Estuarine zooplankton responses to flood pulses and hypoxic blackwater event

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Abstract

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

Introduction

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

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

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

Research on how zooplankton may respond to hypoxia associated with flood events in estuaries is rare. There is however a great deal of knowledge related to more persistent low-oxygen conditions. Research on zooplankton in the oxygen minimum zones in the oceans, have found zooplankton are generally not present in water with < 0.29 mg L^{-1} dissolved oxygen (Marcus 2001). In estuaries and coasts that experience persistent oxygen stratification, copepods are less likely to be found below the oxycline, and may alter vertical migration patterns to avoid such areas (Cervetto et al. 1995; Roman et al. 1993). Experimental studies have found wide ranging effects of low oxygen conditions on zooplankton including increased mortality, lowered growth, and reduced copepod egg production (Sedlacek and Marcus 2005; Stalder and Marcus 1997).

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

Materials and methods

Study location and sampling stations

The Hunter estuary, NSW Australia is a wave dominated barrier estuary with a permanently trained open mouth. It drains a large catchment of 21,367 km^2, where the dominant land use is agriculture, alongside areas of mining, small urban centres, and forest. The tidal limit of the estuary is Maitland, and its major estuarine tributaries are the Patterson and Williams Rivers. Land use in the upper estuary region consists of extensive floodplains used primarily for grazing, and in the lower estuary there are wetlands, industry, and urban areas. Discharge in the Hunter River is regulated by a series of large to medium dams in the middle catchment, and a weir on the Williams River. Three sampling stations in the estuary were used during this study (Fig. 1 A). These stations were chosen to represent different salinity zones; during low inflows the salinity at stations 1, 2, and 3, was 12, 23, and 36 respectively. Station 1 is 23 km downstream of the tidal limit and 10 km upstream from Station 2, which is 12 km upstream from Station 3, which is 18km upstream from the mouth of the estuary.

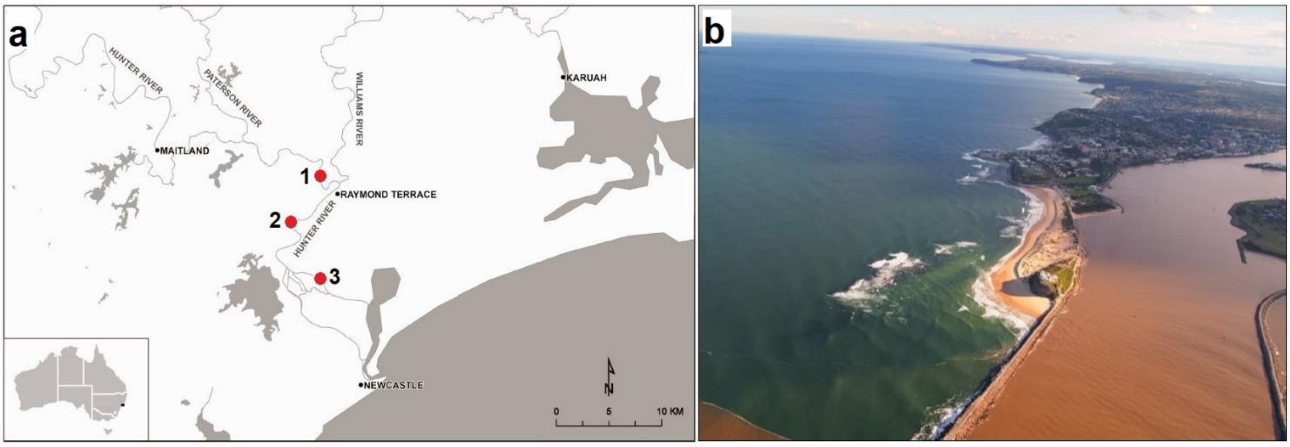

Figure 1. Hunter River estuary, NSW, Australia. a) Sampling stations 1, 2, and 3, b) Photograph showing the mouth of the estuary near Newcastle taken two days after the peak of the flood event on 4 March 2013 that led to extensive hypoxia within the estuary (photo courtesy NSW Department of Industry).

