsus sanguineus* to New Jersey
29 (McDermott 1991), and the zebra mussel (*Dreissena polymorpha*) from Eurasia to the
30 Great Lakes (e.g. Griffiths et al. 1991).

31 To provide alternatives to mid-ocean ballast exchange, chemical biocides and
32 physical treatments are being developed and tested for their ability to reduce NIS prior to
33 ballast water discharge (Hallegraeff 1998). Examples of these alternatives include
34 treatment with hydrogen peroxide (e.g. Hallegraeff et al. 1997), electric shock (Montani
35 et al. 1995), heat (Bolch and Hallegraeff 1993; Hallegraeff et al. 1997) and biocides such
36 as SeaKleen (Sano et al., 2004). Another method receiving increased attention is the use
37 of ozone to generate total residual oxidant (TRO) concentrations in ballast water that are
38 lethal to organisms. Ozone reacts with bromide ion in seawater to form bromine
39 (HOBr/OBr^-) and bromate ion (BrO_3^-), both of which are toxic to marine organisms (e.g.
40 Gensemer et al. 2005, this issue; Herwig et al. unpublished data). A preliminary study by
41 Cooper et al. (2002) tested ozone delivery to ballast water tanks onboard the *S/T Tonsina*,

42 a 265 m oil tanker carrying 41,365,000 L of ballast water. Ozone treatment for 5-10 h
43 eliminated 71 to ≥ 99 % of selected marine phytoplankton, zooplankton, and bacteria.

44 In a companion study, Gensemer et al. (2005) simulated the efficacy of the ozone
45 treatment system from the *S/T Tonsina* in the laboratory using adult mysid shrimp
46 (*Americamysis bahia*), juvenile topsmelt (*Atherinops affinis*), and sheepshead minnows
47 (*Cyprinodon variegatus*), and adults of two amphipod species (the benthic *Leptocheirus*
48 *plumulosus* and pelagic *Rhepoxinius abronius*). Batch ozonation in aquaria containing
49 artificial seawater (ASW) showed that with the exception of *L. plumulosus*, all species
50 tested died well within a typical 5 h ozonation period as a result of TRO formation (LT50
51 ≤ 5 h, LC50s = 0.31 to 0.94 mg TRO/L as Br₂). *L. plumulosus* was not affected by any
52 TRO concentration over the time periods tested (LC50 > 5.63 mg TRO/L as Br₂).
53 Further experiments revealed that juvenile *Americamysis bahia* experienced continued
54 mortality over 48 h after short term ozone exposure (1.5 h) and subsequent removal to
55 untreated ASW. *Americamysis bahia* were also susceptible to ozonated seawater that had
56 been stored in the dark for 48 h before beginning toxicity testing.

57 To further characterize the effective TRO concentrations required for removal of
58 NIS from seawater, measurements of oxidant formation over time with ozone treatment
59 in different coastal waters is necessary to consider variations in factors that may influence
60 the buildup or loss of TRO such as salinity and dissolved organic carbon. Toxicity
61 experiments in different seawaters could also reveal any impact of water characteristics
62 on species sensitivity to TRO. This paper expands on the results presented in Gensemer
63 et al. (2005) by using ASW and natural waters from four locations to compare TRO
64 loading rates over time. The toxicity of TRO towards juvenile *Americamysis bahia*,

65 *Atherinops affinis*, and *Cyprinodon variegatus* in ASW and four coastal seawaters was
66 also assessed. In addition, toxicity experiments were conducted following treatment with
67 a chemical reductant to confirm that oxidant toxicity could be removed if necessary to
68 ensure environmental safety of the ozonated seawater.

