Dear Referee #1,

Thank you very much for your very insightful and positive comments on our manuscript “Can mussels be used as sentinel organisms for characterisation of pollution in urban water systems?” by E. S. Reichwaldt and A. Ghadouani (hess-2015-523). They provide very important feedback to improve this manuscript.

Below is a proposed revision report on how we would like to address your comments.

We will first reply to your overall comments, before we will address the specific comment in more detail.

**1) Overall comments**

| No. | Comments | Response |
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| 1 | I think the objectives could be improved | We agree and have restated the objectives using your suggestion from your comment #14. The objectives will now read as follows: “Specifically, we anticipated that (1) a higher input of nitrogen-rich waters upstream would lead to a higher isotopic signatures of nitrate, (2) spatial differences in the level of nitrates in the water would lead to spatial differences in mussel isotopic signature, and (3) the increased distance from the mouth would lead to an elevated $^{15}N$ values in mussels due to elevated $^{15}N$ inputs from nitrogen-rich waters upstream. “ |
| 2 | Some of the introduction/discussion regarding water management approaches streamlined to move the reader more quickly to the meat of the paper. | This comment is a short version of your comment #11 and we will reply in more detail later. In brief, we will rework the introduction along your suggestions, but would like to point out that a second reviewer found this introduction very useful. As such, we will not delete the complete first paragraph of the introduction, but at this stage consider deleting some sentences (e.g., Page 2 Lines 9 – 13 and the first sentence of the second paragraph) and exchanging the words “nutrient” and “pollutant” with “nitrogen” to make it more specific in the third paragraph. For more details, please see our reply to your comment #11. |

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| 3 | 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 15N composition and therefore a larger focus on the N-cycle may be needed to explain the results observed here. | We agree that this needs clarification. The analysis of the nitrogen signature in general has proven to be a powerful tool as an indicator of anthropogenic contamination. Our study looks specifically at the stable isotope signature of nitrate. Then, to additionally test if mussels can be used as bioindicators of nitrogen pollution, we broaden our objectives and look at nitrogen.<br><br>We will make this clearer throughout the manuscript by exchanging the word "nutrients" with "nitrogen" where appropriate by restating the objectives: "The main aim of this study was to identify the variability of nitrogen concentration in an urban estuary over time and space and to ascertain the suitability of the isotopic signature ($\delta^{15}N$) of mussel tissue as an indicator of nitrogen pollution in urban water systems. Specifically, we anticipated that (1) a higher input of nitrogen-rich waters upstream would lead to a higher isotopic signatures of nitrate, (2) spatial differences in the level of nitrates in the water would lead to spatial differences in mussel isotopic signature, and (3) the increased distance from the mouth would lead to an elevated $^{15}N$ values in mussels due to elevated $^{15}N$ inputs from nitrogen-rich waters upstream. |
| 4 | 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. | We agree and we will include a paragraph on page 12 Line 21as follows: "An alternative explanation would be that POM could originate upstream where nitrate might have had higher $\delta^{15}N$ values (not quantified in this study). Upon entering the estuary, POM mixes with estuarine POM, uncoupling the within-estuary $\delta^{15}N$ nitrate and POM $\delta^{15}N$ values. This could also explain the strong relationship between $\delta^{15}N$ in mussels and the distance from the estuary mouth found in our study. Such a strong relationship can be expected in estuaries with low pollution levels due to the aforementioned mixing, while little spatial variability in $\delta^{15}N$ values of primary consumers can be expected in heavily polluted estuaries due to the dominance of upstream POM, as was shown by Oczkowski et al. (2008)."<br><br>Please also see our reply to your comment #48. |

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| 5 | 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. | We agree with the reviewer. Because NO3, NH4, or TDIN concentrations are very similar for many of the sites they cannot completely explain $\delta^{15}N$ variability in mussels. We will include this in<br><br>i) the discussion (Page 12 Line 31):" Site MC was closest to the ocean, was one of the deepest sites and had a higher TDIN concentration compared to all other sites, which were not different with regard to TDIN concentration between themselves. This emphasises that the differences in mussel $\delta^{15}N$ between sites might be due to site-specific nutrient cycling processes in our estuary and might not reflect nitrogen pollution itself."<br><br>ii) the conclusion (Page 14 Line 5):" The negative trends between mussel $\delta^{15}N$ values and nitrate concentration or nitrate $\delta^{15}N$ values emphasize that mussels might not be good indicators for $NO_3$ sources in systems with low pollution levels. Instead, the small differences in mussel stable isotope signatures might reflect differences in site specific nutrient cycling caused by physicochemical conditions or biological factors rather than nitrogen pollution."<br><br>Please also see our reply to your comment #50. |