Sampling procedures

Discharge was obtained from two gauging stations operated by the NSW Department of Primary Industries (waterinfo.nsw.gov.au), the Hunter River at Greta, and Patterson River at Gostwyck. A combined daily discharge from these stations is used to represent inflow to the estuary. Discharge on the Williams River was excluded due to the operation of a weir that regulates inflow to the estuary. Daily water temperatures were recorded by the Hunter River at Greta gauging station. Sampling occurred approximately and was conducted by boat sampling up the most downstream station first within 2 hours of high tide. Conductivity, dissolved oxygen, pH and site specific temperature were measured with a Hydrolab Surveyor 4 and MS5 Sonde probe. Depth profiles were recorded at 1 m intervals between the surface and bottom at all stations. As all stations were well mixed at all times only surface data is presented here (depth profiles have been included as Supplementary material.). Salinity was calculated using the electrical conductivity method (APHA 2005). Turbidity samples were measured using a Hach 2100 turbidimeter.

Triplicate samples for dissolved organic carbon (DOC), nitrate/nitrite (NOx), ammonia (NH_4), soluble reactive phosphorus (SRP), silica (Si), and chlorophyll *a* were taken by hand at 20 cm below the water surface. All nutrient samples were collected in 50ml PET bottles stored on ice before being frozen until analysis. Samples for dissolved nutrients were filtered in the field with 0.45 µm polycarbonate filters. Organic carbon samples were analysed in the laboratory by the High Temperature Combustion Method (APHA 2005). Nitrogen and phosphorus samples were analysed using a segmented flow analyser (OI Analytical Model FS3100) according to standard methods (APHA 2005). Samples for chlorophyll *a* were determined by filtering 500 ml of water onto GF/F filters. Filters were frozen until subsequent determination by Standard Methods (APHA 2005) using the grinding technique and acetone as a solute with correction for phaeophytin. A detection limit of 1 µg L^{-1} was used for chlorophyll *a* analysis.

Samples for zooplankton enumeration were taken in duplicate at each station via vertical tows with as 37 µm plankton net and preserved with >50% ethanol. The volume of water was determined accounting for the depth of water filtered, diameter of net, and assumed filtration efficiency of 75%. In the laboratory samples were homogenised and split with 50% of each sample reserved for future analysis. Zooplankton density (individuals m^3) was estimated by counting consecutive aliquots in a Sedgewick rafter counting chamber until 100 specimens of each (copepods, rotifers, nauplii) taxon were counted or until the entire subsample counted. Zooplankton were identified to highest taxonomic level feasible via keys of Shiel (1995) and

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

Statistical analysis

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

Redundancy analysis (RDA) was performed using CANOCO ver 4.5 to analyse the environmental factors that influenced zooplankton density and assemblage structure (Braak and Šmilauer 2002).Separate analysis was performed for major taxa of mesozooplankton (copepodites and adult copepods) and microzooplankton (nauplii and rotifers) at each sampling station. All samples and variables were subjected to Shaprio-Wilk’s normality test and all samples with a non-normal distribution were log transformed (Ln x+1). The explanatory environmental variables were selected using automatic forward selection. To ensure models were not over-fitted we eliminated factors with variable inflation factors >10 and where there was strong co-linearity. We tested discharge measures of Q (discharge on day of sampling) and then average antecedent measures (average discharge over the preceding number of days) for 5, 10, 20, 30 and 40 days. The measure that provided the most explanatory power was included in the model. Discharge, all nutrient and physio-chemical parameters that were measured were tested. For microzooplankton, copepod abundance was included as they are potential predators exerting top-down control. For mesozooplankton, rotifers were included as a potential prey item. Monte-Carlo permutation (999 permutations without restriction) was used to test the significance of canonical axis and environmental variables on zooplankton communities.

