

1 **Estuarine zooplankton responses to flood pulses and a hypoxic blackwater**
2 **event**

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15

16 **Abstract**

17 Flood pulses in estuaries following storms and rainfall events, are short-lived but important
18 moments for a range of ecosystem processes including the delivery of resources and
19 promoting productivity. Conversely some flood pulses can lead to adverse outcomes such as
20 poor water quality conditions. The aim of this study was to determine how zooplankton
21 abundance and community composition responded to flood pulses and if they responded
22 differently during a flood pulse that led to hypoxic conditions. To do this we conducted a
23 two-year observational study in the Hunter River estuary, Australia, monitoring zooplankton
24 communities monthly for a period that covered two major flood pulse events including one
25 that caused widespread hypoxia and a major fish kill. The results showed zooplankton
26 abundance was higher or no different following the 2012 flood when dissolved oxygen
27 remained stable compared to pre-flood conditions. During the 2013 flood when hypoxia
28 occurred the abundance of copepods, nauplii and rotifers were at their lowest for the study
29 period. Zooplankton assemblages were not distinctly different following the 2012 flood pulse
30 compared to the pre-flood period but were different during the hypoxic 2013 flood, though
31 quickly returned to resemble pre-flood conditions in the proceeding months. The study
32 provides useful insights in how zooplankton populations may respond to flood events and
33 recover after hypoxic conditions in estuarine ecosystems.

34 **Introduction**

35 Flood events play an important role in estuarine ecosystems. These events may be
36 conceptualised freshwater inflows that rise to or above the banks of the upstream river
37 (Puckridge et al. 1998). Flood pulses lead to increased hydrological connectivity between
38 aquatic and terrestrial environments, and result in pulses of organic matter entering estuaries
39 (Ford et al. 2005). Whilst short lived, these ‘hot moments’ are intense periods of
40 biogeochemical cycling (McClain et al. 2003) and can account for a sizeable portion of
41 annual organic carbon loads delivered to coasts (Hitchcock and Mitrovic 2015; Raymond and
42 Saiers 2010).

43 Floods have long been conceptualised as a crucial part of food webs in many aquatic
44 environments (Junk et al. 1989). The organic carbon and nutrients delivered during these
45 events constitutes a major resource pulse and can subsidise the growth of heterotrophic
46 microbes, and in-turn protists and mixotrophic organisms (Lennon and Cottingham 2008).
47 Increased biomass at these lower levels of the food web can be consumed by zooplankton
48 which form a crucial part of aquatic food webs as important intermediaries between lower
49 trophic levels and fish (Murphy et al. 2012). Meta-analysis of a wide range of studies
50 examining resource pulses have shown that estuarine and marine communities regularly
51 respond to resource pulses with successional booms and busts in productivity (Nowlin et al.
52 2008; Yang et al. 2010). There is also tight coupling between zooplankton and the other
53 biogeochemical changes associated with floods (Sugimoto and Tadokoro 1997). Floods can
54 also act as broader disturbance events and have the potential to rapidly alter the composition
55 of planktonic communities through changes in hydrologic forcing (Kobayashi et al. 1998),
56 salinity (Primo et al. 2009) and by altering top-down relations (Hoover et al. 2006; Scheffer
57 et al. 2008).

58 Food web responses to flood pulses in estuaries are dependent on the timing, magnitude, and
59 frequency of rainfall events (Hitchcock et al. 2016a; Hitchcock et al. 2016b), catchment
60 characteristics such as soil types, land use, and geomorphology (Dodson et al. 2005). During
61 extreme flood events the organic matter subsidy delivered to estuaries may be so large that
62 heterotrophic metabolism and respiration quickly outpaces photosynthesis and reaeration
63 leading to hypoxia (Wong et al. 2010). Under these circumstances the ability of secondary
64 consumers such as zooplankton to utilise enhanced microbial or primary production may be
65 stymied (Marcus 2001). Under worse case scenarios prolonged hypoxic conditions may lead
66 to fish kills and the death other animals.

67 Research on how zooplankton may respond to hypoxia associated with flood events in
68 estuaries is rare. There is however a great deal of knowledge related to more persistent low-
69 oxygen conditions. Research on zooplankton in the oxygen minimum zones in the oceans,

70 have found zooplankton are generally not present in water with $< 0.29 \text{ mg L}^{-1}$ dissolved
71 oxygen (Marcus 2001). In estuaries and coasts that experience persistent oxygen
72 stratification, copepods are less likely to be found below the oxycline, and may alter vertical
73 migration patterns to avoid such areas (Cervetto et al. 1995; Roman et al. 1993).
74 Experimental studies have found wide ranging effects of low oxygen conditions on
75 zooplankton including increased mortality, lowered growth, and reduced copepod egg
76 production (Sedlacek and Marcus 2005; Stalder and Marcus 1997).

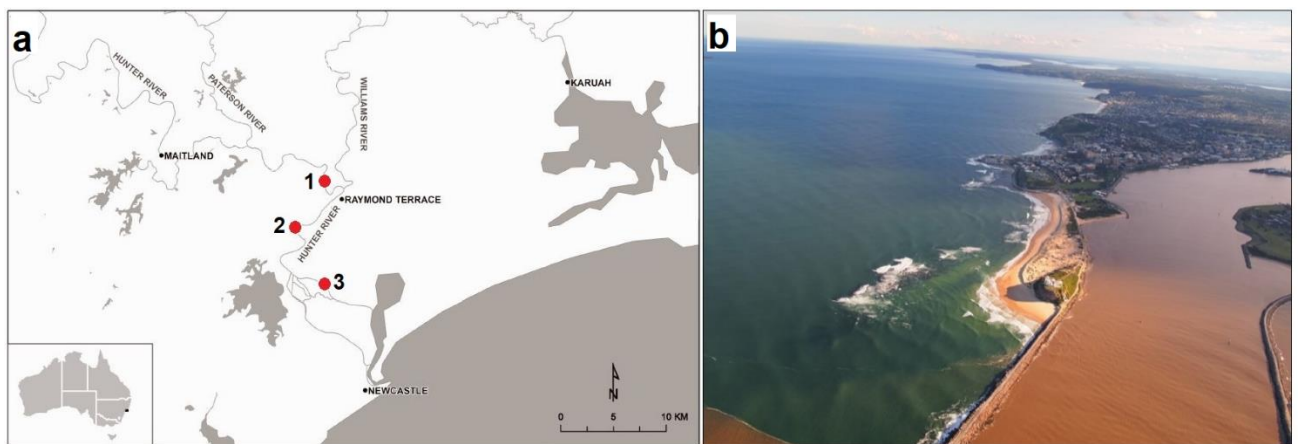
77 It is likely zooplankton communities will undergo major changes following flood pulses in
78 estuaries. However, it remains unclear how zooplankton abundance and community structure
79 may vary in response to conditions associated with different flood pulses. In particular, will
80 zooplankton respond differently during flood pulses that lead to hypoxia compared to those
81 that do not? The aim of this study is to understand how estuarine zooplankton responded to
82 flood pulse events. To address this aim we conducted a two-year observational study of
83 zooplankton populations in the Hunter River Estuary, Australia, during which time two major
84 flood pulse events occurred. Flood pulses occurred at similar times of consecutive years, with
85 one leading to hypoxic conditions. We hypothesised that a) zooplankton abundance would be
86 higher following flood pulses but not when hypoxia is occurring, and b) that zooplankton
87 community composition would be different following flood pulses compared to low flow
88 conditions and c) following hypoxia zooplankton would take longer to resemble pre-flood
89 community composition. These hypothesis will be tested by comparing zooplankton
90 abundance and community composition before and after flood-pulse events.