69

70 **MATERIALS AND METHODS**

71

72 *Water samples and organisms*

73

74 Waters used for oxidant loading included artificial seawater (ASW; Red Sea Salt
75 and deionized water, 30 ppt), and samples collected from the Cape Fear River Estuary,
76 NC (33 ppt), Shannon Point, WA (Shannon Point Marine Center, 32 ppt), Vallejo, CA
77 (California Maritime Academy, 13 ppt), and Yaquina Bay, OR (Northwest Aquatic
78 Sciences, 31 ppt). Total dissolved organic carbon content (DOC) of the site waters was
79 analyzed using a Shimadzu TOC-5050A total organic carbon analyzer with an ASI-
80 5000A autosampler (Kyoto, Japan). Total dissolved nitrogen (TDN) of the site waters
81 was measured using an Antek 9000 series total dissolved nitrogen analyzer (Houston,
82 Texas). Juvenile mysid shrimps (*Americamysis bahia*, 3 to 4 d), sheepshead minnows
83 (*Cyprinodon variegatus*, 3 to 4 d) and topsmelt (*Atherinops affinis*, 11 to 12 d) were
84 obtained from Aquatic Biosystems (Fort Collins, CO). All organisms were shipped
85 overnight to the testing laboratory and acclimated for 24 h before test initiation.

86

87 *Oxidant loading rates*

88 Ozone was dispensed at a concentration of 23.2 mg O₃/L/min using a model SC-
89 10 ozone generator (Nutech 03 Inc., McLean, VA) to seawater placed into three, 20-L
90 glass aquaria. Flow to each chamber was maintained at 40.5 mL O₃/min using N012-10

91 flow meters with sapphire floats (Gilmont Instruments, Barrington, IL). Ozone was
92 distributed to the chambers using Kynar tubing and ozone tolerant diffusers (Aquatic
93 Ecosystems). A control 20-L chamber received compressed air at 40.5 mL/min.

94 To characterize the ozone loading kinetics for each seawater type, total residual
95 oxidant (TRO) measurements were obtained from each chamber at 0.5 h intervals from 0
96 to 6 h with a Pocket Colorimeter using a *N,N*-diethyl-*p*-phenylenediamine/potassium
97 iodide (DPD/KI) indicator (Hach, Loveland, CO). This procedure is equivalent to
98 USEPA Method 330.5 for wastewater and Standard Method 4500-Cl₂ G for drinking
99 water. TRO concentration (mg/L) measurements were calculated and expressed as
100 equivalent concentrations of bromine (Br₂, 1 mol Cl₂ = 0.44 mol Br₂) and averaged for
101 the three chambers treated with ozone.

102

103 *Oxidant toxicity*

104 For each toxicity experiment with *Americamysis bahia*, waters were treated with
105 ozone until a TRO concentration of approximately 2 mg/L as Br₂ was reached. Serial
106 dilutions with non-ozonated water were achieved by mixing ozonated and non-ozonated
107 waters. Concentrations used were 100 % (ozonated water only), 50 %, 25 %, 12.5 %,
108 6.25 %, and 0 % (non-ozonated water only). TRO measurements were taken from each
109 dilution to verify the accuracy of the concentrations. Four 250 mL replicates of each
110 concentration in 500 mL beakers were used for each test and maintained at 20°C in an
111 environmental chamber. Ten juvenile *Americamysis bahia* were used in each replicate,
112 and were fed 0.1 mL *Artemia franciscana* at test initiation and twice daily. The mysids
113 were examined at 24 h for mortality, and dead organisms were removed. Surviving

114 organisms were again examined for mortality at 48 h after the beginning of the test.
115 Conductivity and ammonium concentrations of the water were measured at test initiation,
116 and pH, dissolved oxygen (DO), temperature, and salinity were monitored daily. All
117 median-lethal concentrations (LC50) and 95% confidence intervals were calculated at 24
118 and 48 h using the Trimmed Spearman-Kärber method (e.g. Hamilton et al. 1977).