Results

Freshwater inflows and hypoxic event

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

The flood events delivered high loads of organic carbon and nutrients to the estuary. Concentrations of DOC and SRP, and measurements of turbidity were all at their highest in the week during the major flood events (Fig. 2d, f, g). Nitrogen concentrations were more variable, with concentrations of both NO_x and NH_4 relatively low during the March 2013 event (Fig. 2e,h). Dissolved oxygen concentrations remained between 60%-120% at all stations at all times with the exception of March 2013 when dissolved oxygen ranged from 1.6 – 2.1 % (or 0.13 – 0.17 mg L^{-1}) and was consistently low throughout the water column

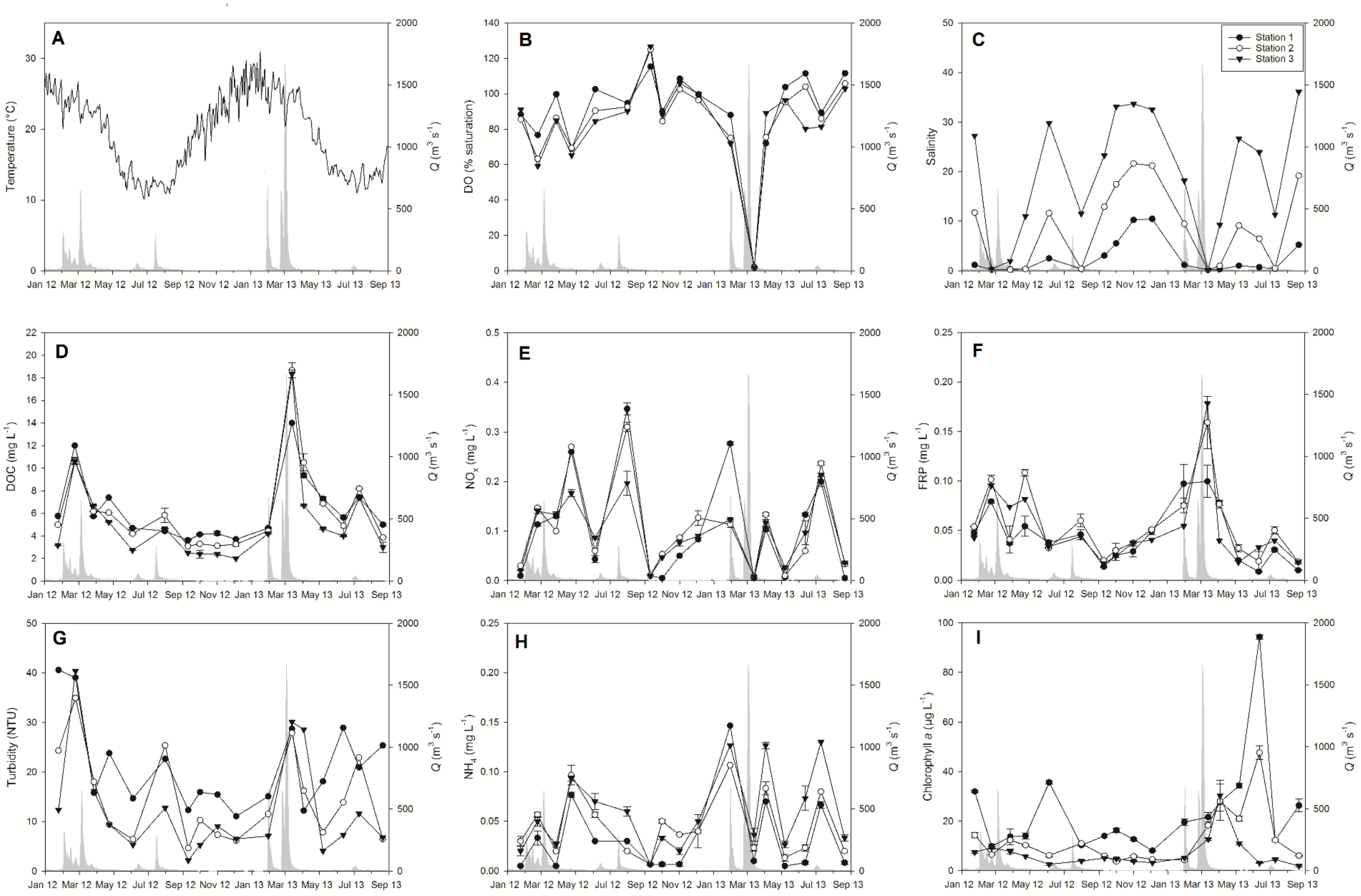

Figure 2: Discharge entering the Hunter estuary October 2012 to October 2013 (right-axis, shaded), along with a) temperature, b) dissolved oxygen (% saturation). C) salinity, D) dissolved organic carbon, E) NO$_x$, F) filtered reactive phosphorus, G) turbidity, H) ammonia, and I) chlorophyll *a*. Error bars are standard error.