91 **Materials and methods**

92 **Study location and sampling stations**

93 The Hunter estuary, NSW Australia is a wave dominated barrier estuary with a permanently
94 trained open mouth. It drains a large catchment of $21,367 \text{ km}^2$, where the dominant land use
95 is agriculture, alongside areas of mining, small urban centres, and forest. The tidal limit of the

106 estuary is Maitland, and its major estuarine tributaries are the Patterson and Williams Rivers.
107 Land use in the upper estuary region consists of extensive floodplains used primarily for
108 grazing, and in the lower estuary there are wetlands, industry, and urban areas. Discharge in
109 the Hunter River is regulated by a series of large to medium dams in the middle catchment,
110 and a weir on the Williams River. Three sampling stations in the estuary were used during
111 this study (Fig. 1 A). These stations were chosen to represent different salinity zones; during
112 low inflows the salinity at stations 1, 2, and 3, was 12, 23, and 36 respectively. Station 1 is
113 23 km downstream of the tidal limit and 10 km upstream from Station 2, which is 12 km
114 upstream from Station 3, which is 18km upstream from the mouth of the estuary.



105
106 **Figure 1.** Hunter River estuary, NSW, Australia. a) Sampling stations 1, 2, and 3, b)
107 Photograph showing the mouth of the estuary near Newcastle taken two days after the peak
108 of the flood event on 4 March 2013 that led to extensive hypoxia within the estuary (photo
109 courtesy NSW Department of Industry).

110 **Sampling procedures**

111 Discharge was obtained from two gauging stations operated by the NSW Department of
112 Primary Industries (waterinfo.nsw.gov.au), the Hunter River at Greta, and Patterson River at
113 Gostwyck. A combined daily discharge from these stations is used to represent inflow to the
114 estuary. Discharge on the Williams River was excluded due to the operation of a weir that
115 regulates inflow to the estuary. Daily water temperatures were recorded by the Hunter River
116 at Greta gauging station. Sampling occurred approximately and was conducted by boat

117 sampling up the most downstream station first within 2 hours of high tide. Conductivity,
118 dissolved oxygen, pH and site specific temperature were measured with a Hydrolab Surveyor
119 4 and MS5 Sonde probe. Depth profiles were recorded at 1 m intervals between the surface
120 and bottom at all stations. As all stations were well mixed at all times only surface data is
121 presented here (depth profiles have been included as Supplementary material.). Salinity was
122 calculated using the electrical conductivity method (APHA 2005). Turbidity samples were
123 measured using a Hach 2100 turbidimeter.

124 Triplicate samples for dissolved organic carbon (DOC), nitrate/nitrite (NO_x), ammonia
125 (NH₄), soluble reactive phosphorus (SRP), silica (Si), and chlorophyll *a* were taken by hand
126 at 20 cm below the water surface. All nutrient samples were collected in 50ml PET bottles
127 stored on ice before being frozen until analysis. Samples for dissolved nutrients were filtered
128 in the field with 0.45 µm polycarbonate filters. Organic carbon samples were analysed in the
129 laboratory by the High Temperature Combustion Method (APHA 2005). Nitrogen and
130 phosphorus samples were analysed using a segmented flow analyser (OI Analytical Model
131 FS3100) according to standard methods (APHA 2005). Samples for chlorophyll *a* were
132 determined by filtering 500 ml of water onto GF/F filters. Filters were frozen until
133 subsequent determination by Standard Methods (APHA 2005) using the grinding technique
134 and acetone as a solute with correction for phaeophytin. A detection limit of 1 µg L⁻¹ was
135 used for chlorophyll *a* analysis.

136 Samples for zooplankton enumeration were taken in duplicate at each station via vertical
137 tows with a 37 µm plankton net and preserved with >50% ethanol. The volume of water was
138 determined accounting for the depth of water filtered, diameter of net, and assumed filtration
139 efficiency of 75%. In the laboratory samples were homogenised and split with 50% of each
140 sample reserved for future analysis. Zooplankton density (individuals m³) was estimated by
141 counting consecutive aliquots in a Sedgewick rafter counting chamber until 100 specimens of
142 each (copepods, rotifers, nauplii) taxon were counted or until the entire subsample counted.
143 Zooplankton were identified to highest taxonomic level feasible via keys of Shiel (1995) and

144 Swadling (2013). We have only included data on copepods, nauplii, and rotifers; other
145 zooplankton present in much smaller numbers are listed in supplementary material.

146 **Statistical analysis**

147 Zooplankton assemblage data was analysed to compare changes through time at each
148 sampling station using e-Primer version 6. Mean abundance for all common genera of
149 copepods and rotifers, were included as well as nauplii. Data was first transformed to
150 presence/absence and a similarity matrix were created for each station using the Bray-Curtis
151 index. Multidimensional scaling plots were created to illustrate changes in zooplankton
152 assemblage structure through time. We applied labels to sampling times in the MDS to
153 represent the two major summer inflow events that occurred in February 2012 and March
154 2013 (labelled flood), the two months after these events (post-flood), and then all others times
155 labelled low-flow. We conceptualised the post flood period as a distinct two-months allowing
156 two to three generations of copepod genera to re-establish if they underwent major changes as
157 a result of the flood event as hypothesised.

158 Redundancy analysis (RDA) was performed using CANOCO ver 4.5 to analyse the
159 environmental factors that influenced zooplankton density and assemblage structure (Braak
160 and Šmilauer 2002). Separate analysis was performed for major taxa of mesozooplankton
161 (copepodites and adult copepods) and microzooplankton (nauplii and rotifers) at each
162 sampling station. All samples and variables were subjected to Shapiro-Wilk's normality test
163 and all samples with a non-normal distribution were log transformed ($\ln x+1$). The
164 explanatory environmental variables were selected using automatic forward selection. To
165 ensure models were not over-fitted we eliminated factors with variable inflation factors >10
166 and where there was strong co-linearity. We tested discharge measures of Q (discharge on
167 day of sampling) and then average antecedent measures (average discharge over the
168 preceding number of days) for 5, 10, 20, 30 and 40 days. The measure that provided the most
169 explanatory power was included in the model. Discharge, all nutrient and physio-chemical

170 parameters that were measured were tested. For microzooplankton, copepod abundance was
171 included as they are potential predators exerting top-down control. For mesozooplankton,
172 rotifers were included as a potential prey item. Monte-Carlo permutation (999 permutations
173 without restriction) was used to test the significance of canonical axis and environmental
174 variables on zooplankton communities.