**2) Specific comments**

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| 6 | Page 1:<br>19 higher nitrogen stable isotope signature. Enriched in 15N is more accurate. Purely preference here, you did well | We agree that both phrases can be used. We prefer to keep "higher nitrogen stable isotope signature" in the abstract to make it easier for readers who are not entirely familiar with stable isotope jargon. |
| 7 | Page 1:<br>23 Can you omit the sentence beginning with "Our results showed a trend. . ." I think the sentence isn't necessary in the abstract. | We agree and will omit this sentence in the abstract. |

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| 8 | Page 1:<br>26 What are natural values? Maybe state within range of observed values within estuaries of W. Australia | We agree that this was ambiguous. We will clarify it by rewriting this sentence to :" … nitrogen stable isotope values of nitrate throughout the estuary were well within natural values of uncontaminated groundwater or organic nitrate from soils, indicating groundwater inflow rather than pollution by human activity was responsible for differences between sites."<br><br>This will and has been described in more detail with references in the discussion on page 11 Lines 15-23.<br><br>Unfortunately no values for Western Australia are available, but we will additionally provide a citation for this statement in the conclusion: Page 14 Line 2: "…were due to a natural input of nitrate uncontaminated groundwater (Xue et al., 2009) rather than human pollution.". |
| 9 | Page 1:<br>28 Delete " which allowed for the detection of spatial difference" | We agree with this comment and will delete this part of the sentence. |
| 10 | Page 1:<br>29 change to 'organisms' | We agree with this comment and will correct this. |
| 11 | Page 2:<br>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).<br>Page 2:<br>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. | We understand your concern and will aim to strike a good balance between "getting to the point quickly" and "giving a broad picture of state of pollution management", which we think is appropriate for this journal that has such a broad range of readers. We therefore consider keeping the first two paragraphs as the general introduction but in a shorter version. We think that by applying the following, we will strike a nice balance between your suggestions and the comment from the second reviewer, who thought our introduction was very valuable:<br><br>i) delete part of the first paragraph (Page 2 Line 9 – 13)<br><br>ii) delete the first sentence of the second paragraph to make sure that we get to the point more quickly<br><br>iii) in the third paragraph, we will consider being more specific by exchanging the words "nutrient" and "pollutant" with "nitrogen". |

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| 12 | Page 3:<br>11 – delete s from ‘urbans’ | We agree with this comment and will correct this. |
| 13 | Page 4:<br>18 Citation for this?<br><br>Would be useful to reader to know how work in polluted waterbodies then relates to concentrations and isotopic composition presented in this paper – were polluted waterbodies exhibiting higher concentrations and heavier 15N values? Over a larger range? Etc | The citations are currently included after the next sentence. We will bring them forward and combine the two sentences. It will then read as: “Earlier studies in polluted freshwater and marine systems found positive relationships between the concentration of nitrogen and the isotopic signature of nitrogen in mussels, and between the isotopic signature of nitrate-N and that of mussels (Cabana and Rasmussen, 1996; McClelland et al., 1997; Costanzo et al., 2001; Anderson and Cabana, 2005; Gustafson et al., 2007; Wen et al., 2010), suggesting that bivalves are suitable indicators of changes in nutrient pollution load to waterbodies.”<br><br>We will also add, as suggested, a brief comparison between nitrogen concentration / $\delta^{15}N$ values found in our study and the values reported in the cited previous studies. We will add this on page 12 Line 3. |
| 14 | Page 4:<br>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 | We agree with his comment and will rephrase objective (2) as follows: “(2) spatial differences in the level of nitrates in the water would lead to spatial differences in mussel isotopic signature” |
| 15 | Page 4:<br>26 change to ‘nitrogen-rich’ | We agree with this comment and will correct this. |
| 16 | Page 4:<br>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 15N composition of mussels reflect observed composition in nitrate. | We agree that this was expressed ambiguously. We will restate objective 2 as suggested above (#14) to “(2) spatial differences in the level of nitrates in the water would lead to spatial differences in mussel isotopic signature” to make this explicit. |
| 17 | Page 4:<br>29 ‘lead to increased anthropogenic signal’. Rephrase, you anticipate that you will observe elevated 15N due to elevated 15N inputs from nitrogen-rich waters, which follows your prediction (1). | We agree and will rephrase objective 3 as follows: “(3) the increased distance from the estuary mouth would lead to elevated $^{15}N$ values in mussels due to elevated $^{15}N$ inputs from nitrogen-rich waters upstream.” |