119 Toxicity tests with *Atherinops affinis* and *C. variegatus* were conducted similarly
120 to *Americamysis bahia* using ASW and water from Yaquina Bay, OR. However,
121 previous experiments suggested both organisms were more sensitive to ozonated
122 seawater (Gensemer et al. 2005) and as a result lower TRO concentrations were used for
123 each organism (*Atherinops affinis*: 100 % = approximately 1 mg/L as Br₂; *C. variegatus*,
124 Yaquina Bay sample, 100 % = 0.25 mg/L as Br₂). Both *Atherinops affinis* and *C.*
125 *variegatus* were tested in 400 mL replicates and fed 0.2 mL of *Artemia franciscana* twice
126 daily. Experiments with *C. variegatus* were conducted for 96 h in accordance with EPA
127 protocol 600/4-90/027F.- After 48 h the water in each replicate was replaced with new
128 water that had been diluted to the same TRO concentrations, and LC50 values were
129 calculated at 24 h intervals from 0 to 96 h.

130 In addition to the toxicity experiments described above, similar tests were
131 conducted in which each dilution series was treated with a solution of 0.005M sodium
132 thiosulfate (Na₂S₂O₃) before organisms were added. TRO measurements were taken after
133 the sodium thiosulfate addition to confirm that oxidants were reduced. Reduction
134 experiments were conducted using ASW and waters from Cape Fear, NC, Shannon Point,
135 WA, and Yaquina Bay, OR on both *Americamysis bahia* and *C. variegatus* employing the
136 same dilution methods as previously described for *Americamysis bahia* toxicity tests (100

137 % = approximately 2 mg/L as Br₂). Reduction experiments with *Atherinops affinis* were
138 limited to addition of sodium thiosulfate to the highest TRO concentration used in
139 toxicity tests (approximately 1 mg/L as Br₂). In all cases, four control replicates were
140 included in tests in which non-ozonated water received the same amount of sodium
141 thiosulfate as the highest concentration.

142

143 **RESULTS**

144 *Oxidant loading rates*

145 DOC concentrations in the site waters ranged from 717.6 µg/L (Shannon Point,
146 WA) to 2382 µg/L (Vallejo, CA). TDN concentrations ranged from 173.6 µg/L (Yaquina
147 Bay, OR) to 481.2 µg/L (Vallejo, CA; Table 1). With the exception of the Vallejo, CA
148 sample, ozonation of all waters caused a steady increase in TRO concentration of
149 approximately 2 mg/L/h during the first 3 h of treatment (Figure 1 a-e). Saturation
150 appeared to occur after 6 h, with TRO concentrations ranging from approximately 8 to 10
151 mg/L as Br₂.

152 In contrast, TRO concentrations in the Vallejo, CA water sample increased more
153 slowly than ASW or the other site waters, with measurements of 1 mg/L after 1 h and 3
154 mg/L as Br₂ after 2 h. The maximum occurred after 3 h at approximately 4 mg/L as Br₂.

155

156 *Oxidant toxicity*

157 LC50 values for *Americamysis bahia* after 24 h in tests using ASW and each of
158 the site waters were not significantly different (i.e., 95 % confidence intervals all
159 overlapped), ranging from 0.55 to 0.62 mg TRO/L as Br₂ (Table 2). After 48 h, LC50

160 values were also similar, ranging from 0.34 to 0.46 mg TRO/L as Br₂, although mysids in
161 waters from Shannon Point, WA (LC50 = 0.46 mg/L as Br₂) were slightly less sensitive
162 than those in water from Cape Fear, NC (LC50 = 0.34 mg/L as Br₂). 48 h data was not
163 available for tests using water from Vallejo, CA due to control failure. In all 48 h tests,
164 higher mortality was observed after 48 h than after 24 h.

165 Toxicity tests with *C. variegatus* in ASW resulted in 100 % mortality at TRO
166 concentrations \leq 0.23 mg/L as Br₂ after 24 h (Table 3; LC50 = 0.17 mg/L as Br₂).
167 However, tests conducted using water from Yaquina Bay, OR (100 % = 0.23 mg/L as
168 Br₂) yielded a comparable LC50 value to that observed in ASW (0.18 mg/L as Br₂ at 24-
169 96 h). Toxicity tests in ASW and water from Yaquina Bay, OR, showed that *Atherinops*
170 *affinis* were less sensitive than *C. variegatus* over 24 and 48 h in both cases (LC50 = 0.34
171 and 0.26 mg/L as Br₂, respectively).