175 **Results**

176 **Freshwater inflows and hypoxic event**

177 Freshwater discharge to the Hunter estuary was low for most of 2012 and 2013 with the
178 exception of two higher flow periods occurring in February-March 2012 and 2013 which
179 culminated in major floods with daily discharge rates in the 99th percentile (Fig. 2). The first
180 flood peaked on the 6th March 2012 at mean daily discharge rate of 646 m³ s⁻¹, whilst the
181 second flood event peaked on the 4th March 2013 with discharge rate of 1039 m³ s⁻¹. The
182 cumulative volume discharged for the main part of the flood events (including the rising limb
183 for two days before the peak of flow and falling limb for seven days after peak) was over
184 two-fold larger for the 2013 flood at 431,925 ML compared to 2012 flood at 192,948 ML.
185 Both flood events resulted in over bank flooding on the on the estuarine floodplains, though
186 floodwaters persisted for a number of weeks following the 2013 flood. Both flood events
187 were preceded by smaller peaks in flow in the four weeks prior to the flood events, however
188 these events did not result overbank flooding.

189 The flood events delivered high loads of organic carbon and nutrients to the estuary.
190 Concentrations of DOC and SRP, and measurements of turbidity were all at their highest in
191 the week during the major flood events (Fig. 2d, f, g). Nitrogen concentrations were more
192 variable, with concentrations of both NO_x and NH₄ relatively low during the March 2013
193 event (Fig. 2e,h). Dissolved oxygen concentrations remained between 60%-120% at all
194 stations at all times with the exception of March 2013 when dissolved oxygen ranged from
195 1.6 – 2.1 % (or 0.13 – 0.17 mg L⁻¹) and was consistently low throughout the water column

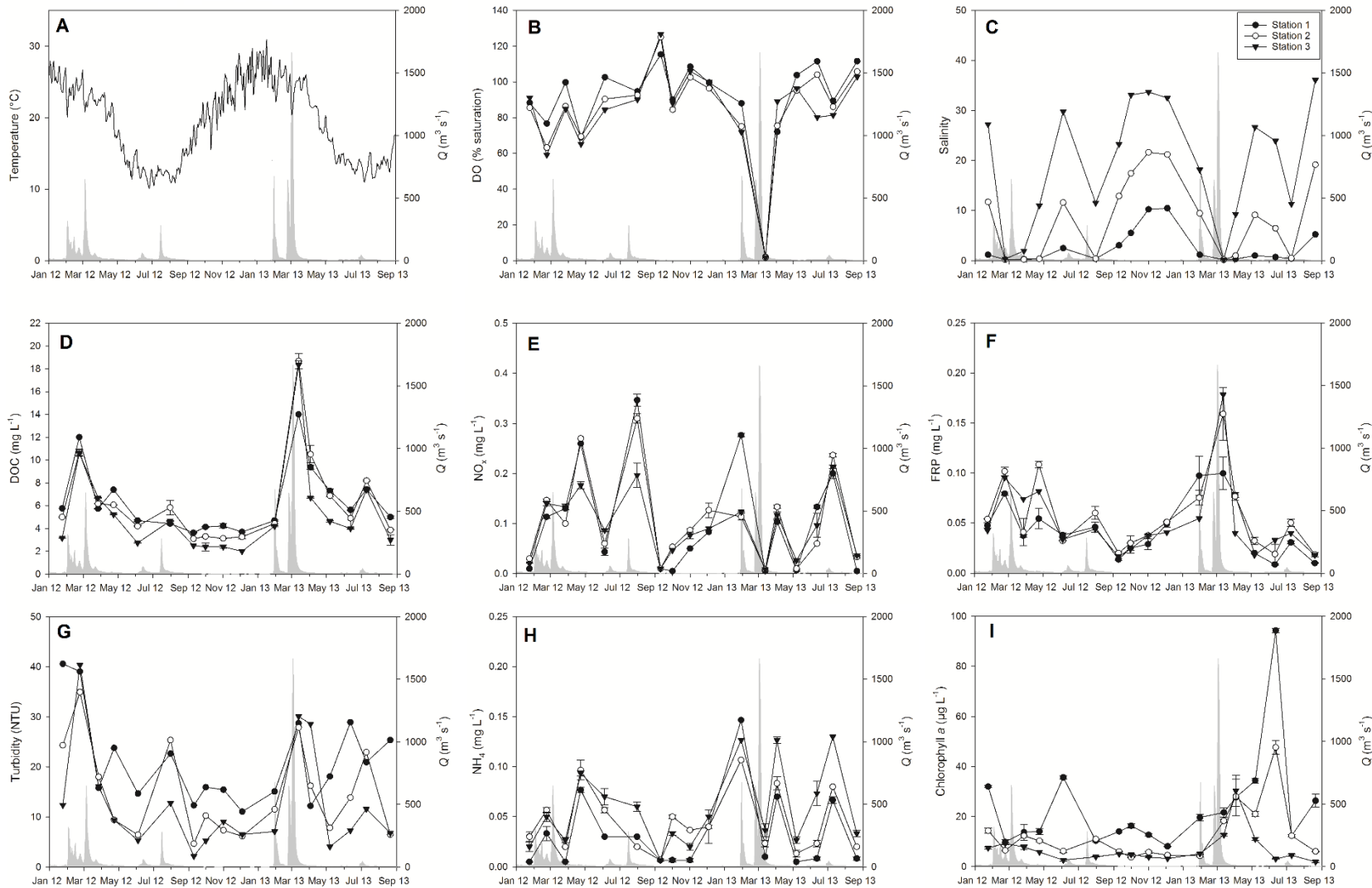


Figure 2: Discharge entering the Hunter estuary October 2012 to October 2013 (right-axis, shaded), along with a) temperature, b) dissolved oxygen (% saturation). C) salinity, D) dissolved organic carbon, E) NO_x , F) filtered reactive phosphorus, G) turbidity, H) ammonia, and I) chlorophyll *a*. Error bars are standard error.

211 (Fig. 2b, Supplementary material). Chlorophyll *a* concentrations remained relatively low at all
212 stations following the February 2012 event, though increased at all stations in the months after the
213 2013 flood event (Fig. 2i). The highest chlorophyll *a* concentrations occurred during May 2013
214 coinciding with blooms of *Cyclotella meneghiniana* at stations 2 and 3 in May 2013 (visible in the
215 zooplankton abundance samples).

216 **Zooplankton abundance and community**

217 Zooplankton abundance varied across the study period and between sampling stations. During the
218 flood in February 2012, where DO remained >60% saturation, zooplankton abundance were higher
219 than during the March 2013 flood pulse. During this period rotifer abundances were slightly higher at
220 station 2, and copepod abundance was higher at station 3 relative to before the flood. In the month
221 after the flood (March 2012) nauplii abundance was relatively high at station 1 and copepod
222 abundance relatively high at station 2. There was however no consistent pattern of response at all
223 sampling stations.

224 During the March 2013 flood, when DO was very low, all major zooplankton groups at all stations
225 recorded their lowest abundance (Fig. 3). During March 2013 calanoid copepodites were absent at all
226 stations and nauplii were absent from Station 3. In the month after, when DO concentrations had
227 recovered, April 2013, nauplii and copepod abundance was high at stations 2 and 3. Rotifer
228 abundance were also higher in the months after the March 2013 flood at stations 1 and 2.