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| 18 | Page 5<br>8 Change to - prone to 'nutrient' pollution | We agree and will add the word "nutrient" into the sentence. |
| 19 | Page 6<br>General – clarify that the 15N 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 15N concentrations you report are relative to the standard and are not absolute concentrations (isotope scientists know this, others may not). Same for 18O. This should be done in the methods section at a minimum, often re-stated in data tables as part of the units of 15N | We agree with this comment and can confirm that both institutions used the same standards ($\delta^{15}$N: air; $\delta^{18}$O: Vienna Standard Mean Ocean Water, VSMOW) and that all values are reported in per mil (‰) with respect to the international standards. We will include the following sentences in the method section:<br><br>- mussels and POM: "All values are reported in per mill (‰) with respect to the international standard (air)."<br><br>- nitrate: "All values are reported in per mill (‰) with respect to the international standards ($\delta^{15}$N: air; $\delta^{18}$O: Vienna Standard Mean Ocean Water, VSMOW)." |
| 20 | Page 7<br>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. | We agree and will change the sentence as suggested to "To determine the isotopic composition of nitrogen in particulate organic matter (POM), the food source for mussels that presents the direct link between nitrate and the mussels, 0.7 – 2.5 L…". |
| 21 | Page 7<br>1b Your hypothesis #2 is that mussel 15N corresponds to nitrate 15N, no? But here you say that mussels feed on POM so the reader is confused by the nitrate 15N hypothesis. You should rectify this earlier in the introduction somehow. Either focus on POM or state how N cycling would link nitrate and POM 15N composition. | We agree that we have made the link between $^{15}$N of nitrate, POM and mussels not clear enough. We will therefore explain this better in the introduction by adding the following sentences:<br><br>Page 4: Line 4: "This signal is then passed on to higher trophic levels up the food chain (Cabana and Rasmussen, 1994; Carvalho et al., 2015): Elevated $\delta^{15}$N signals in nitrate have been shown to lead to elevated $\delta^{15}$N signals in organisms that directly take up nitrate from the water, such as phytoplankton and microbes (Harrington et al., 1998). These organisms form an important part of particulate organic matter (POM), which serves as food for filter feeders (e.g., mussels). Mussels that ingest POM with elevated $\delta^{15}$N signal will then also show a higher $\delta^{15}$N signal." |

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| | | Page 7 Line 1: “To determine the isotopic composition of nitrogen in particulate organic matter (POM), the food source for mussels that presents the direct link between nitrate and the mussels,…” |
| 22 | Page 7<br>1c You state that mussel 15N and POM 15N are linked but you don’t show in a figure. And the link between 15N POM and 15N NO3 is also not discussed in the results. | We believe that you refer to Page 9.<br>We agree and will include the figure showing the significant, positive relationship between mussel and POM $\delta^{15}N$ and we think it will be helpful for the reader, because in the improved manuscript version we will discuss more results regarding POM and its relationship to mussel nitrogen stable isotope values. (see also your comment #48)<br>We will also include a sentence on the relationship between $\delta^{15}N$ of POM and nitrate. This will read as follows: “The relationship between $\delta^{15}N$ of POM and nitrate was not significant; however this calculation was based on only five data points where simultaneous measurements of the two $\delta^{15}N$ values were available, making this result arguable.” |
| 23 | Page 7<br>4 Change to “Harvested mussels were measured and dissected to obtain the foot tissue. . .” | We agree and will change it as suggested to: “Harvested mussels were measured and dissected to obtain the foot tissue for stable isotope analysis.” |
| 24 | Page 7<br>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. | We agree that the description was unclear. We will therefore change it to:<br>“The feet of three individuals per site were combined, dried at 60°C for at least 24 h, fully homogenized with mortar/pestle, and stored in a desiccator until a subsample was analysed for mussel $\delta^{15}N$ and C:N ratio.” |
| 25 | Page 8:<br>4 long term average based on how many years? Citation? | We agree and will add the years the average was based on and the citation. The sentence will now read “Rainfall was below average in 2010 with 421 mm for the entire sampling period, while the average for this period for the previous 17 years was 690 mm (1993-2009; Bureau of Meteorology, 2016).” |

