

Interactive comment on “Can mussels be used as sentinel organisms for characterisation of pollution in urban water systems?” by E. S. Reichwaldt and A. Ghadouani

Anonymous Referee #1

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Comments regarding paper Review of “Can Mussels be used as sentinel organisms for characterization of pollution in urban water systems?”

Overall

Well written and with an easy to follow set-up and results. I think the objectives could be improved and some of the introduction/discussion regarding water management approaches streamlined to move the reader more quickly to the meat of the paper.

I was a bit confused by exactly what was meant by pollution – nitrate or nitrogen generally. There is a strong focus on nitrate but the results don't point strongly to mussels reflecting nitrate concentration or ^{15}N composition and therefore a larger focus on the

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N-cycle may be needed to explain the results observed here.

I think with some slight reorganisation, streamlining and expansion of N-cycling within the estuary this manuscript will be much improved. More attention should be paid to POM and how/why or whether POM is decoupled from NO₃ and how this relates to the 15N of the mussels. Your strongest figure is 15N-mussel vs the distance from estuary (some of others are strongly influenced by one site, MC) and this is not fully explained in the discussion.

There is a strong emphasis on site-specific characteristics influencing mussels however, aside from MC, the concentration of NO₃ and NH₄ were fairly low and not correlated across sites. So, NO₃ and NH₄ not likely to explain site-specific 15N-mussel variability. This should be addressed quickly in the discussion section.

With some tightening and expansion in some areas (my edits below) I think this paper constitutes a nice addition to the applicability of food webs and biological indicators of nutrient sources.

Page 1:

19 higher nitrogen stable isotope signature. Enriched in 15N is more accurate. Purely preference here, you did well 23 Can you omit the sentence beginning with “Our results showed a trend...” I think the sentence isn’t necessary in the abstract. 26 What are natural values? Maybe state within range of observed values within estuaries of W. Australia 28 Delete “ which allowed for the detection of spatial difference” 29 change to ‘organisms’

Page 2:

First paragraph doesn’t relate well to abstract or title. I would introduce this paper with the current state of affairs regarding nitrogen in urban water systems, then identify the problem – the quantification of a spatial and temporally varying regulated chemical species (nitrogen).

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Second paragraph starts from the point of restoration and then proceeds to the problem – limited understanding of temporal and spatial variability of pollution (I would state nitrogen here, it is your focus)

I suggest deleting most of paragraph 1 and improving paragraph 2 to more concisely state your research problem, question etc. Get to the point of the paper very quickly.

Page 3:

Good. Paper is fully into the nitrogen sources, how to characterize variability etc.

11 – delete s from ‘urbans’

Page 4:

18 Citation for this? Would be useful to reader to know how work in polluted water-bodies then relates to concentrations and isotopic composition presented in this paper – were polluted waterbodies exhibiting higher concentrations and heavier ^{15}N values? Over a larger range? Etc

Make sure objectives use same phrasing. “Would lead to. . .” is good and used in 2 of 3. Keep it uniform to help the reader. (2) is more of a conclusion

26 change to ‘nitrogen-rich’

27 ‘(2) distinct spatial difference in mussels. . .’ This doesn’t quite make sense. Do you mean to say that the number of mussels relates to the nitrate concentration? Or that the ^{15}N composition of mussels reflect observed composition in nitrate.

29 ‘lead to increased anthropogenic signal’. Rephrase, you anticipate that you will observe elevated ^{15}N due to elevated ^{15}N inputs from nitrogen-rich waters, which follows your prediction (1).

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8 Change to - prone to ‘nutrient’ pollution.

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General – clarify that the ^{15}N composition is reported in units relative to an international standard (air usually). I assume the standard is the same for both isotope facilities used in this paper. Report it and clarify that the ^{15}N concentrations you report are relative to the standard and are not absolute concentrations (isotope scientists know this, others may not). Same for ^{18}O . This should be done in the methods section at a minimum, often re-stated in data tables as part of the units of ^{15}N

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1 Change to “To determine the isotopic composition of nitrogen in particulate organic matter (POM), a source of food for mussels, 0.7 – 2.5 L. . .” Avoid using ‘signature’ unless you’ve determined that the isotopic composition of POM is unique, particularly if you’re only using one isotope for characterization.

1b Your hypothesis #2 is that mussel ^{15}N corresponds to nitrate ^{15}N , no? But here you say that mussels feed on POM so the reader is confused by the nitrate ^{15}N hypothesis. You should rectify this earlier in the introduction somehow. Either focus on POM or state how N cycling would link nitrate and POM ^{15}N composition.

1c You state that mussel ^{15}N and POM ^{15}N are linked but you don’t show in a figure. And the link between ^{15}N POM and ^{15}N NO_3 is also not discussed in the results.

4 Change to “Harvested mussels were measured and dissected to obtain the foot tissue. . .”

6 Was the foot tissue homogenized before isotope analysis or was the entire sample of 3 combined foot tissue used in the mass-spectrometer? If the entire sample was used, state so, if the sample was fully homogenized with mortar/pestle state that. As it is it seems there were 3 distinct pieces of foot tissue were dried together.

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4 long term average based on how many years? Citation?

