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Interactive comment on “Contribution and pathways of diazotroph derived nitrogen to zooplankton during the VAHINE mesocosm experiment in the oligotrophic New Caledonia lagoon” *by* B. P. V. Hunt et al.

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Anonymous Referee #2

General comments Several studies have indicated DDN can significantly contribute to the food web base of zooplankton in systems where diazotrophs are important. Using a stable isotope approach, Montoya et al. (2002) found that the contribution of DDN to the food web base in the oligotrophic North Atlantic Ocean ranged from 0 – 67%. Rolff (2000) also found utilization of fixed N (DDN) by the zooplankton community in summer in the Baltic Sea. However questions remain as to the exact mechanisms

whereby DDN enters the zooplankton food web. Many studies consider indirect paths, that is, diazotroph release of DIN and DON (Capone et al., 1994; e.g., Ploug et al., 2011) and uptake of this N by the microbial loop, to be the major mechanism of DDN contribution to zooplankton. Evidence of direct grazing on diazotrophs has been more elusive, and has been considered limited due to a number of factors including toxicity of cyanobacteria (Sellner, 1997). The study by Hunt et al. represents an advance in that it demonstrates using qPCR that zooplankton ingest many diazotrophs (at least the Trichodesmium spp., het-1, het- 2, and UCYN-C present in their experiments). They also demonstrate for the first time using 15N labeling experiments the direct ingestion and assimilation of DDN from UCYN-C, but little assimilation of DDN from Trichodesmium spp. or het-1. Unicellular cyanobacteria (e.g., UCYN-C) can have abundances and N2 fixation rates greater than the more traditionally considered Trichodesmium spp. (Moisander et al., 2010), but few studies have examined the potential transfer of this new nitrogen to zooplankton. Thus this study indicates grazing of UCYN-C by zooplankton may be an important mechanism for transfer of DDN up the marine food web.

Hunt et al. also quantify the contribution of DDN to the base of the zooplankton food web using a two-endmember mixing model based on zooplankton _15N values throughout the mesocosm experiment. This is a powerful approach, and has been used successfully in several studies, however there are a few issues.

First, errors should be considered in the mixing model. The model makes several assumptions concerning endmembers (page 10 lines 17-22). Namely, TEF is assumed to be 2.2‰ the N isotope composition of diazotrophs is assumed to be -2‰ and a δ15N value for zooplankton assuming a solely nitrate-based food web assumed to be 4.5‰ (nitrate) + 2.2‰ (TEF) = 6.7‰ What are the errors on these estimates and how do they propagate into the final %DDN contribution? Diazotroph δ15N values range between -1 to -2‰ for example (Montoya et al., 2002). The TEF of consumers raised on plant and algal diets is 2.2 ± 0.3‰ (McCutchan Jr. et al., 2003). However no errors are reported for %ZDDN (Figure 5), and thus the significance of the increase %ZDDN over the experiment (page 16 lines 30-31) is not clear. Similarly, what are the errors associated with the calculation of % daily DDN production ingested (Figure 5)?

Author: Calculation of error margins for our estimates of 1. diazotroph nitrogen contribution to zooplankton biomass and 2. % daily DDN production ingested consumed, is an important point. This will certainly improve the quality of our estimates. We have calculated the min and max values for both 1 and 2 taking into account the error in TEF estimate (2.2± 0.3, following McClutchan et al 2003), and a range of diazotroph δ15N values between -1 and -2‰ (following Montoya et al., 2002), using -1.5 as the mean value. The min and max values will be included as error bars for measures 1 and 2 in Figure 5.

A more difficult issue is in the choice of the reference endmember for the mixing model. The reference endmember is the δ15N value for zooplankton assuming a solely nitrate based food web, here assumed to be 4.5‰ (the δ15N value of nitrate entering the system) + 2.2‰ (TEF) = 6.7‰ for reference zooplankton. However the study site in New Caledonia is a LNLC system where recycled nutrients, e.g., NH4+, are likely important for production. Thus the actual reference endmember should be zooplankton δ15N values assuming recycling of new NO3- entering the system. This recycling will result in 15N depleted NH4+ and consequently zooplankton δ15N values that are lower than the assumed δ15N -NO3- + TEF = 6.7‰ËŹ .g., reference zooplankton δ15N values in Montoya et al. (2002) ranged from 4.3 – 6.4‰ŹThe authors need to address how their choice of reference endmember affect %ZDDN, given recycling within the system.