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| 26 | Page 8:<br>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. | We agree with this comment and will report the average discharge for 1993-2009 and the minimum and maximum values within this period. These values are taken from the Department of Water data base, which we will cite. It will now read as follows: "This resulted in a lower than usual discharge from the tributaries into the estuary with a mean discharge from the Swan River of 1.2 x $10^5$ $m^3$ $d^{-1}$ in 2010 compared to an average discharge of 8.4 x $10^6$ $m^3$ $d^{-1}$ for the period of 1993-2009 for the same season (min. – max: 1.99 x $10^6$ $m^3$ $d^{-1}$ (2002) – 2.21 x $10^7$ $m^3$ $d^{-1}$ (1996) (Department of Water, 2016)." |
| 27 | Page 8:<br>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).<br><br>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. | We agree with this comment and will add a citation from a previous study, which reports on salinity in this estuary. We will also add that seawater has a salinity of 35. The section will now read: "This might have contributed to higher salinities throughout the entire estuary during this study than previously reported (Stephens and Imberger 1997) and no relationship between salinity and distance to the estuary mouth was detected. During high tide, the salinity at all sites was between 24.2 and 32.4 and there was no difference in salinity between sites which can be considered brackish to saline (salinity of seawater is 35)."<br><br>We believe that this information together with the description of the Swan River Estuary (2.1 Study site) will now be sufficient to understand the dynamics of salinity in this estuary.<br><br>We would like to note that salinity does not have a unit as it is a ratio of the conductivity of a seawater sample and a standard potassium chloride solution (see UNESCO (1985): The international system of units (SI) in oceanography, UNESCO Technical Papers No. 45, IAPSO Pub. Sci. No. 32, Paris, France.) We will therefore not include a unit. |
| 28 | Page 8:<br>31 Change to "while nitrogen from $NH_4^+$ was greater at all other sites (Fig. 2)". | We agree and will change it as suggested. |

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| 29 | Page 8:<br>31 Can omit sentence starting with "This is supported by significant. . .". It doesn't add much value compared to previous sentence. | We agree and this section will now read: "On average, $NO_x$ was the dominant N source at MC, SCC and WO, while nitrogen from $NH_4^+$ was greater at all other sites (Fig. 2) (Kruskal Wallis one-way ANOVA, H = 59.0, df = 6). |
| 30 | Page 9:<br>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). | We agree and we will change this to: "The TN:TP ratio (weight) was between 0 and 6.5 with 84% of the samples in our study being below 2.2, indicating a high possibility of nitrogen limitation in this system (Redfield 1958; Geider and La Roche, 2002). |
| 31 | Page 9:<br>18 "Analysis of stable isotope composition of NO3. . ."<br>Change 'signature' throughout unless you're really talking about the uniqueness of a component's isotopic composition. | We agree and will change this as suggested here and elsewhere throughout the manuscript (e.g., Page 12 Line 30). |
| 32 | Page 9:<br>19 restate minimum concentration requirements | We agree and will include the minimum concentration requirement. It will now read as: "Analysis of the stable isotope composition of $NO_3$ was limited to a total of 25 samples that fulfilled nutrient concentration requirements for the analysis (0.71 µM $NO_3$-N)." |
| 33 | Page 10:<br>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. | We agree that this was unclear. We will delete the part about the fractionation, as it is not relevant for the message of the paper. |
| 34 | Page 10:<br>5 Move this sentence second in the paragraph. Move second sentence to the first sentence position. | We agree and will change the position of these two sentences. |