172 All toxicity tests conducted with *Americamysis bahia*, *Atherinops affinis*, and *C.*
173 *variegatus* after the addition of sodium thiosulfate resulted in no mortality at any tested
174 TRO concentration (Tables 2 and 3).

175

176 **DISCUSSION**

177 Variations in salinity, DOC, and TDN of seawater are important factors to include
178 in measurements of oxidant formation over time during ozone treatment. The amount of
179 available bromide and organic materials with which ozone can react could impact the
180 TRO saturation limit of the seawater and reduce rates of oxidant loading. Our results
181 indicated that salinity had a direct influence on TRO saturation from ozonation. Waters
182 of comparable salinities (ASW, Cape Fear, NC, Shannon Point, WA, Yaquina Bay, OR;

183 salinity 30-33 PPT) all saturated between 8 and 10 mg TRO/L as Br₂ after 6 h, and water
184 from Vallejo, CA (13 PPT) saturated at approximately 4 mg/L as Br₂ after 3 h.

185 Although water from Vallejo, CA had the highest DOC and TDN of all site
186 waters, these water quality parameters did not appear to have any effect on the highest
187 concentration of TRO reached. The DOC content of water from Cape Fear, NC was
188 similar to that of the Vallejo, CA sample (2186.4 and 2382 µg/L, respectively) but the
189 Cape Fear, NC water showed a comparable rate of oxidant accumulation to the water
190 with the lowest DOC content tested (Shannon Point, WA, 717.6 µg/L). TDN content
191 also varied among the site waters with salinities above 30 ppt (173.6 – 354.3 µg/L).
192 However, Perrins et al. (unpublished data) did suggest that increased dissolved organic
193 carbon content can significantly reduce oxidant formation with ozonation. The apparent
194 discrepancy is likely due to the difference in ozone loading rates for the two tests.
195 Perrins et al. used a much slower rate than the experimental design employed in the
196 present study. Higher loading rates could oxidize DOC more efficiently and therefore not
197 show the oxidant demand by the DOC that was observed by Perrins et al.

198 Toxicity experiments showed the effect of TRO from ozone treatment on
199 *Americamysis bahia* was not greatly affected by the type of water used during testing.
200 There was no significant difference between ASW and any of the site waters after 24 h
201 (LC50s = 0.55 - 0.62 mg TRO/L as Br₂), and LC50 values were only slightly higher in
202 water from Shannon Point, WA (0.46 mg/L as Br₂) than ASW or the other site waters
203 (0.34 to 0.42 mg/L as Br₂) after 48 h. Thus, the effective dose of ozone-produced
204 oxidants in seawater will likely be the same regardless of the site-specific chemical ozone
205 demand properties of individual seawater samples.

206 Mortality in *A. bahia* was greater after 48 h than after 24 h, in accordance with the
207 observations of Gensemer et al. (2005) that oxidants from ozonation can persist in
208 solution for at least 2 d, and that mortality can take 1 to 2 d to be expressed even after
209 short-term exposures. Mysids exposed to oxidants in water from Vallejo, CA
210 experienced a control failure after 48 h that prevented determination of an LC50 value,
211 but this may have been due to the lower salinity of the water.

212 Both *Atherinops affinis* and *C. variegatus* were more sensitive to ozone-produced
213 oxidants than *Americamysis bahia* (average LC50s = 0.30 mg/L as Br₂ at 48 h and 0.18
214 mg/L at 96 h, respectively). As mentioned above, while *Atherinops affinis* was less
215 sensitive than *C. variegatus* in both ASW and water from Yaquina Bay, OR, *Atherinops*
216 *affinis* had a faster median lethal time (LT50) than *C. variegatus* (Gensemer et al. 2005).
217 Fish may experience faster mortality due to increased contact with oxidants via their gills
218 (Richardson et al. 1983). In fact, several *Atherinops affinis* and *C. variegatus* died in
219 lethal TRO concentrations before test preparation was completed (≤ 0.5 h).