7 The comparison is between discharge during the winter of 2010 and the winter of 1994 and the conclusion is that 2010 discharge was lower than usual. Is there a published mean discharge value you can compare to? Or is the discharge of '94 the only published value for comparison? To state discharge is lower than usual you should have an average or trend of some sort for comparison

10 Unusually high salinity? Is this relative to a published average salinity value for the estuary? Need citation or cleaner text. Either state the salinity was high throughout the area or high relative to a specific mean value (with citation if possible).

10b What are the units for salinity? I suggest adding the salinity recorded for the ocean water in the nearby area (or salinity of ocean water generally) for the reader to compare.

31 Change to “while nitrogen from NH_4^+ was greater at all other sites (Fig. 2)”.

31 Can omit sentence starting with “This is supported by significant. . .”. It doesn't add much value compared to previous sentence.

Page 9

4 change to “The TN:TP ratio (weight) was between 0 and 6.5, with 84% of the ratios (by site?) below 2.2”. Move the rest of the paragraph to appropriate place in discussion OR condense to simple sentence that cites published thresholds for determining nitrogen limitation (7.2 or 2.2).

18 “Analysis of stable isotope composition of NO_3 . . .” Change ‘signature’ throughout unless you're really talking about the uniqueness of a component's isotopic composition.

19 restate minimum concentration requirements

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1 Clarify sentence findings – I understood that POM 15N and mussel 15N collected at each site had a significant, positive relationship to one another. By fractionation effect of 0.6 do you mean that mussel 15N composition was on average 0.6 greater than POM 15N composition at same site? Clarify this for the reader, particularly if you're not including a figure.

5 Move this sentence second in the paragraph. Move second sentence to the first sentence position.

7 'smaller than range seen in 15N nitrate' (... to ...) restate range of nitrate 15N to make it easier for the reader to compare the relative ranges of each.

8 use lower case δ , not Δ . It would be better to rephrase the sentence so you are not starting with a greek letter.

9 "no temporal trend" sentence starts with a non-trend and ends with a significant (?) trend between 15N and distance to estuarine mouth, connect the two clauses with a 'though'.

Figures 3, 5 and 7 all strongly influenced by MC site.

Figure 5. You show scenarios with and without CI or MC sites, was WO site included in regressions?

31 avoid using 'site-specific' twice in same sentence. Restructure.

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It would be easier for the reader if the discussion followed directly from the 3 objectives stated in the introduction – nitrogen and 15N conc upstream; 15N mussel by site and nitrate conc; distance from mouth = anth signal.

24 What do you mean by this sentence. Expand more. How does the fraction of NO_x in the DIN pool explain site-specific variation in 15N? It's stated here but the reader doesn't understand how simply from the sentence.

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First two sentences are redundant, simplify and merge. Sentence 1 is cumbersome with overuse of “15N values”.

Trend between mussel 15N and nitrate 15N strongly driven by site MC. As is relationship with TDIN. Without MC site, there is little to no trend. You should address this head-on in your discussion section.

21 Relationship can't be 'higher'. The r^2 value can be higher, the relationship can be stronger etc. Though the slope of the line doesn't change much with removal of CI site, the fit improves. I mention earlier but you should also clarify if you keep the WO site in the regression.

21b Good explanation of N cycling dynamics at this site. Could you include something similar for the MC site, even if it's conjectural it would be useful given how different the site was relative to the others.

POM and mussel 15N are linked but nitrate 15N negatively linked to mussel 15N (driven by MC site). Could it be that POM sources are not within-estuary? If you're estuary is N-limited then production should be low, could be that POM is all sourced outside (upstream I imagine) and within-estuary nitrate 15N and nitrate concentrations aren't important to POM production. This could explain uncoupled 15N between POM and NO_3 . Do you have evidence of this? This would still be in line with the overall story here, reinforcing need for site-specific information and management approaches.

18 but your nitrogen sources of nitrate and ammonium were not different between sites (except MC) so this seems unlikely to explain differences in mussel 15N, no? More likely differences in POM 15N drove differences in mussel 15N and is reflected in relationship between mussel 15N and distance from mouth.

It seems like there are other n cycling effects that are occurring here and could help

to explain the negative (or lack of) correlation between 15N-NO₃ and 15N-mussel (or TDIN and 15N-mussel. Your MC site may be influencing interpretation too much. If you had to interpret these data without the MC site, how would you do so? Does it change your overall conclusions? I would like to see more results regarding the POM and its connection to N-cycling. You have a fairly strong trend between 15N-mussel and distance from estuary mouth. What is driving this?

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4 correlated to nitrogen concentrations. But these were all negative correlations, no? TDIN is shown, not POM or NO_x.

15N-mussel negatively correlated to 15N-NO₃. 15N-NO₃ positively (though MC weighs heavily) related to NO_x concentration. High NO₃ reflected in 15N-NO₃ does not appear in 15N-mussels as sites with high 15N-NO₃ and NO₃ have low 15N-mussels, no? So mussels don't appear to be good indicators of NO₃ sources as they don't reflect 15N-NO₃, no?

In the discussion and conclusion sections you refer to mussels reflecting nitrate pollution but the link is weak, dependent on MC, and negative. Explain how these connections interact or simplify your message in the discussion and conclusion. The emphasis appears to be on nitrate but the linkages between nitrate and mussel tissue are unclear.

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