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

Zooplankton abundance and community

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

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

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

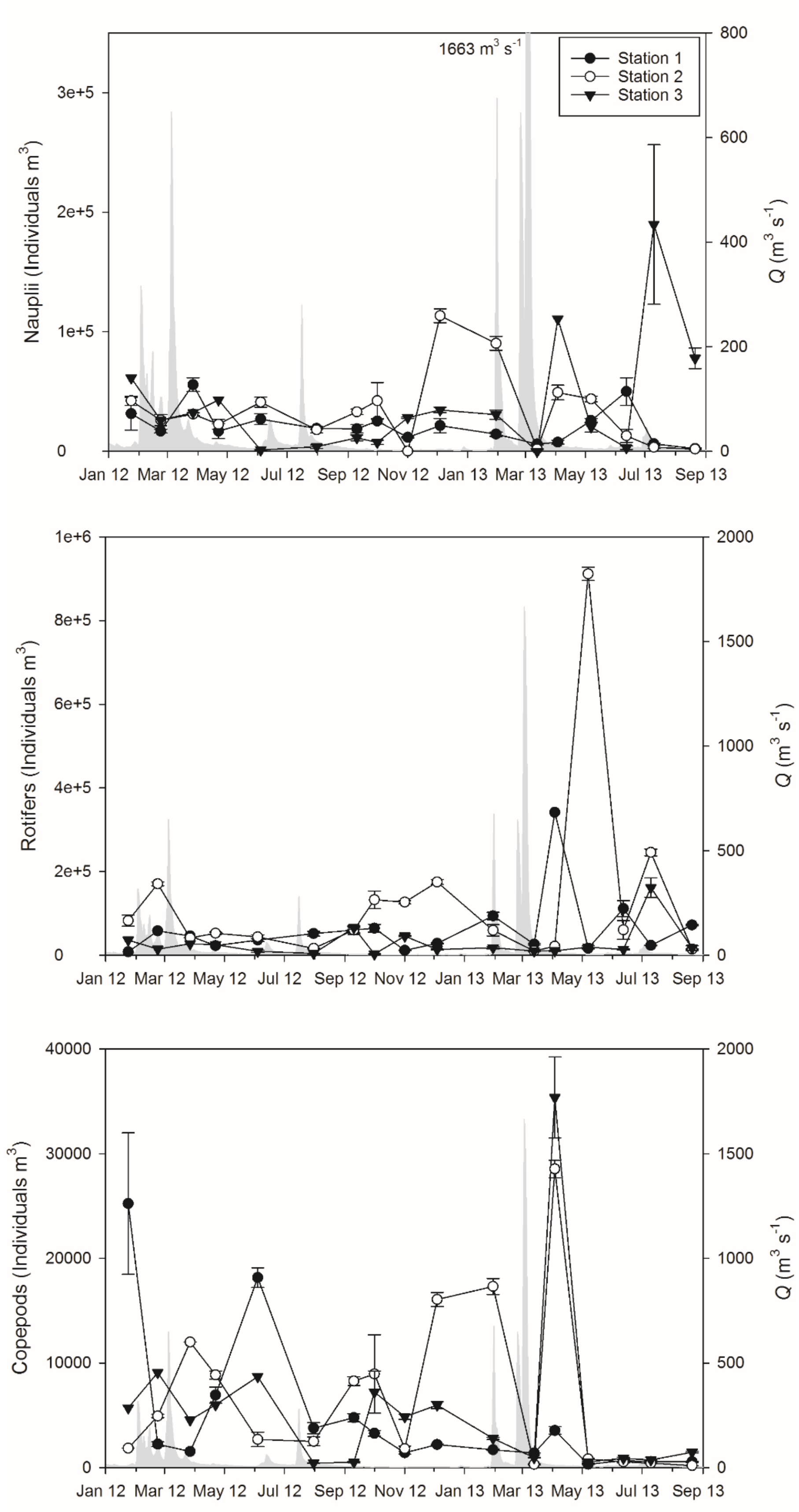

Figure 3. Zooplankton abundance on the Hunter estuary, October 2012 to October 2013. a) Nauplii, b) Rotifers, and c) Copepods. Error bars are standard error.