220 The addition of sodium thiosulfate (Na₂S₂O₃) to solutions in toxicity tests
221 removed all of the TRO and was effective in preventing mortality from occurring in any
222 species in this study. Successful incorporation of chemical reductants such as Na₂S₂O₃ in
223 ballast water treatment protocols could provide a reliable method of oxidant elimination
224 in addition to sunlight degradation of oxidants after ballast water release (Treinin 1970;
225 Macalady et al. 1977; Subhani and Lodhi 1980; Klaning and Wolff 1985; Amichai et al.
226 1989).

227 The findings of both the present study and those of Gensemer et al. (2005)
228 suggest that although TRO saturation in brackish and marine waters may take several

229 hours to occur during ozone treatment using gas diffusers, concentrations effective at
230 eliminating planktonic invertebrates and fish may be achieved in ≤ 1 h with sufficient
231 ozone delivery rates (20 to 25 mg O₃ /L /h). All pelagic organisms used in both studies
232 were susceptible to TRO concentrations ≤ 0.62 mg/L as Br₂, in addition to other
233 organisms including phytoplankton (LC50s = 0.04 – 0.20 mg TRO/L as Br₂; Toner and
234 Brooks 1975) crab larvae (LC50s = 0.04 – 0.06 mg TRO/L as Br₂; Toner and Brooks
235 1975) and striped bass larvae (LC50s = 0.04 – 0.07 mg TRO/L Br₂ (Hall et al. 1981).[†]
236 Thus, delivering at least 0.5 – 1 mg TRO/L as Br₂ should be sufficient in most cases to
237 eliminate a wide range of pelagic organisms from marine ballast water. Higher TRO
238 concentrations may be necessary for benthic organisms (Gensemer et al., 2005) that limit
239 exposure to oxidants by means of their physiology or by remaining in sediment carried by
240 ballast tanks.

241 Toxicity data from the present studies and from the literature could also be used to
242 determine what oxidant levels are environmentally safe upon ballast discharge. Risk-
243 based decisions would need to be made to set maximum acceptable discharge
244 concentrations, and potentially take into account dilution or degradation following
245 discharge into receiving waters. While mixing and sunlight exposure are known to
246 diminish TRO concentrations, complete removal of TRO may be difficult in constrained
247 harbor spaces or areas with vulnerable indigenous species. Our results show that
248 chemical treatments such as Na₂S₂O₃ may provide a fast and effective means of reducing
249 TRO concentrations without the risk of endangering organisms in the vicinity of the
250 vessel undergoing ozonation.

[†] While LC50 values expressed in these cases were originally reported as mg Cl₂/L, we have converted them to mg Br₂/L.

251

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Table 1. Seawater characteristics. Analyses of total dissolved organic carbon and total dissolved nitrogen included three replicate measurements per sample.

Water	Salinity (ppt)	Total dissolved organic carbon ($\mu\text{g/L}$) (St Dev)	Total dissolved nitrogen ($\mu\text{g/L}$) (St Dev)	Ammonia (mg/L)
Artificial Seawater (ASW)	30	n/a	n/a	< 1
Cape Fear, NC	33	2186.4 (\pm 56.6)	215.5 (\pm 3.1)	< 1
Shannon Point, WA	32	718.0 (\pm 179.6)	354.3 (\pm 4.5)	< 1
Vallejo, CA	13	2382 (\pm 124.1)	481.2 (\pm 3.1)	< 1
Yaquina Bay, OR	31	1069.1 (\pm 68.2)	173.6 (\pm 4.9)	< 1

Table 2. Median-lethal concentrations (LC50) for juvenile *Americamysis bahia* after 24 and 48 h of exposure to ozonated seawater.