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| 35 | Page 10:<br>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. . | We agree and have changed this to: "Values of $\delta^{15}N$ of mussels varied between 6.8 and 10.3 ‰ and the range was therefore smaller than the range seen in nitrate $\delta^{15}N$ (-1.3 and 10.4 ‰)." |
| 36 | Page 10:<br>8 use lower case , not _. It would be better to rephrase the sentence so you are not starting with a greek letter. | We agree and will restate the sentence. It will now read as: "Mussel $\delta^{15}N$ was significantly different between sites (one-way ANOVA; $\delta^{15}N$: $F_{6,98}$ = 42.53) (Fig. 5) and increased significantly with distance from the estuary mouth ($r^2$ = 0.563, y = 0.12x+7.74, $F_{1,110}$ = 141.65) (Fig. 6)." |
| 37 | Page 10:<br>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'. | We believe that this comment refers to the following sentences (page 10 Lines 7-10): "No temporal trend in mussel $\delta^{15}N$ was detected (Fig. 4). $\Delta^{15}N$ of mussels was significantly different between sites (one-way ANOVA; $\delta^{15}N$: $F_{6,98}$ = 42.53) (Fig. 5) and mussel $\delta^{15}N$ increased with increasing distance from the estuary mouth (Fig. 6)."<br><br>We will change the second sentence as suggested in the previous comment (#36) and also add that the increase with distance from the estuary mouth was significant. As the two sentences are two separate analyses(the first is a temporal, the second a spatial analysis), we will not combine these sentences. It will now read as: "No significant relationship between mussel length and mussel $\delta^{15}N$ (linear regression; $F_{1,13}$ = 2.235) was found. No temporal trend in mussel $\delta^{15}N$ was detected (Fig. 4). Mussel $\delta^{15}N$ was significantly different between sites (one-way ANOVA; $\delta^{15}N$: $F_{6,98}$ = 42.53) (Fig. 5) and increased significantly with distance from the estuary mouth ($r^2$ = 0.563, y = 0.12x+7.74, $F_{1,110}$ = 141.65) (Fig. 6)." |
| 38 | Page 10:<br>Figures 3, 5 and 7 all strongly influenced by MC site. | We agree and will discuss the strong influence of MC on relationships at various places throughout the manuscript. For instance,<br><br>Page 9 Line 11: "The concentrations of total dissolved inorganic nitrogen …. were higher towards the estuary mouth (Fig. 2), although these relationships were weak …. and were driven by site MC only."<br><br>Page 11 Line 13: "In the Swan River estuary, $NO_3$ was enriched and there was a positive relationship between nitrate $\delta^{15}N$ and the concentration of |

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| | | $NO_x$ throughout the estuary, although this was strongly driven by site MC."<br><br>Page 12 Line 7: "Our study showed a positive relationship between food (POM) and mussel $\delta^{15}N$, but a negative relationship between nitrate $\delta^{15}N$ and consumers (mussels), which was strongly affected by site MC."<br><br>Page 12 Line 21: "The relationship between mussel $\delta^{15}N$ and TDIN concentration within the estuary was much stronger when omitting site Cl and not significant when omitting site MC."<br><br>In addition, we will include a paragraph in which we will interpret the data without site MC (Page 12 Line 31):" Site MC was closest to the ocean, was one of the deepest sites and had a higher TDIN concentration compared to all other sites, which were not different with regard to TDIN concentration between them. This emphasises that the differences in mussel $\delta^{15}N$ between sites might be due to site-specific nutrient cycling processes in our estuary and might not reflect nitrogen pollution itself."<br><br>This will not change our overall conclusion that mussels can be used as indicators for site-specific differences in pollution or nutrient cycling, which is "…important information for local management, but would have gone undetected at high pollution levels as the larger deviations of nitrogen stable isotope values would have made such small differences in mussel values invisible." (page 14 Line 8) |
| 39 | Page 10:<br>Figure 5. You show scenarios with and without CI or MC sites, was WO site included in regressions? | We did not include the marine site (WO) in the regression. We agree that this has not been described clearly and we will now include the following sentence in the figure legend: "WO was not included in the regressions." There are two reasons why we did not include WO in the regressions: 1) the N-cycle is likely to be different in the estuary compared to the marine environment; 2) The purpose of this paper is to identify if mussels can be used as bioindicators within a system, which would be the estuary in our case. As such, including the marine site is not relevant but would rather confound trends and findings. The purpose of showing WO is purely to |