229 High abundances of zooplankton also occurred during low flow periods; nauplii and copepod
230 abundance was higher during the summer months December 2012 and January 2013 at station 2, and
231 copepod abundance was high during January 2012 at station 1. The highest nauplii abundance
232 occurred at station 3 during July 2013.

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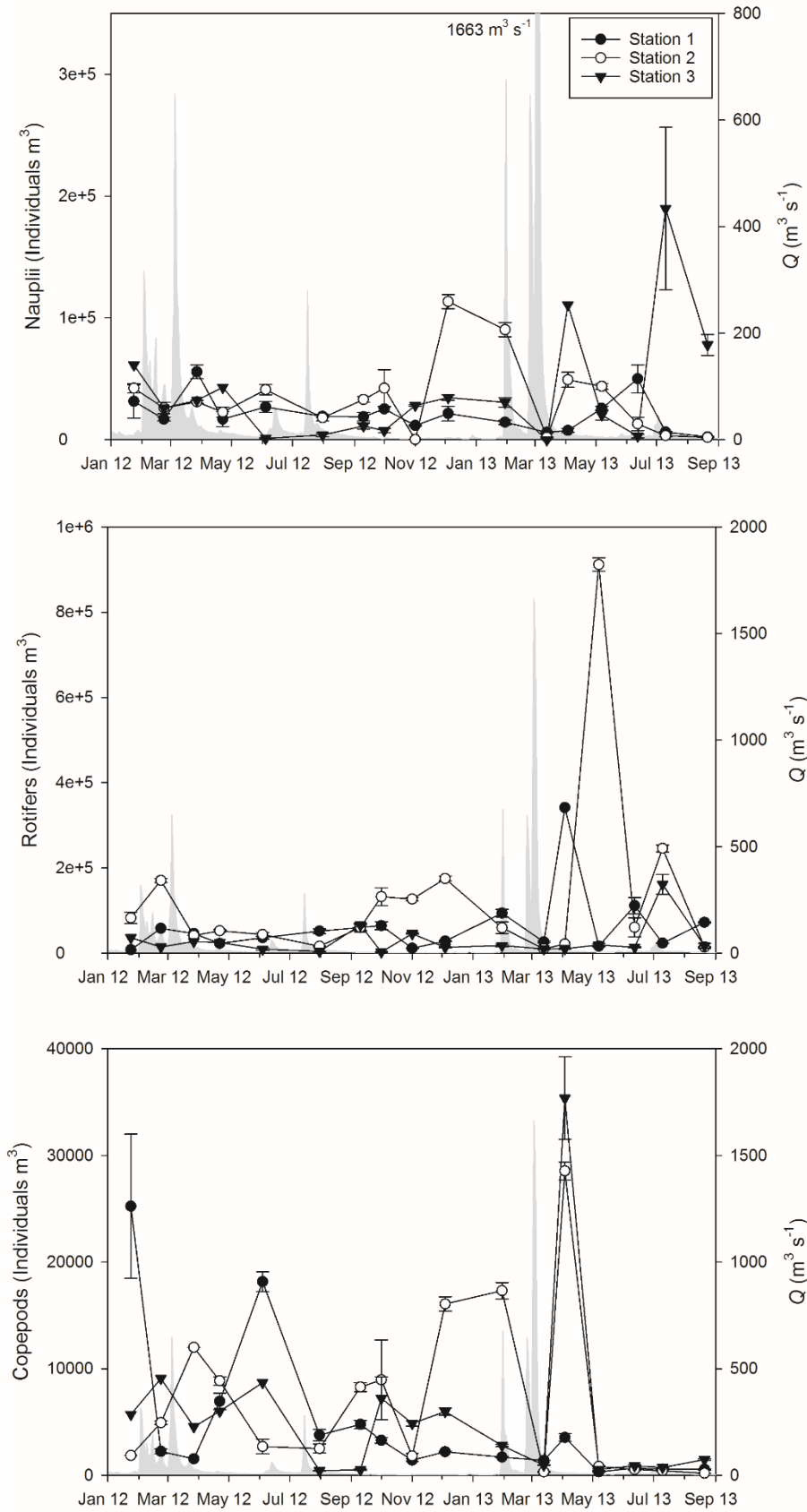


Figure 3. Zooplankton abundance on the Hunter estuary, October 2012 to October 2013. a) Nauplii, b) Rotifers, and c) Copepods. Error bars are standard error.

245 MDS plots based on the presence/absence of zooplankton taxa demonstrated there was no clear
246 distinction in zooplankton communities before after the February 2012 flood. Zooplankton
247 populations were distinctly different at stations 2 and 3 during the March 2013 flood pulse compared
248 to low flow periods. By the next month, April 2013, community composition returned to one similar
249 to the pre-flood low flow period. At station 1 zooplankton communities appeared somewhat distinct
250 during the flood and post-flood period during both events, but was less distinct than the differences
251 at station 2 and 3.

252

253 RDA for meso- and microzooplankton at each site demonstrated that different environmental factors
254 helped explain between 46%-66% variation in zooplankton populations (Fig. 5, Table 1). When
255 building RDA models nutrients and turbidity were removed from models at most stations, and DO
256 removed at station 1, as they were highly collinear with discharge. In testing which discharge
257 variable to include in each model, 30-day average antecedent rainfall explained the most variation in
258 zooplankton at station 1, whilst 20-day average antecedent discharge explained the variation at
259 stations 2 and 3. Abundance of the cyclopoid *Mesocyclops* sp. was positively related to discharge
260 and temperature and negatively related to salinity at all stations (Fig. 5a,c,e). The other dominant
261 cyclopoid *Oithona* sp. was positively related to salinity. The calanoid *Sulcanus conflictus* was
262 positively related to salinity at the upstream stations 1 and 2. Copepodites were generally negatively
263 related with discharge at all stations. Nauplii abundances were always positively related to adult
264 copepod abundances (Fig. 5b, d, f). Most rotifer taxa were negatively related to salinity and
265 positively related to 30-day antecedent discharge at station 1 with the exception off Asplanchnidae
266 (Fig. 5b). Brachionidae that was negatively related to copepod abundance and positively related to
267 DOC at all stations. Colurellidae was positively related to discharge and DOC at stations 1 and 2,
268 and Trichoceridae positively related to DOC at station 1. At stations 2 and 3 Synchaetidae and
269 Epiphanidae were positively related to temperature.