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

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

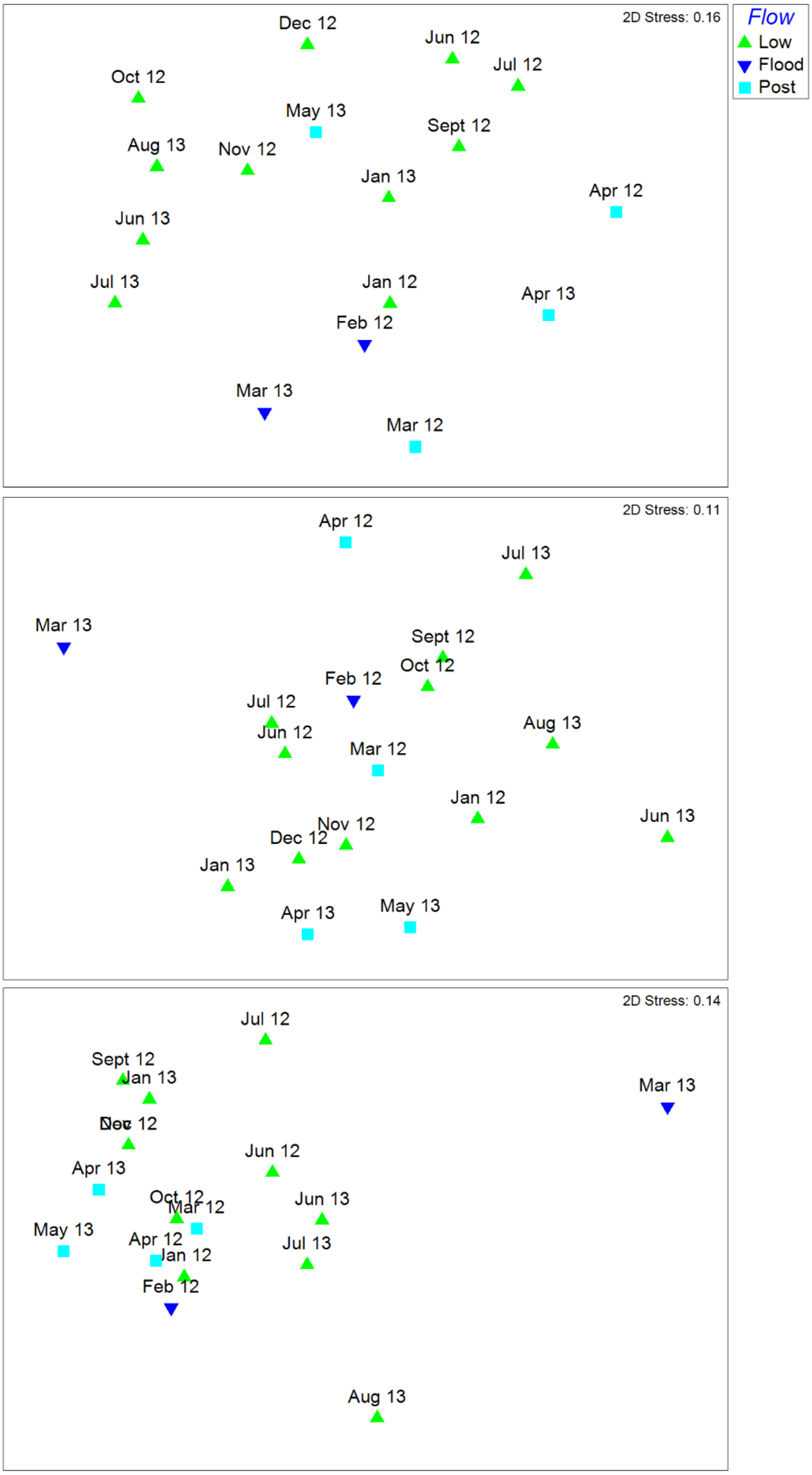

Figure 4.