C.I. = Confidence Interval. TRO = total residual oxidant. ^ano C.I. available.

Site Water	24 h LC50 (95% C.I.) mg TRO/L as Br ₂	48 h LC50 (95% C.I.) mg TRO/L as Br ₂	LC50 with 0.005 M <i>Na₂S₂O₃</i> addition
Artificial seawater (ASW)	0.58 (0.52-0.64)	0.42 (0.37-0.48)	<i>No mortality</i>
Cape Fear, NC	0.55 (0.49-0.61)	0.34 (0.30-0.37)	<i>No mortality</i>
Shannon Point, WA	0.61 (0.56-0.66)	0.46 (0.39-0.53)	<i>No mortality</i>
Vallejo, CA	0.62 ^a	n/a	<i>No mortality</i>
<i>Yaquina Bay, OR</i>	<i>0.58 (0.51-0.66)</i>	<i>0.37 (0.33-41)</i>	<i>No mortality</i>

Table 3. Median-lethal concentrations (LC50) for juvenile *Atherinops affinis* and *Cyprinodon variegatus* after 24, 48, 72, and 96 h of exposure to ozonated seawater.

Species	Seawater	24 h LC50 (95% C.I.) mg TRO/L as Br ₂	48 h LC50 (95% C.I.) mg TRO/L as Br ₂	72 h LC50 (95% C.I.) mg TRO/L as Br ₂	96 h LC50 (95% C.I.) mg TRO/L as Br ₂	LC50 with 0.005 M Na ₂ S ₂ O ₃ addition
<i>Atherinops affinis</i>	Artificial Seawater (ASW)	0.34 (0.32- 0.36)	0.34 (0.32- 0.36)	n/a	n/a	No mortality
<i>Atherinops affinis</i>	Yaquina Bay, OR	0.26 (0.25- 0.27)	0.26 (0.25- 0.27)	n/a	n/a	No mortality
<i>Cyprinodon variegatus</i>	Artificial Seawater (ASW)	0.17 ^a	0.17 ^a	0.17 ^a	0.17 ^a	No mortality
<i>Cyprinodon variegatus</i>	Yaquina Bay, OR	0.18 (0.17- 0.19)	0.18 (0.17- 0.19)	0.18 (0.17- 0.18)	0.18 (0.17- 0.18)	No mortality

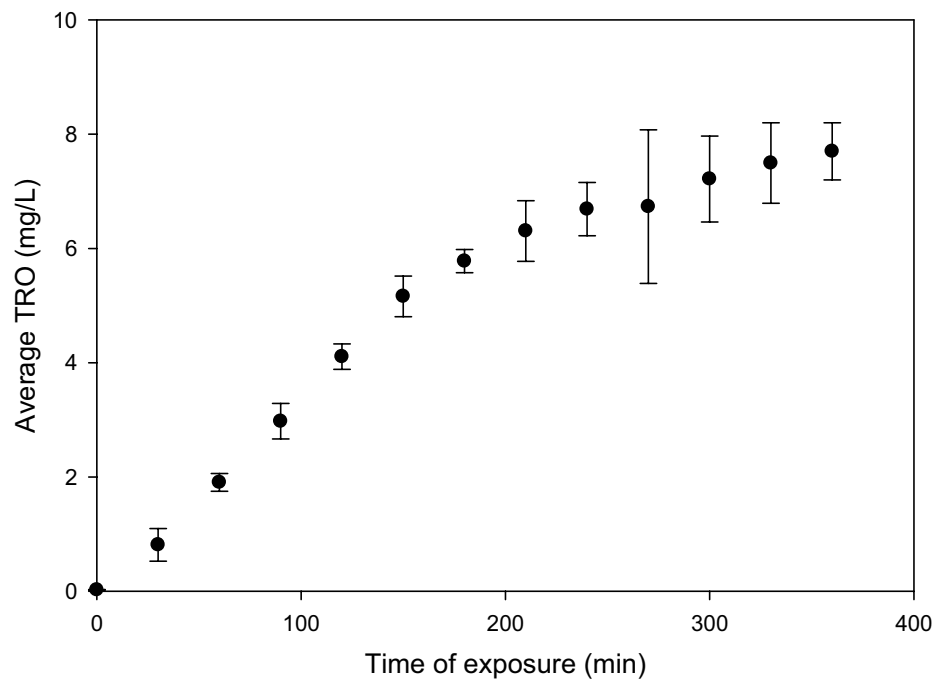
C.I. = Confidence Interval. TRO = total residual oxidant. ^aNo C.I. available.