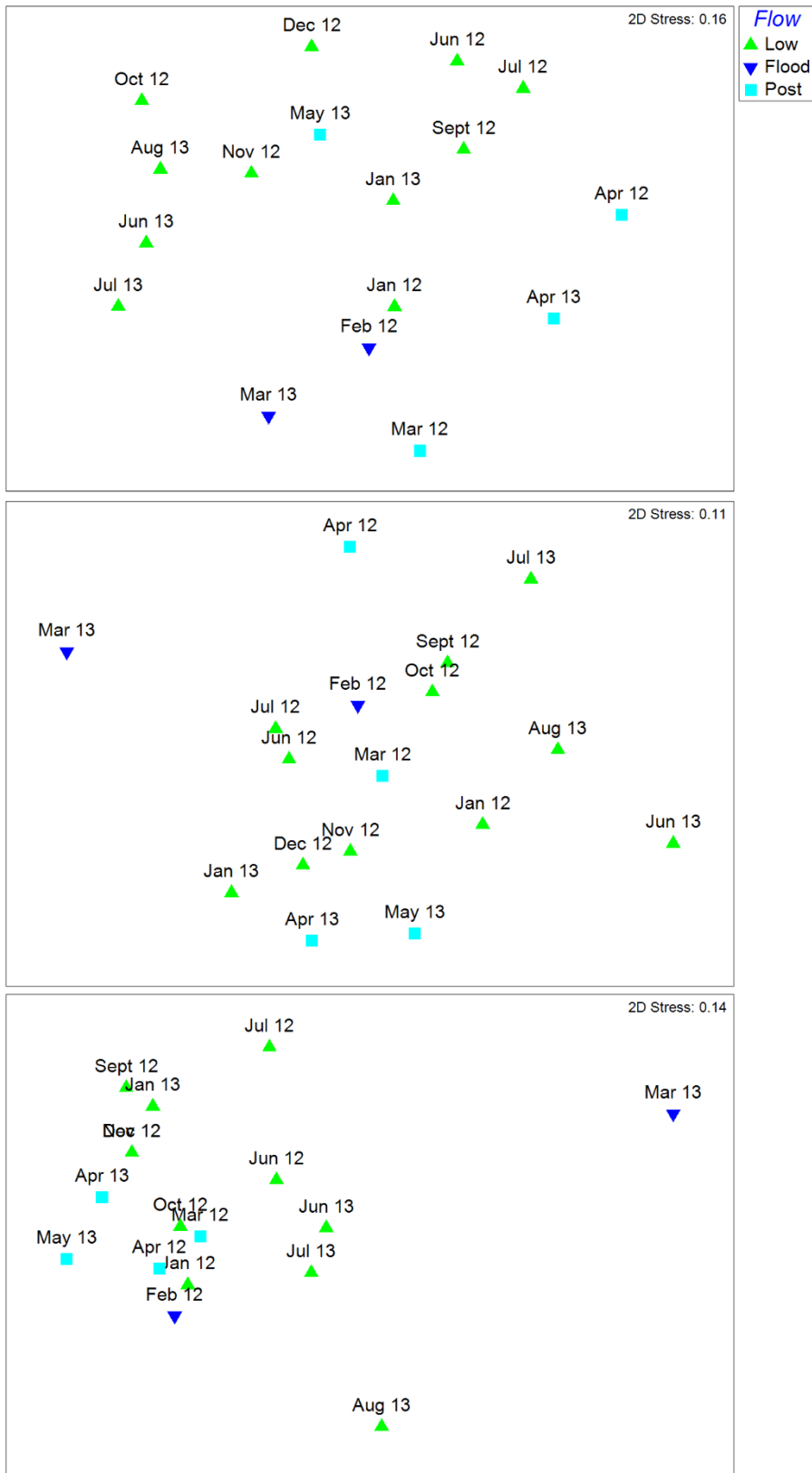


Figure 4. Multidimensional scaling plots constructed from Bray-Curtis resemblance matrix of dominant zooplankton taxa on the Hunter estuary January 2012 to August 2013 for a) Station 1, b) Station 2, and c) Station 3

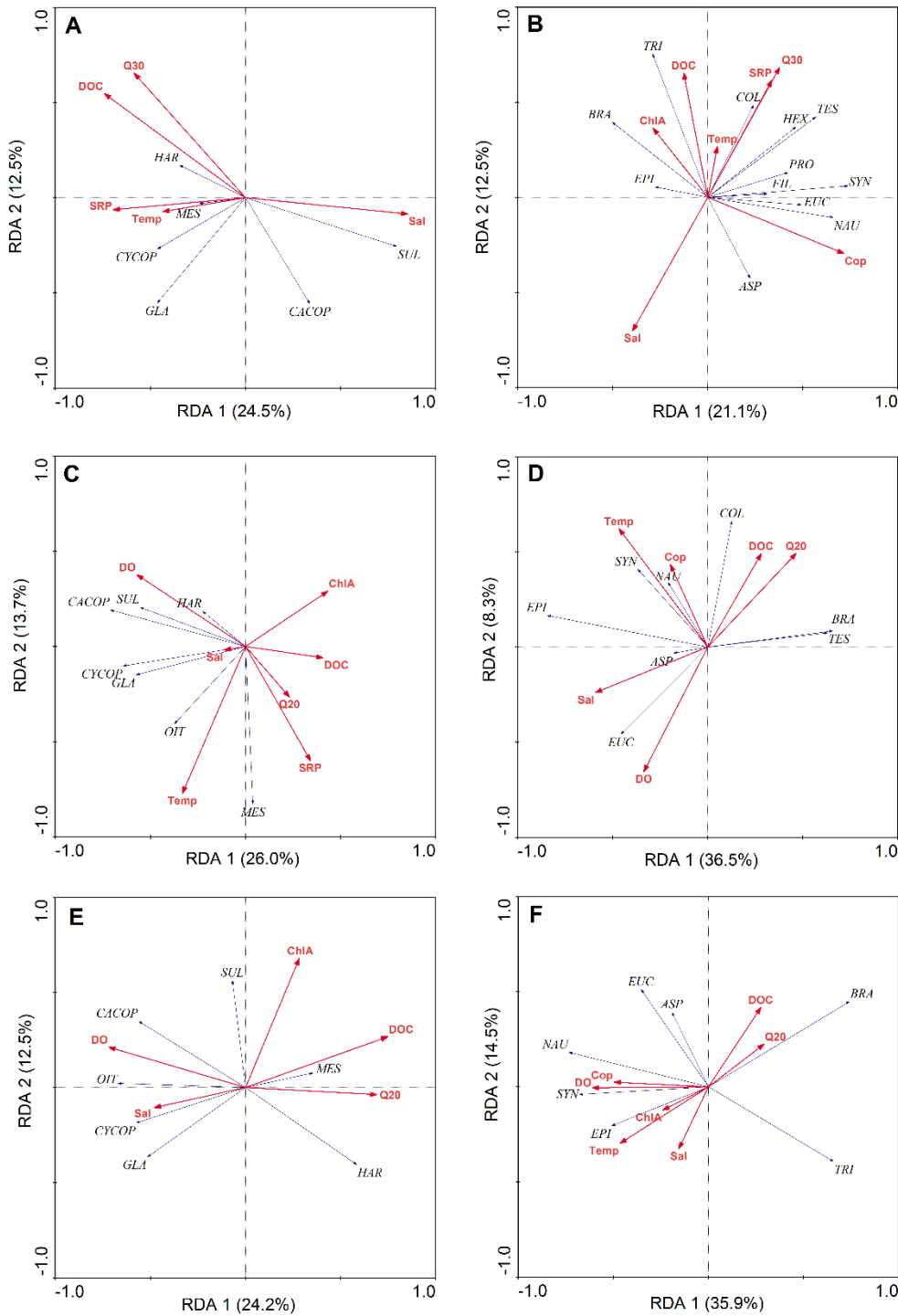


Figure 5. Redundancy analysis for major zooplankton taxa for Hunter River estuary January 2012 to August 2013. A) copepods at Station 1, B) nauplii and rotifers at Station 1, C) copepods at Station 2, D) nauplii and rotifers at Station 2, E) copepods at station 3, and F) nauplii and rotifers at Station 3.