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

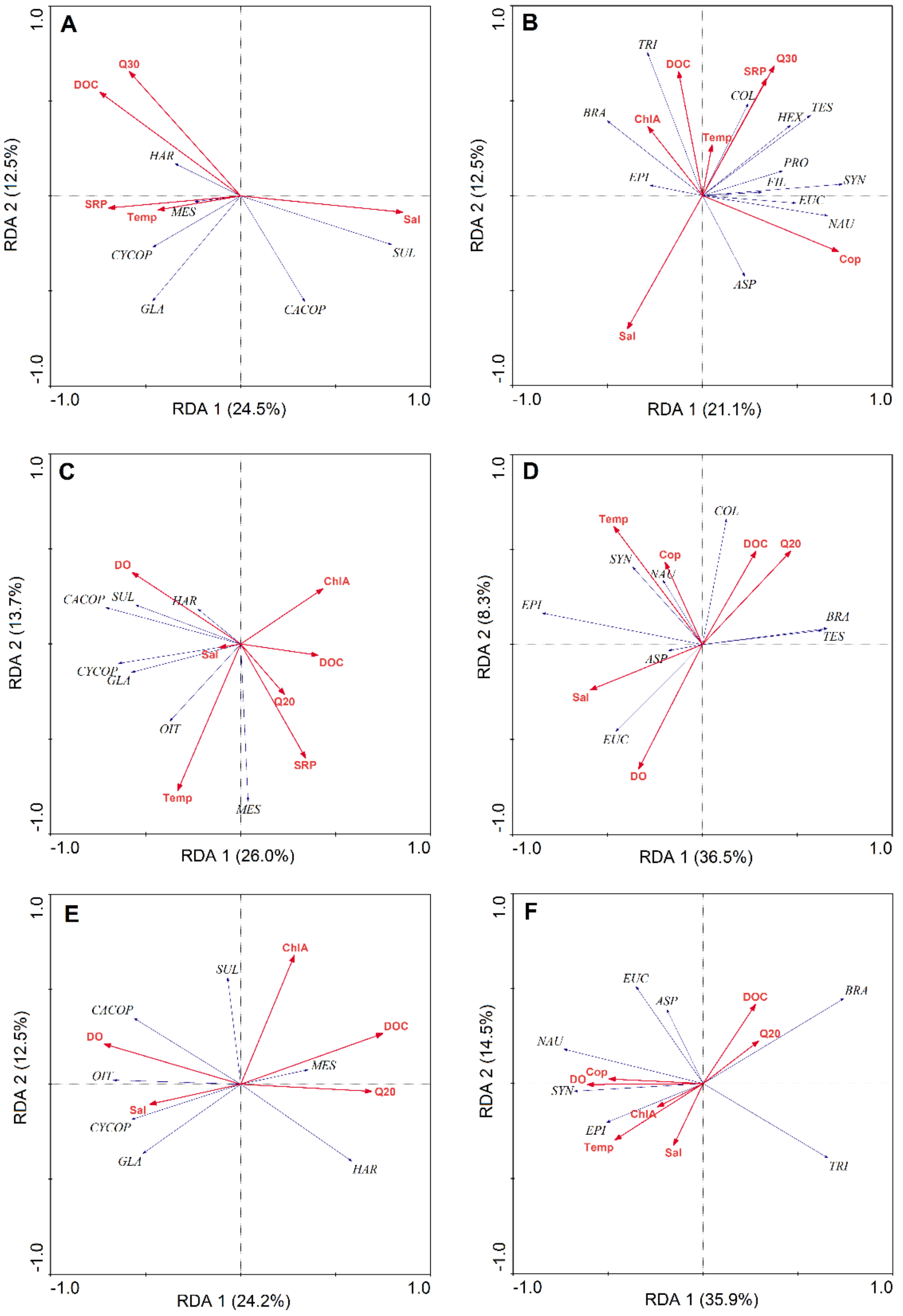

Figure 5. Redundancy analysis for major zooplankton taxa for Hunter River estuary January 2012 to August 2013. A) copepods at Station 1, B) nauplii and rotifers at Station 1, C) copepods at Station 2, D) nauplii and rotifers at Station 2, E) copepods at station 3, and F) nauplii and rotifers at Station 3. Abbreviations: ASP= *Asplanchna*, BRA= *Brachionus*, CACOP= calanoid copepodite, ChA= chlorophyll *a*, COL= *Colurella*, Cop= copepod, Clad= cladocera, CYCOP= cyclopoid copepodite, EPI= *Epiphanes*, FIL=Filinia, GLA= *Gladioferens pectinatus*, HAR= *Harpacticoida*, HEX= Hexarthra, MES= *Mesocyclops sp.*, NAU= nauplii, OIT= *Oithona* sp, PRO=Proalidae, *Q*10/20= mean 10 or 20 day antecedent discharge, ROT= rotifers, Sal= salinity, SUC= *Sulcanus conflictus*, SYN= *Synchaeta*, Temp= temperature, TES= *Testudinella*, TRI= *Trichocera*.

Discussion

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

Whilst the March 2013 flood pulse led to an initial decrease in zooplankton abundance across the estuary, high numbers of nauplii and copepods were observed in the month after once dissolved oxygen levels had returned to normal in April 2013 at stations 2 and 3. This appears more typical of the response expected from secondary consumers to resource pulses in aquatic environments (Yang et al. 2010). The boom in zooplankton production is likely explained by a combination of additional food resources and potentially reduced top-down pressure as a result of hypoxia in the preceding weeks. The March 2013 flood delivered substantial basal resources in the form of organic carbon and nutrients (Fig. 2) which were utilised by heterotrophic bacteria (Carney et al. 2015) and primary producers (Fig. 2i). Though we did not enumerate protists or phytoplankton directly in this study, our observations whilst counting zooplankton indicated high numbers of tintinid ciliates and mixotrophic taxa such as Cryptomonads and Euglenoids in the period after the March 2013 flood. It is likely there was a high abundance of food available for the very small numbers of zooplankton present during the hypoxic period and for those repopulating in the following months. We have previously shown in mesocosm experiments that additions of DOC mimicking inputs of DOC received during flood can provide a substantial resource subsidy to zooplankton production (Hitchcock et al. 2016b; Hitchcock et al. 2010; Mitrovic et al. 2014).