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| | | provide a baseline for a marine environment. |
| 40 | Page 10:<br>31 avoid using 'site-specific' twice in same sentence. Restructure | We agree and will substitute the second "site-specific" by "spatial". |
| 41 | Page 11<br>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. | We agree and by adopting changes from this and your other comments, the discussion of our data will be structured as follows:<br>1) nitrogen concentrations in the estuary (spatial; upstream/downstream)<br>2) discussion of nitrate $\delta^{15}N$ values (site specific; processes that lead to differences between these values).<br>3) Mussel $\delta^{15}N$ between sites and relationship between nutrient concentrations<br>4) Mussels and distance from estuary mouth<br>5) Mussel $\delta^{15}N$ over time and suitability as indicators |
| 42 | Page 11:<br>24 What do you mean by this sentence. Expand more. How does the fraction of NOx 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 | We agree that this paragraph was unclear and needed expanding. We will restate it as follows: "The fraction of NOx of the TDIN pool (%) was significantly different between sites (data not shown; $y = 0.15x-6.9$, $r^2 = 0.215$, $F_{1,23} = 6.30$, $P < 0.05$), with site MC having a higher mean fraction (mean = 62.5%) compared to all other sites, except for SCC. An earlier study by Sugimoto et al. (2009) also found a positive relationship between nitrate $\delta^{15}N$ values and the nitrate fraction in TDIN which they explained by *in situ* isotopic effects during nitrification. However whether higher $\delta^{15}N$ values of nitrate at MC are related to site specific nitrification rates in our estuary needs further investigation, because the $\delta^{18}O$ and $\delta^{15}N$ values of nitrate are rather representative of atmospheric $NO_3$ deposition values (Durka et al., 1994; Fang et al., 2011) and nitrification is likely to play a minor role at ammonium concentrations <5 µM (Day et al., 1989) that prevail in the Swan River estuary. |
| 43 | Page 12<br>First two sentences are redundant, simplify and merge. | We agree and will restated these two sentence as following: "Earlier studies indicated that nitrogen $\delta^{15}N$ values are reflected in higher trophic levels in a |

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| | 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. | predictable way with a positive relationship between $\delta^{15}N$ of nitrate, primary producer and primary consumer (e.g., mussels) (Cabana et al., 1994; Cabana and Rasmussen, 1996; Harrington et al., 1998; Oczkowski et al., 2008; Carvalho et al., 2015)."<br><br>We will mention the fact that the trends are strongly driven by site MC in the following places (please also see our reply to your comment #38):<br><br>Page 9 Line 11: "The concentrations of total dissolved inorganic nitrogen …. were higher towards the estuary mouth (Fig. 2), although these relationships were weak …. and were driven by site MC only."<br><br>Page 11 Line 13: "In the Swan River estuary, $NO_3$ was enriched and there was a positive relationship between nitrate $\delta^{15}N$ and the concentration of $NO_x$ throughout the estuary, although this was strongly driven by site MC."<br><br>Page 12 Line 7: "Our study showed a positive relationship between food (POM) and mussel $\delta^{15}N$, but a negative relationship between nitrate $\delta^{15}N$ and consumers (mussels), which was strongly affected by site MC."<br><br>Page 12 Line 21: "The relationship between mussel $\delta^{15}N$ and TDIN concentration within the estuary was much stronger when omitting site Cl and not significant when omitting site MC." |
| 44 | Page12:<br>21 Relationship can't be 'higher'. The r2 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. | We agree and will exchange the word "higher" with "stronger" and will also add the word "within the estuary" so that the sentence will read as follows: "The relationship between mussel $\delta^{15}N$ and TDIN concentration within the estuary was much stronger when omitting site Cl and not significant when omitting site MC." |
| 45 | Page12:<br>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 | We agree that we have to discuss site MC in more detail, even if it can only be speculative only. Rather than having a trend within the estuary, it could be that mussel isotope values are affected by different processes that are happening on a spatial scale within the estuary. This would blur a clear interpretation of the data. The two sites that strongly affect any relationship |