Abbreviations: ASP=

Asplanchna, BRA=

Brachionus, CACOP=

calanoid copepodite,

ChA= chlorophyll *a*,

COL= *Colurella*, Cop=

copepod, Clad=

301 cladocera, CYCOP= cyclopoid copepodite, EPI= *Epiphanes*, FIL=Filinia, GLA= *Gladioferens*
 302 *pectinatus*, HAR= *Harpacticoida*, HEX= Hexarthra, MES= *Mesocyclops sp.*, NAU= nauplii, OIT=
 303 *Oithona sp*, PRO=Proalidae, Q10/20= mean 10 or 20 day antecedent discharge, ROT= rotifers, Sal=
 304 salinity, SUC= *Sulcanus conflictus*, SYN= *Synchaeta*, Temp= temperature, TES= *Testudinella*, TRI=
 305 *Trichocera*.

306 **Discussion**

307 Flood pulses to estuaries act as semi-regular resources pulses. As such we predicted that zooplankton
308 abundance would increase following the flood events in response to increased basal food resources.
309 The results however did not show a clear response, for example during the 2012 flood, where
310 increasing organic carbon loads were delivered to the estuary, copepod abundance increased at one
311 sampling station and decreased at another. Zooplankton response to flood pulses in the literature are
312 also mixed. For example we have previously found bottom-up responses and increased zooplankton
313 biomass following a flood pulse in the Bega River estuary, Australia (Hitchcock et al. 2016a).
314 Hoover et al. (2006) observed similar rapid bottom-up responses from zooplankton following storm
315 flows in a coastal bay in Hawaii, USA. Other authors however have physical and biogeochemical
316 changes to be stronger influence such as advection (Kobayashi et al. 1998) and changing salinity
317 conditions (Madhupratap and Haridas 1975). The effect of salinity and flow appeared to more
318 directly explain the abundance of many zooplankton taxa across the two-year period. Salinity
319 appeared a to play an important structuring force, for example *Sulcanus Conflictus* was positively
320 related to increases in salinity at station 1; the copepod is typically restricted to salinities between 3
321 and 34 (Ough and Bayly 1989). Similarly most rotifer taxa were negatively related to salinity
322 indicative of most species being restricted to only freshwater.

323 Whilst the March 2013 flood pulse led to an initial decrease in zooplankton abundance across the
324 estuary, high numbers of nauplii and copepods were observed in the month after once dissolved
325 oxygen levels had returned to normal in April 2013 at stations 2 and 3. This appears more typical of
326 the response expected from secondary consumers to resource pulses in aquatic environments (Yang
327 et al. 2010). The boom in zooplankton production is likely explained by a combination of additional
328 food resources and potentially reduced top-down pressure as a result of hypoxia in the preceding
329 weeks. The March 2013 flood delivered substantial basal resources in the form of organic carbon and
330 nutrients (Fig. 2) which were utilised by heterotrophic bacteria (Carney et al. 2015) and primary

331 producers (Fig. 2i). Though we did not enumerate protists or phytoplankton directly in this study, our
332 observations whilst counting zooplankton indicated high numbers of tintinid ciliates and mixotrophic
333 taxa such as Cryptomonads and Euglenoids in the period after the March 2013 flood. It is likely there
334 was a high abundance of food available for the very small numbers of zooplankton present during the
335 hypoxic period and for those repopulating in the following months. We have previously shown in
336 mesocosm experiments that additions of DOC mimicking inputs of DOC received during flood can
337 provide a substantial resource subsidy to zooplankton production (Hitchcock et al. 2016b; Hitchcock
338 et al. 2010; Mitrovic et al. 2014).

339 Hypoxia would have also relieved top-down pressure on zooplankton for a period of time. Grazing
340 pressure is a dominant structuring force for zooplankton communities much of the time (Gliwicz and
341 Pijanowska 1989). More persistent hypoxic zones in stratified waters are potentially used by some
342 zooplankton to escape predation (Vanderploeg et al. 2009). Faithfull et al (2011) demonstrated this
343 potential interacting effect of bottom-up and top-down forces in a Swedish Lake, showing that rotifer
344 populations increased in response to additions of organic matter, but only when juvenile fish were
345 not present. This potential effect would only have lasted as long as it took fish to repopulate once
346 hypoxia ended. Importantly these results demonstrate that within a few weeks of hypoxia ending in
347 the estuary there was a large amount of zooplankton that could be used by larval or juvenile fish as
348 they potentially began repopulating the estuary after the fish kill.

349 We predicted that zooplankton assemblages would be distinctly different following flood pulses but
350 this was only evident for the March 2013 flood which caused dramatic changes to zooplankton
351 abundance and composition. During the hypoxia event zooplankton abundances were their lowest at
352 any point during the study. At stations 2 and 3 copepodites were absent entirely, as were nauplii at
353 station 3 (Fig. 3). Whilst advection is likely to have played a role in disrupting populations, we
354 suggest the low numbers were also likely influenced by hypoxia as during the February 2012 flood
355 zooplankton number remained much higher. Laboratory studies testing copepod mortality under

356 different oxygen conditions, including genera present in the hunter estuary such as *Macrocylops*,
357 *Eucyclops* (Tinson and Laybourn-Parry 1985), and *Acartia* (Marcus et al. 2004; Stalder and Marcus
358 1997), have found that most species are unable to survive in conditions where DO is $<0.29 \text{ mg L}^{-1}$
359 (Marcus 2001 and references therein). There are some species of Calanoid and Cyclopoid copepods
360 that can survive for short periods in anoxic waters through anaerobic respiration, though they appear
361 to be highly specialised to areas of persistent hypoxia such as oxygen minimum zones and bottom
362 waters of lakes and embayment's (Chaston 1969; Childress 1976). The community composition of
363 zooplankton at stations 2 and 3 was distinctly different during the hypoxic flood, which was less due
364 to different taxa being present but more the absence of a number of regular occurring zooplankton.

365 As well as direct mortality, the low presence of nauplii in the Hunter estuary may in part be
366 explained by hypoxic conditions suppressing the emergence of copepods from egg banks (Ning et al.
367 2015). Laboratory studies have shown the production of eggs and hatching is greatly reduced under
368 hypoxic conditions. Invidia et al. (2004) tested hatching and survival of *Acartia tonsa* eggs under
369 near anoxic conditions, finding that short-term exposure had no impacts, however > 15 days
370 exposure led to reduced hatchings and increased mortality. The survival of copepod eggs under low-
371 oxygen is higher for species that produce diapause eggs such as *Sulcanus conflictus* (Marcus and
372 Lutz 1998).