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

We predicted that zooplankton assemblages would be distinctly different following flood pulses but this was only evident for the March 2013 flood which caused dramatic changes to zooplankton abundance and composition. During the hypoxia event zooplankton abundances were their lowest at any point during the study. At stations 2 and 3 copepodites where absent entirely, as were nauplii at station 3 (Fig. 3). Whilst advection is likely to have played a role in disrupting populations, we suggest the low numbers were also likely influenced by hypoxia as during the February 2012 flood zooplankton number remained much higher. Laboratory studies testing copepod mortality under different oxygen conditions, including genera present in the hunter estuary such as *Macrocylops*, *Eucyclops* (Tinson and Laybourn-Parry 1985), and *Acartia* (Marcus et al. 2004; Stalder and Marcus 1997), have found that most species are unable to survive in conditions where DO is <0.29 mg L^{-1} (Marcus 2001 and references therein). There are some species of Calanoid and Cyclopoid copepods that can survive for short periods in anoxic waters through anaerobic respiration, though they appear to be highly specialised to areas of persistent hypoxia such as oxygen minimum zones and bottom waters of lakes and embayment's (Chaston 1969; Childress 1976). The community composition of zooplankton at stations 2 and 3 was distinctly different during the hypoxic flood, which was less due to different taxa being present but more the absence of a number of regular occurring zooplankton.

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

Some zooplankton taxa may be better placed to survive hypoxic events. The Cyclopoid copepod *Mesocylops sp.* had the highest abundance of adult copepods during the hypoxic period and was present at relatively low numbers at each sampling station (Fig. 5 d-f). Other genera of Cyclopoid copepods present were *Oithonia sp*. at station 2 in small numbers. We have previously found *Mesocyclops sp*. present during and after flood events in another estuary (Hitchcock et al. 2016a). Part of Mesocylcops' resilience to these disturbances appears to be its ability to withstand high flow velocities and maintain its position in the water column (Richardson 1992). Mesocyclops may also have a physiological advantage in low oxygen conditions. The relatively small size of *Mesocyclops* sp., and the other cyclopoid present *Oithona* sp., means their surface area to volume ratio is large which may be an advantage when dissolved oxygen is low. Small Cyclopoids such as *Oithon*a have lower metabolism and respiration rates compared to equivalent sized Calanoid genera (Almeda et al. 2011; Castellani et al. 2005). Species of *Mesocyclops* have been found inhabiting anoxic bottom waters in other environments, for example Lake Nkuruba, Uganada (Kizito 1998).

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

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

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

Incidents of hypoxia have dramatically increased over the last 50 years (CENR 2010; Diaz 2001). Hypoxia occurs as heterotrophic microbes consume organic matter and microbial respiration outpaces primary production. The growing occurrence of hypoxia is most commonly linked to high organic matter loads associated with algal blooms, stimulated by nutrient inputs to coastal water (Howarth et al. 2011). Other incidences, such as this in the Hunter estuary, are associated with the direct input of organic matter, be it diffuse sources from the catchment during flood events or via point source inputs from industry or wastewater. Whilst the biogeochemical processes behind hypoxia can be considered natural, anthropogenic activities, such modification of the landscape and flow regime likely increased severity of hypoxia in the Hunter River estuary.

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

Conclusion

Resource pulses result from a process of accumulation over time and sporadic release. In the Hunter estuary the importance of flood pulses as important resource pulses are recognised in existing water policy; rules that aim to protect a portion of high inflows reaching the estuary are in place with the goal of maintaining and enhancing ecosystem health and productivity. It is important then for natural resource managers to consider this complex set of biogeochemical relations, and how they may be influenced by different hydrological regimes. As illustrated in this study the response of secondary consumers such as zooplankton to flood pulse was mixed, with increase in abundance or no change following one flood event and a significant drop in abundance during the hypoxic flood event. These results indicate that over a short to medium time scale zooplankton communities may be relatively resilient to large scale hypoxic flood events. Important avenues for further researchers will be understanding a more detailed view of the successional changes that occur during events, how in-situ dynamics may relate to experimental physiology and food web studies, and the role that booms in zooplankton abundance after such floods may play in providing a energetic resources to larval and juvenile fish.

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