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| | 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 NO3. 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. | are Cl and MC, therefore the manuscript will include the following:<br>- Cl: A likely explanation for why Cl is different is already described in detail in the discussion (Page 12 Line 21)<br>- MC: We already discussed the idea that the low $\delta^{15}N$ of mussels at MC (and therefore the negative relationship with TDIN) is due to the fact that at higher nitrogen concentrations can lead to primary producers being choosier which leads to a negative relationship between nutrient concentration and mussel (Page 12 Line 1-20). We will further explore the idea that MC is different by adding (Page 12 Line 31): "Site MC was closest to the ocean, was one of the deepest sites and had a higher TDIN concentration compared to all other sites, which were not different with regard to TDIN concentration between them. This emphasises that the differences in mussel $\delta^{15}N$ between sites might be due to site-specific nutrient cycling processes in our estuary and might not reflect nitrogen pollution itself."<br>We would further like to thank the reviewer for their idea that POM is originating from outside the estuary (upstream). This is a very interesting speculation and we will add this into the discussion on page 12 Line 21as follows: "An alternative explanation would be that POM could originate upstream where nitrate might have had higher $\delta^{15}N$ values (not quantified in this study). Upon entering the estuary, POM mixes with estuarine POM, uncoupling the within-estuary $\delta^{15}N$ nitrate and POM $\delta^{15}N$ values. This could also explain the strong relationship between $\delta^{15}N$ in mussels and the distance from the estuary mouth found in our study. Such a strong relationship can be expected in estuaries with low pollution levels due to the aforementioned mixing, while little spatial variability in $\delta^{15}N$ values of primary consumers can be expected in heavily polluted estuaries due to the dominance of upstream POM, as was shown by Oczkowski et al. (2008)." |
| 46 | Page 13<br>18 but your nitrogen sources of nitrate and ammonium were not different between sites (except MC) so this seems | We agree that differences in POM $^{15}N$ might drive differences in mussel $^{15}N$ and that this could be reflected in relationship between mussel $^{15}N$ and distance from the estuary mouth. We will therefore delete this section and |

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| | 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. | incorporate parts of it earlier within the discussion, specifically where we will now discuss the strong relationship between mussel $^{15}N$ and distance to estuary mouth (page 12 Line 2): "An alternative explanation would be that POM could originate upstream where nitrate might have had higher $\delta^{15}N$ values (not quantified in this study). Upon entering the estuary, POM mixes with estuarine POM, uncoupling the within-estuary $\delta^{15}N$ nitrate and POM $\delta^{15}N$ values. This could also explain the strong relationship between $\delta^{15}N$ in mussels and the distance from the estuary mouth found in our study. Such a strong relationship can be expected in estuaries with low pollution levels due to the aforementioned mixing, while little spatial variability in $\delta^{15}N$ values of primary consumers can be expected in heavily polluted estuaries due to the dominance of upstream POM, as was shown by Oczkowski et al. (2008)." |
| 47 | Page 13<br>18 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? | We agree and will weaken the dependency of the discussion using relationships only. We will do this by<br><br>i) Acknowledging that the relationships are strongly driven by MC (see also our reply to your comment #38 & #43): - Page 9 Line 11: "The concentrations of total dissolved inorganic nitrogen …. were higher towards the estuary mouth (Fig. 2), although these relationships were weak …. and were driven by site MC only." - Page 11 Line 13: "In the Swan River estuary, $NO_3$ was enriched and there was a positive relationship between nitrate $\delta^{15}N$ and the concentration of $NO_x$ throughout the estuary, although this was strongly driven by site MC." - Page 12 Line 7: "Our study showed a positive relationship between food (POM) and mussel $\delta^{15}N$, but a negative relationship between nitrate $\delta^{15}N$ and consumers (mussels), which was strongly affected by site MC." - Page 12 Line 21: "The relationship between mussel $\delta^{15}N$ and TDIN concentration within the estuary was much stronger when omitting site Cl and not significant when omitting site MC."<br><br>ii) Interpreting the data without site MC (Page 12 Line 31):" Site MC was closest to the ocean, was one of the deepest sites and had a higher TDIN |