373 Some zooplankton taxa may be better placed to survive hypoxic events. The Cyclopoid copepod
374 *Mesocyclops sp.* had the highest abundance of adult copepods during the hypoxic period and was
375 present at relatively low numbers at each sampling station (Fig. 5 d-f). Other genera of Cyclopoid
376 copepods present were *Oithonia sp.* at station 2 in small numbers. We have previously found
377 *Mesocyclops sp.* present during and after flood events in another estuary (Hitchcock et al. 2016a).
378 Part of *Mesocyclops*' resilience to these disturbances appears to be its ability to withstand high flow
379 velocities and maintain its position in the water column (Richardson 1992). *Mesocyclops* may also
380 have a physiological advantage in low oxygen conditions. The relatively small size of *Mesocyclops*

381 sp., and the other cyclopoid present *Oithona* sp., means their surface area to volume ratio is large
382 which may be an advantage when dissolved oxygen is low. Small Cyclopoids such as *Oithona* have
383 lower metabolism and respiration rates compared to equivalent sized Calanoid genera (Almeda et al.
384 2011; Castellani et al. 2005). Species of *Mesocyclops* have been found inhabiting anoxic bottom
385 waters in other environments, for example Lake Nkuruba, Uganada (Kizito 1998).

386 Rotifers may be more tolerant to hypoxia than other zooplankton (Esparcia et al. 1989).

387 Rotifer abundance was generally low in March during the hypoxic event, however the number of
388 rotifer families present during hypoxia was not greatly different from the months either side of the
389 flood at stations 1 and 2. At station 3 there were more rotifer families present during the hypoxic
390 period, though this is likely due to the dominance of freshwater during these weeks as there are many
391 more freshwater rotifer species than estuarine and marine species. Few studies have assessed
392 estuarine or marine rotifer responses to hypoxia compared to freshwater systems. The exception is
393 for *Brachionus plicatilis*, a saline tolerant rotifer and common commercial live food in aquaculture,
394 whose growth and metabolism is reduced, but survival unaffected by $DO < 1 \text{ mg L}^{-1}$ (Esparcia et al.
395 1989). Rotifers are observed in hypoxic areas more commonly than other zooplankton. Mikschi
396 (1989) found that the distribution of *Filinia* and *Keratella* (genera of Brachionidae) in Lake Obersee,
397 Austria, to be unaffected by low-oxygen concentrations, however other genera belonging to
398 Syncheatidae only survived in higher oxygen conditions.

399 Whilst the zooplankton assemblage was distinctly different in March 2013 compared to other times,
400 by April a few weeks after dissolved oxygen concentrations had returned to normal, the zooplankton
401 assemblages resembled that of the pre-flood population. This rapid change is likely due to the
402 biophysical nature of estuaries, with zooplankton able to quickly repopulate environments due to
403 continued inflow of freshwater zooplankton from upstream, and the arrival of zooplankton from
404 marine water during incoming tides. Understanding these changes at finer temporal scale is not
405 possible due to the nature of sampling regime which was restricted to sampling every three to four

406 weeks. Increased sampling frequency during such events is needed to understand the dynamics of
407 zooplankton repopulation and successional changes following flood pulses and hypoxic events.
408 Incidents of hypoxia have dramatically increased over the last 50 years (CENR 2010; Diaz 2001).
409 Hypoxia occurs as heterotrophic microbes consume organic matter and microbial respiration
410 outpaces primary production. The growing occurrence of hypoxia is most commonly linked to high
411 organic matter loads associated with algal blooms, stimulated by nutrient inputs to coastal water
412 (Howarth et al. 2011). Other incidences, such as this in the Hunter estuary, are associated with the
413 direct input of organic matter, be it diffuse sources from the catchment during flood events or via
414 point source inputs from industry or wastewater. Whilst the biogeochemical processes behind
415 hypoxia can be considered natural, anthropogenic activities, such modification of the landscape and
416 flow regime likely increased severity of hypoxia in the Hunter River estuary.

417 In the Hunter River catchment organic matter loads are affected by anthropogenic activities in
418 different ways. Conversion of forests to agricultural land is associated with increased erosion and
419 suspended sediment loads, as well as increased nutrient inputs. Extraction and damming of water has
420 reduced the occurrence of over-bank flows on the estuarine floodplain, allowing greater organic
421 matter loads to accumulate. This means when the floodplain is inundated, as during this event, the
422 loads of organic matter entering the water column are much higher than they potentially would have
423 been. Concomitantly floodplain modification such as channels draining backswamps and areas of
424 the Hunter floodplain with acid sulphate soils can lead to rapid deoxygenation of waters (DPI NSW
425 2008; Johnston et al. 2003; Ruprecht et al. 2018).

426 **Conclusion**

427

428 Resource pulses result from a process of accumulation over time and sporadic release. In the Hunter
429 estuary the importance of flood pulses as important resource pulses are recognised in existing water
430 policy; rules that aim to protect a portion of high inflows reaching the estuary are in place with the

431 goal of maintaining and enhancing ecosystem health and productivity. It is important then for natural
432 resource managers to consider this complex set of biogeochemical relations, and how they may be
433 influenced by different hydrological regimes. As illustrated in this study the response of secondary
434 consumers such as zooplankton to flood pulse was mixed, with increase in abundance or no change
435 following one flood event and a significant drop in abundance during the hypoxic flood event. These
436 results indicate that over a short to medium time scale zooplankton communities may be relatively
437 resilient to large scale hypoxic flood events. Important avenues for further researchers will be
438 understanding a more detailed view of the successional changes that occur during events, how in-situ
439 dynamics may relate to experimental physiology and food web studies, and the role that booms in
440 zooplankton abundance after such floods may play in providing a energetic resources to larval and
441 juvenile fish.

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445 analysis.