Author: We agree with Reviewer 2 that the choice of model end member is difficult issue, and that we did not detail this sufficiently in the first version of our manuscript. Perhaps the most challenging aspect of this is that although the New Caledonia lagoon is a LNLC environment it is also an environment apparently strongly influenced by nitrogen fixation. It is therefore not possible to confidently select zooplankton samples from the lagoon that will not reflect at least some influence of diazotrophic nitrogen. Indeed, Montoya et al (2002) noted this specifically as an issue in their study. Although

they used a δ15N range of 4.3 – 6.4‰ as their zooplankton reference value, they noted:

“Because the reference zooplankton used in Eq. 2 may reflect some inputs of recently fixed nitrogen, the values shown in Table 2 are a conservative estimate of the role of diazotroph nitrogen in supporting zooplankton biomass production. In fact, measurements of the δ15N values of individual amino acids isolated from zooplankton collected at selected stations of leg 2 of cruise SJ9603 are consistent with a higher diazotroph contribution, approaching 100% at times, to the zooplankton in the western part of the transect (McClelland et al. pers. comm.).” page 1625, paragraph 1.

In a previous paper (Hunt et al. 2015) we recorded mean zooplankton grazer δ15N of 5.94‰ in the Low Nitrate Low Chlorophyll region east of New Caledonia. Given that there was likely some influence of diazotrophy in that region, the zooplankton end member value of 6.7‰ used in this study does seem to be a realistic estimate of δ15N not influenced by diazotrophic nitrogen.

Specific comments

1. P.2 line 15 – I find the phrase “% contribution of DDN to zooplankton biomass” somewhat confusing as it sounds like DDN is increasing zooplankton biomass. However this has been used in several studies (Montoya et al., 2002). The authors may want to consider if there is another phrase that may be more appropriate.

Author: We will clarify this as “% contribution of DDN to zooplankton nitrogen biomass”.

2. P.2 line 17 – What is BNF?

Author: This is a typo and has been removed from the abstract.

3. P.2 lines 21-24 – Consider rewriting this to make it more clear that all diazotrophs were ingested but only UCYN-C was assimilated significantly by zooplankton.

Author: We have re-worded these lines as “qPCR analysis targeting four of the common diazotroph groups present in the mesocosms (Trichodesmium, het-1, het-

2, UCYN-C) demonstrated that all four were ingested by copepod grazers, and that their abundance in copepod stomachs generally corresponded with their in situ abundance. 15N2 labeled grazing experiments therefore provided evidence for direct ingestion and assimilation of UCYN-C-derived N by the zooplankton, but not for het-1 and Trichodesmium, supporting an important role of secondary pathways of DDN to the zooplankton for the latter groups,. . .”

4. P.3 line 7 – What is sustaining 50% of primary productivity? I think they mean N2 fixation, but it sounds like they mean upwelled NO3-.

Author: We will clarify this sentence as follows:

“In the oligotrophic tropical and subtropical oceans, where strong stratification limits the upward mixing of nitrate replete deep water into the photic zone, this new N is particularly important, sustaining $\sim$50 % of primary productivity (Karl et al., 1997).

5. P.3 line 14 – Here and throughout the manuscript “δ15N” should be “δ15N value”.

Author: We will make this change.

6. P.3 line 17 – This would be true only in systems where N2 fixation is important. Clarify this. Which systems?

Author: This refers to phytoplankton δ15N where nitrate is the primary nitrogen source, i.e., “By comparison, the average ocean nitrate δ15N is $\sim$ 5 ‰ (Sigman et al., 1999; Sigman et al., 1997), leading to higher δ15N for primary producers using this source.”

7. P.4 line 19 – Reference for “reduced feeding and egg production: : : : :when fed a mixed cyanobacteria diet”?

Author: Sellner et al (1996) Phycologia, 35, 177-182. We will add this reference.

8. P.6 line 25-26 – Which poecilostomatoid copepods do you refer to? Do you mean all cyclopoids? E.g., http://copepodes.obs-banyuls.fr/en/?

Author: Poecilostomatoid are a separate order, previously included with the Cyclopoids.

http://www.marinespecies.org/aphia.php?p=taxdetails&id=155879

9. P.7 line 11 – Report all _15N values at the same sig fig throughout the study, e.g., 0.1‰ and 0.2‰Ź

Author: Yes. This will be done.

10. P.10 line 18 – Report TEF as 2.2‰Ź

Author: Yes. This will be done.

11. P.10 line 19 – Sig fig of -2‰

Author: Yes. This will be done.

12. P.11 line 29 – P.12 line 1 – What do you mean? N2 fixation in lagoon lower than mesocosm? Clarify.

Author: This is clarified in the text as follows:

“N2 fixation rates measured in the lagoon waters were significantly ($p<0.05$) lower than those measured in lagoon waters (9.2±4.7 nmol N L-1 d-1) over the 23 days of the experiment.”

13. P.12 line 2 – What did not differ?

Author: This will be clarified as follows:

“N2 fixation rates measured in the lagoon waters (average = 9.2±4.7 nmol N L-1 d-1) were significantly ($p<0.05$) lower than those measured in the mesocosm over the 23 days of the experiment.”

14. P.13 line 7 - Do you mean cyclopoid?

Author: No, we mean poecilostomatoid.

15. P.13 line 24 – Sig figs. 16. P.16 line 26 – Do you mean δ15N values of zooplankton?

Author: Correct, we will amend this to δ15N values.

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