List of Figures

Figure 1. Total residual oxidant (TRO, as Br₂) measurements over 6 h during ozone treatment in a) artificial seawater (ASW) and waters from b) Cape Fear River Estuary, NC c) Shannon Point, WA d) Vallejo, CA and e) Yaquina Bay, OR. Ozone was dispensed at a concentration of 23.2 mg O₃/L/min to three replicate 20 L chambers for each of the waters.

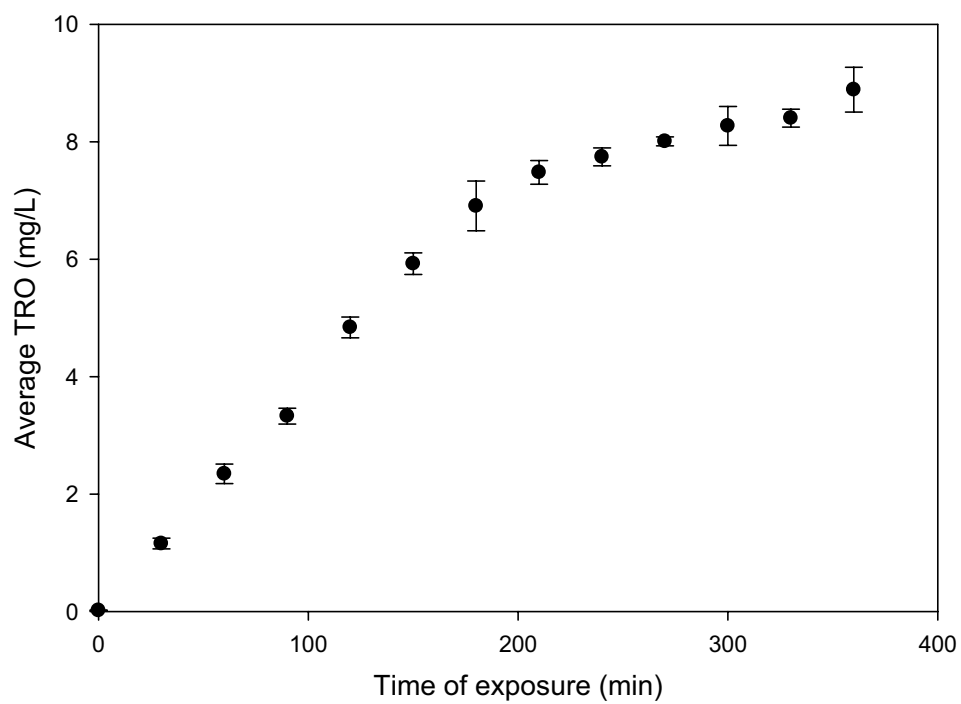
A

ASW



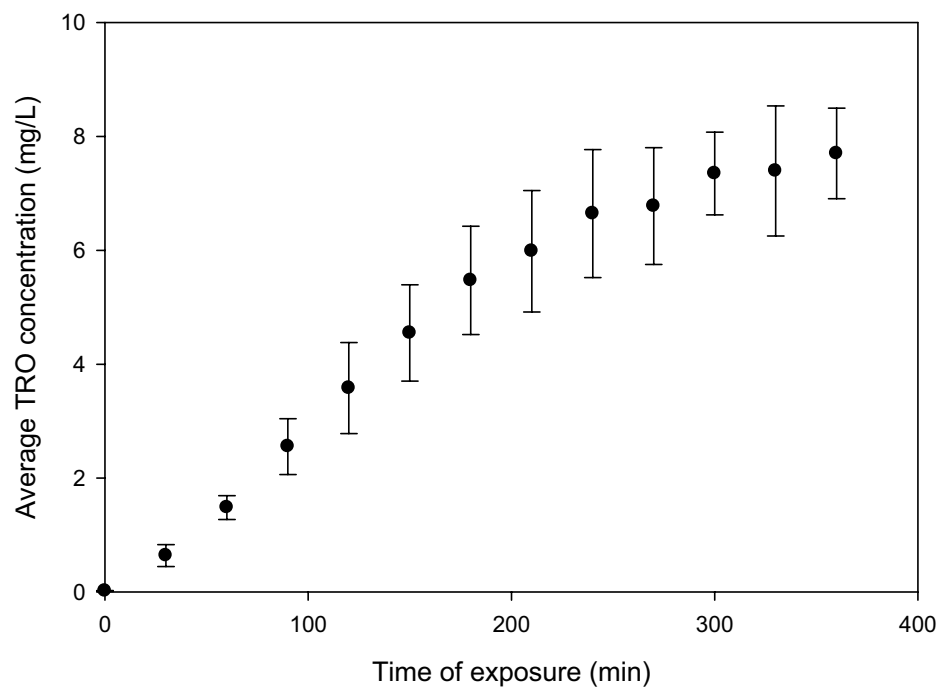
B

Cape Fear NC



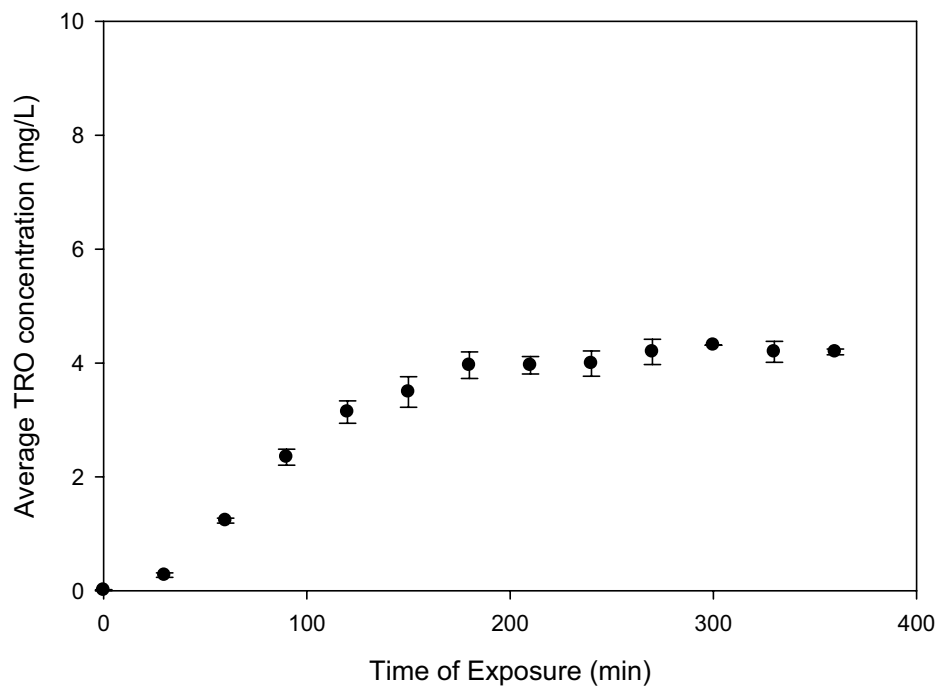
C

Shannon point



D

Vallejo CA



E

Yaquina Bay OR