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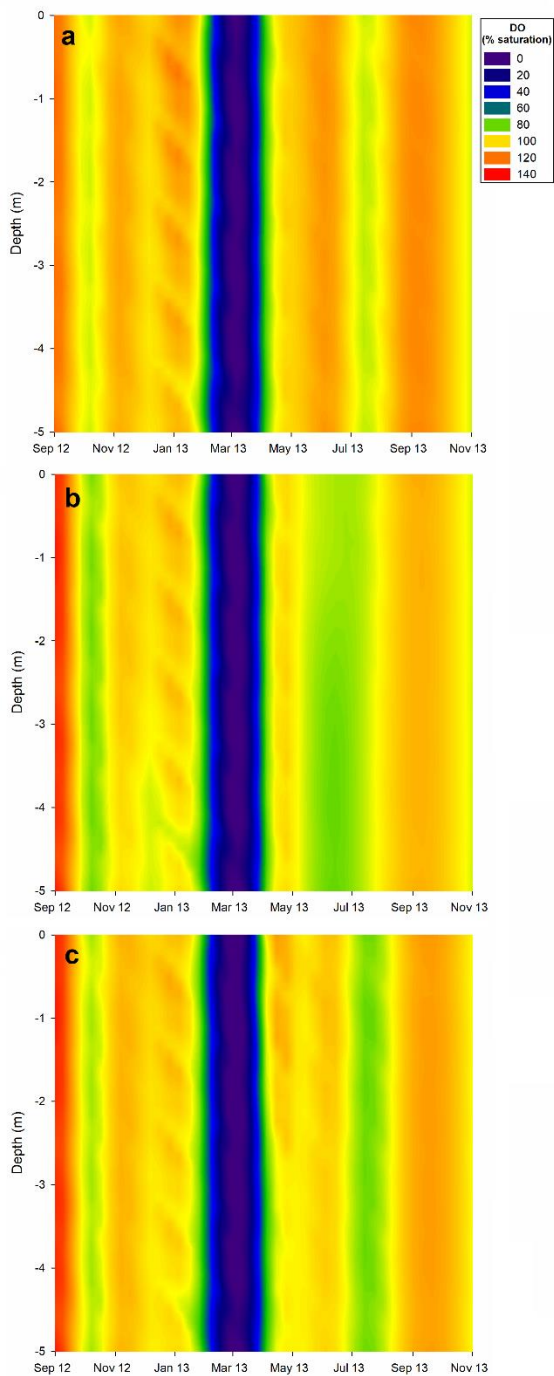
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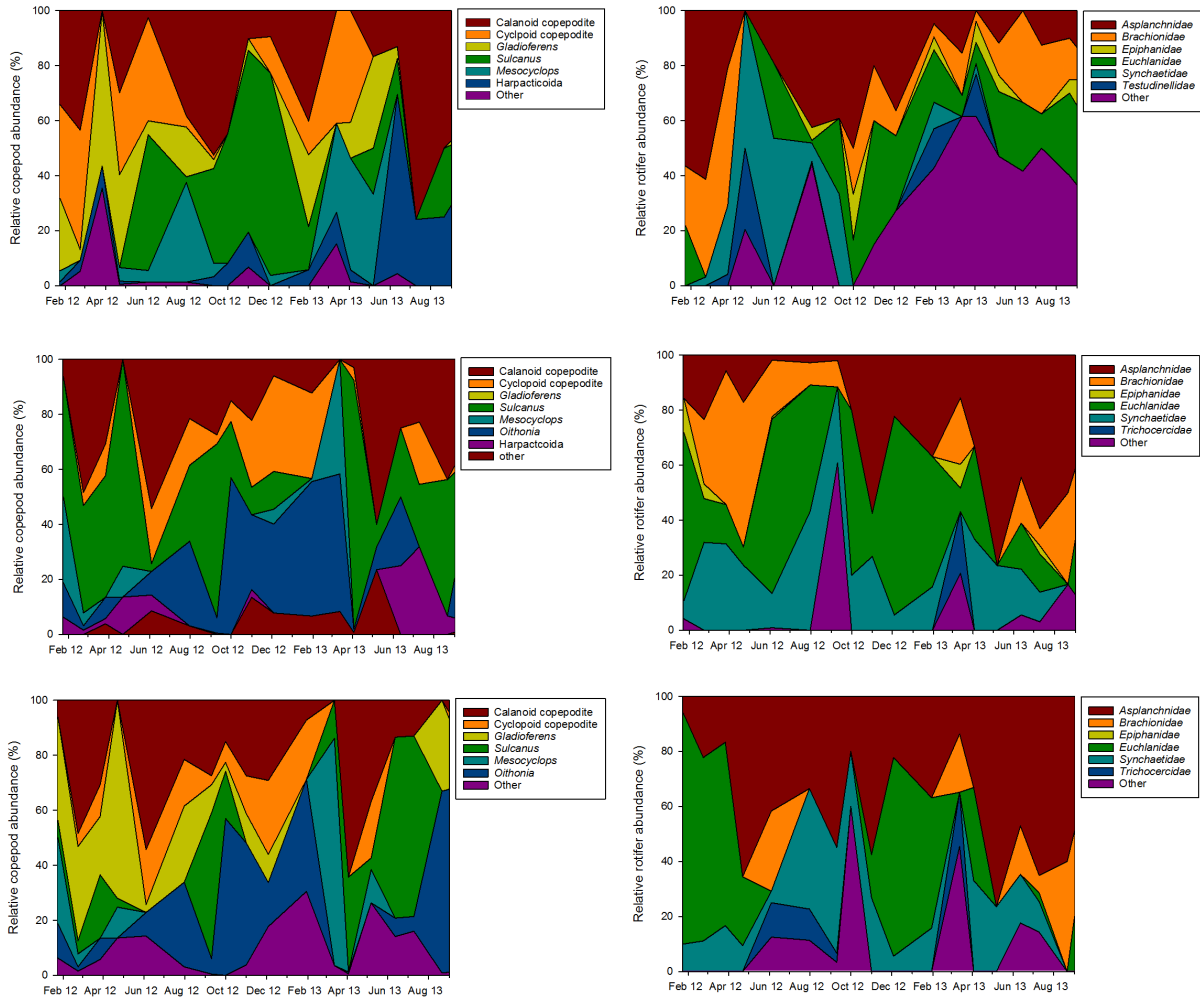
586



588

589 Supplementary figure 1. Dissolved oxygen (% saturation) for the Hunter estuary September 2012 to
590 November 2013 for a) Station 1, b) Station 2, c) Station 3. Dissolve oxygen was measured
591 approximately monthly at 1 m depth interval. Graphs were made using Sigmaplot Ver. 12.6, and
592 using linear interpolation between data points.

593



594

595 Figure S2. Relative zooplankton community composition for the Hunter River Estuary January 2012
 596 to August 2013. A) copepods at Station 1, B) rotifers at Station 1, C) copepods at Station 2, D)
 597 rotifers at Station 2, E) copepods at station 3, and F) rotifers at Station 3.

598

599 Supplementary table 1. List of all zooplankton taxa on the Hunter estuary October 2012 to October
 600 2013.

Zooplankton	601
Rotiferera	Copepoda
Ascomorpha sp.	<i>Acartia sp.</i>
Asplanchna sp.	<i>Boeckella Triarticulata</i>
Brachionus sp.	<i>Calamoecia lucasi</i>
Colurella sp.	<i>Eucyclops australiensis</i>
Dicranopphorus sp.	<i>Gladioferens pectinatus</i>
Epiphanes sp.	Harpacticoida
Euchlanis sp.	<i>Macrocyclops Albidus</i>
Filinia sp.	<i>Oithonia sp.</i>
Hexarthra sp.	<i>Paracyclops sp.</i>
Keretella sp.	<i>Sulcanus conflictus</i>
Lecane sp.	Nauplii
Lindia sp.	Calanoid copepodite
philodinidae	Cyclopoid copepodite
Proalinopsis	Cladocera
Polyarthra sp.	<i>Bosmina meridionalis.</i>
Testudinella sp.	<i>Ceriodaphnia sp</i>
Trichocerca sp	<i>Chydorus sp.</i>
Other	<i>Daphnia carinata</i>
Barnacle nauplii	<i>Moina tenuicornis</i>
Bivalve	
Chironomidae larvae	
Cnidaria	
Collembolla	
Crab zoea	
Hyperiid (juvenile)	
Isotomidae larvae	
Mysidacea	
Oligochaeta	
Ostracoda	
Tardigrada	

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