Dear Referee #2,

Thank you very much for your very helpful 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 and improve the clarity of this manuscript.

We have now prepared replies to all of your comments and would here like to present how we will incorporate them into the next version of the manuscript.

We will first reply to your general comments, before we will discuss how we will address each of your specific comment in more detail.

1) General comments

No.	Comments	Response
1	[Structure] The article is well written and structured. When going first through the manuscript, I had the impression that the introduction was pretty long (it is almost 0.25% of the text). Having said that, there is a lot of useful information and references included. One option could be to shorten a bit the introduction, or to introduce a few sub-headings in order to make it an easier read: basically it is about (1) increasing human impact on aquatic ecosystems, (2) the need for a better understanding of the spatial and temporal variability of pollution levels with a view to better manage these often irreversibly impacted systems, (3) the focus on nutrient pollution, (4) the use of stable isotopes (especially of N) for investigating anthropogenic nutrient pollution, and (5) the introduction of mussels as a sentinel organism in that specific context.	We agree that the introduction is long and will shorten it. We will i) delete part of the first paragraph (Page 2 Line 9 – 13) and ii) delete the first sentence of the second paragraph to make sure that we get to the point more quickly. By this, we hope to achieve 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 large community of readers.

2) Specific comments

2	1-Introduction [pages 2-4]: When reading the introduction, and more specifically the paragraphs to the end where mussels are introduce as sentinel organisms, I was surprised (unless I am mistaken) not to learn about what species have eventually been used for this study. I think this is a very important aspect that the authors have not taken into consideration for their manuscript. In an area where they expect living organisms to be a living archive of the local average environmental conditions it is essential to know a minimum about the metabolism of that organism. Especially in a journal that has a large community of readers from hydrological sciences, we cannot necessarily expect them to know much about this topic. Moreover, since this is a kind of proof-of-concept study, the authors should carefully describe the organisms, growth rates, sensitivity to changing environmental conditions etc. These aspects are likely to be crucial when it comes to eventually understand and discuss the isotopic signatures of N in the mussel's foot tissue. As mentioned further down in this assessment, there is existing literature in this respect and it would certainly be of value to take this into consideration in a revised version of the manuscript.	We agree and will include the species that we used (i.e. blue mussel, *Mytilus edulis*) in various places within the manuscript. The sentences will now read as follows: Abstract: Page 1 Line 20: "The main aim of this study was to assess the suitability of nitrogen stable isotope as measured in mussels (*Mytilus edulis*), as an indicator able to resolve spatial and temporal variability of nitrogen pollution in an urban, tidally influenced estuary (Swan River estuary; Western Australia)." Introduction: Page 4 Line 9: "Bivalves on the other hand, which include the blue mussel are primary consumers with limited movement, and have been suggested as suitable site-specific bioindicators of time-averaged persistence of nutrient pollutants, because their isotopic signature fluctuates less than that of their food sources due to longer tissue turnover rates (Raikow and Hamilton, 2001; Post, 2002; Fukumori et al., 2008; Fertig et al., 2010)." Introduction: Page 4 Line 21: "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 blue mussel (*Mytlius edulis*) tissue as an indicator of nitrogen pollution in urban water systems." Materials and methods: Page 5 Line 19: "Seven sites within the Lower Swan River estuary were sampled 6 times for blue mussels and 9 times for nutrients,…" Materials and Methods: Page 6 Line 10: "Nine blue mussels per site were randomly taken from the pylons of the jetties at each site from between 20 and 40 cm depth and brought into the laboratory on ice in bags containing

		water from the respective site.: We will further include a short paragraph to introduce this mussel species (Page 4 Line 13) in the introduction: "The blue mussel, *Mytilus edulis*, is a common sessile bivalve in estuarine and marine environments that is able to adapt to a wide range of environmental conditions, such as food concentration, temperature and salinity (e.g., Thompson and Bayne, 1974; Widdows et al., 1979; Zandee et al., 1980; Almadavillela, 1984), and that shows low sensitivity to anthropogenic pressures (Mainwaring et al., 2014). As such, this species is able to thrive at different pollution levels and has therefore been used as an indicator species for pollution (Phillips, 1976) and as a model organism for physiological, genetic and toxicological studies (Luedeking and Koehler, 2004) for some time."
3	2-Material and methods [page 5 study sites & 6 sampling and analyses]: When reading the changing conditions in the Swan River estuary, one could expect differences between mussel species that are exposed to these fluctuations in salinity (between high tide and low tide). Is there only one mussel species in the studied area? If not (what is very likely), what are the other species that are present – what species has the sampling protocol been targeting – was it a mix of species – how sure can we be that different sensitivities to changing environmental conditions (including pollution) can lead to differences in metabolic activity?	We believe that this comment directly links to your previous comment #2 and by clarifying that we only used one species (blue mussel, *Mytilus edulis*) we believe this comment has been addressed by our previous reply. We would like to note that by using only one species we made sure that differences in metabolisms are restricted to within-species variability.
4	3-Results [page 8 physicochemical parameters]: given that the study was carried out during rather dry conditions, the prevailing environmental parameters measured in the investigated area have also been rather unusual as stated in the manuscript. Here again, it would be interesting to see how the mussels populations have responded to that (if at all) – is there any information available on that?	We agree that conditions were unusually dry during our study. Unfortunately there is no previous data on this mussel population (e.g., abundance, physiology) that could be used for comparison with our study. However, we would like to emphasise that blue mussels are known to adapt well to varying conditions (will be state in the new version of the manuscript on Page 4 Line 13) and that, because the mussels within the estuary all experienced the same conditions, these dry conditions will not