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| | | concentration compared to all other sites, which were not different with regard to TDIN concentration between them. This emphasises that the differences in mussel $\delta^{15}N$ between sites might be due to site-specific nutrient cycling processes in our estuary and might not reflect nitrogen pollution itself."<br><br>This will not change our overall conclusion that mussels can be used as indicators for site-specific differences in pollution or nutrient cycling, which is "…important information for local management, but would have gone undetected at high pollution levels as the larger deviations of nitrogen stable isotope values would have made such small differences in mussel values invisible." (page 14 Line 8) |
| 48 | Page 13<br>18 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? | - We agree and will add more results for POM in the results section (3.4. Stable isotope values of POM). This will then read as: "POM $\delta^{15}N$ values were between 6.2 and 9.9 ‰ with no significant difference between sites ($F_{6,25}$= 1.327). A weak but significant negative relationship between POM $\delta^{15}N$ values and TDIN concentration was detected ($r^2$ =0.163, y = -0.044x + 9.37, $F_{1,28}$ = 5.44), while a significant positive relationship between nitrogen stable isotope signatures of POM and mussels was found ($r^2$ =0.303, y = 0.20x + 7.40, $F_{1,14}$ = 6.08) (Fig. 4 NEW). The relationship between $\delta^{15}N$ of POM and nitrate was not significant; however this calculation was based on only five data points where simultaneous measurements of the two $\delta^{15}N$ values were available, making this result arguable."<br><br>- We will also include the figure showing the positive relationship between mussel and POM $\delta^{15}N$ (new Figure 4) (please also see your comment #22)<br><br>- We will also discuss the strong trend between $^{15}N$ of mussel and distance from estuary mouth as follows:"…. An alternative explanation would be that POM could originate upstream where nitrate might have had higher $\delta^{15}N$ values (not quantified in this study). Upon entering the estuary, POM mixes with estuarine POM, uncoupling the within-estuary $\delta^{15}N$ nitrate and |

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| | | POM $\delta^{15}N$ values. This could also explain the strong relationship between $\delta^{15}N$ in mussels and the distance from the estuary mouth found in our study. Such a strong relationship can be expected in estuaries with low pollution levels due to the aforementioned mixing, while little spatial variability in $\delta^{15}N$ values of primary consumers can be expected in heavily polluted estuaries due to the dominance of upstream POM, as was shown by Oczkowski et al. (2008)" |
| 49 | Page 14<br>- 4 correlated to nitrogen concentrations. But these were all negative correlations, no? | - We agree that this sentence might have been misleading for the reader. To avoid this we will delete "…that correlated to differences in nitrogen concentrations...". |
| 50 | Page 14<br>15N-mussel negatively correlated to 15N-NO3. 15N-NO3 positively (though MC weighs heavily) related to NOx 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? | - We agree with your comment that in our system mussels are not good indicators for NO3 sources. We believe that this is due to the fact that this estuary showed low nitrogen pollution during the study period (e.g., page 31 Line 31). However, because there are stable differences between sites, we like to argue that mussels are still good indicators for site specific nutrient cycling. Please also see our reply to your comment #5.<br><br>To reflect what we have just said, we will rewrite this section as follows: "The stable spatial differences in mussel $\delta^{15}N$ values over time highlight the value of this organism as a bioindicator of spatial water quality assessment. The negative trends between mussel $\delta^{15}N$ values and nitrate concentration or nitrate $\delta^{15}N$ values emphasize that mussels might not be good indicators for $NO_3$ sources in systems with low pollution levels. Instead, the small differences in mussel stable isotope signatures might reflect differences in site specific nutrient cycling caused by physicochemical conditions or biological factors rather than nitrogen pollution." |
| 51 | Page14:<br>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 | We agree with this comment and will simplify or conclusion to: " The stable spatial differences in mussel $\delta^{15}N$ values over time highlight the value of this organism as a bioindicator of spatial water quality assessment. The negative trends between mussel $\delta^{15}N$ values and nitrate concentration |

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| | 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. | or nitrate $\delta^{15}N$ values emphasize that mussels might not be good indicators for $NO_3$ sources in systems with low pollution levels. Instead, the small differences in mussel stable isotope signatures might reflect differences in site specific nutrient cycling caused by physicochemical conditions or biological factors rather than nitrogen pollution.” |