		affect our conclusions.
5	3-Results [page 8 physicochemical parameters]: On page 8, line 10 units should be added to salinity.	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.
6	3-Results [page 8 physicochemical parameters]: On page 10 the delta symbol should be homogenised.	We agree and will homogenised delta symbols by avoiding using them as a capital at the beginning of a sentence.
7	4-Discussion [page 13]: In lines 6 to 8 I would be careful when stating that stable isotope signatures in mussels of tidally influenced estuaries are less impacted by seasonal changes in watershed input and chemistry compared to large rivers. This statement make sense considering the results of this study, but given the particularly dry conditions that prevailed during this investigation and the proof-of-concept character of this study, there need most probably to be more investigations before a strong statement in this sense.	We agree with this and have weakened this statement by restating it as follows: “Our results therefore indicate that while high seasonal variations of stable isotope signature in mussels can be connected to seasonal changes in watershed input and chemistry in large rivers (Fry and Allen, 2003), this is less pronounced in tidally influenced estuaries or during drier conditions with low freshwater input.”
8	5-Conclusion [page 13]: A similar comment as for the point above can be made for the 1st paragraph of the conclusion.	We agree and will rewrite the second sentence of the first paragraph as: “As such, stable isotope analysis of a model organism, such as the blue mussel can deliver essential information for future decentralised water management practices that are focused on local process understanding.”
9	5-Conclusion [page 13]: Of interest could also be to see if there are differences in signatures between species.	We only analysed stable isotope signature of one species, blue mussel, which we now clarified throughout the manuscript as show in our reply to your comments #2 and #3.
10	5-Conclusion [page 13]: In the conclusion it is stated that the future studies should contribute in similar (low) polluted systems to better understand the baseline of spatial natural isotopic variability in urban aquatic systems. I was wondering if	We agree that this was misleading and we will therefore restate this sentence, which is an additional suggestion for future studies to gain a better understanding of systems with varying and partly low pollution levels. We will rewrite it as follows: “In addition, we advocate future studies in similarly (low) polluted systems that include stable isotope

	this is not somehow contradictory with what is announced in the title – are mussels then really used in the sense of sentinels of pollution or rather as indicators of the baseline of 'spatial natural isotopic variability in urban aquatic systems'. Again here I am possibly confused by the fact that no information is given on how sensitive those organisms are eventually to pollution.	analysis of other food web end-members and nutrients of the groundwater, to develop baselines of spatial natural isotopic variability in urban aquatic systems which will help identifying the importance of local biogeochemical processes for pollution control." We believe that this is reflected in the title. We agree that this information has been missing and will include that blue mussels are not very sensitive to pollution by human activities. As such, this organism is able to thrive at different pollution levels indicating that their stable isotope signature should be an ideal indicator to identify differences in pollution levels. We will include this in the introduction (Page 4 Line 13): "The blue mussel, *Mytilus edulis*, is a common sessile bivalve in estuarine and marine environments that is able to adapt to a wide range of environmental conditions, such as food concentration, temperature and salinity (e.g., Thompson and Bayne, 1974; Widdows et al., 1979; Zandee et al., 1980; Almadavillela, 1984), and that shows low sensitivity to anthropogenic pressures (Mainwaring et al., 2014). As such, this species is able to thrive at different pollution levels and has therefore been used as an indicator species for pollution (Phillips, 1976) and as a model organism for physiological, genetic and toxicological studies (Luedeking and Koehler, 2004) for some time."
11	5-Conclusion [page 13]: As a last comment, one could also say that nutrient pollution is not really an urban problem or at least the origin of it can most of the time be found further upstream in agricultural parts of the catchments. In urban environments, one could also be targeting other sources of pollution, such as heavy metals, xenobiotics, etc.	We agree with this and we have mentioned its future application as sentinels for non-nutrient co-occurring pollutants (such as oils, heavy metals) in the abstract (last sentences), and the conclusion (Page 13 Line 22).
12	Concluding remarks: This manuscript is certainly a very interesting contribution for the readers of this journal and I enjoyed very much reading it. It is an interesting case study – or more specifically a proof-of-concept study –	We agree that using mussels as an indicator for pollution is not new and we will include citations for that (e.g. see our reply to your comment #2 and #10) to weaken the innovative aspect of this study. We further agree with you that the use of this approach in an urban, tidally influences estuary

	introducing mussels as a sentinel organism for investigating nutrient pollution in an urban aquatic environment. Since existing literature on similar applications/studies is not much referred to in the manuscript, the innovative character of this study might however be slightly overrated.	makes this study novel and interesting and that it presents a proof-of-concept study.