

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.

Anonymous Referee #2

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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

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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 ^{15}N 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 N_2 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values range between -1 to -2‰ for example (Montoya et al., 2002). The TEF of consumers raised on plant and algal diets is $2.2 \pm 0.3\text{‰}$ (McCutchan Jr. et al., 2003). However no errors are reported for %ZDDN (Figure 5), and thus the significance of the increase %ZDDN

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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)?

A more difficult issue is in the choice of the reference endmember for the mixing model. The reference endmember is the $\delta^{15}\text{N}$ value for zooplankton assuming a solely nitrate-based food web, here assumed to be 4.5‰ (the $\delta^{15}\text{N}$ 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., NH_4^+ , are likely important for production. Thus the actual reference endmember should be zooplankton $\delta^{15}\text{N}$ values assuming recycling of new NO_3^- entering the system. This recycling will result in ^{15}N depleted NH_4^+ and consequently zooplankton $\delta^{15}\text{N}$ values that are lower than the assumed $\delta^{15}\text{N}\text{-NO}_3^- + \text{TEF} = 6.7\text{‰}$. E.g., reference zooplankton $\delta^{15}\text{N}$ values in Montoya et al. (2002) ranged from 4.3 – 6.4‰. The authors need to address how their choice of reference endmember affect %ZDDN, given recycling within the system.

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.
2. P.2 line 17 – What is BNF?
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.
4. P.3 line 7 – What is sustaining 50% of primary productivity? I think they mean N_2 fixation, but it sounds like they mean upwelled NO_3^- .
5. P.3 line 14 – Here and throughout the manuscript “ $\delta^{15}\text{N}$ ” should be “ $\delta^{15}\text{N}$ value”.
6. P.3 line 17 – This would be true only in systems where N_2 fixation is important. Clarify this. Which systems?
7. P.4 line 19 – Reference for “reduced feeding and egg production. . . .when fed a mixed cyanobacteria diet”?
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/>?
9. P.7 line 11 – Report all $\delta^{15}\text{N}$ values at the same sig fig throughout the study, e.g.,

0.1‰ and 0.2‰. P.10 line 18 – Report TEF as 2.2‰. P.10 line 19 – Sig fig of -2‰. P.11 line 29 – P.12 line 1 – What do you mean? N₂ fixation in lagoon lower than mesocosm? Clarify. P.12 line 2 – What did not differ? P.13 line 7 - Do you mean cyclopid? P.13 line 24 – Sig figs. P.16 line 26 – Do you mean $\delta^{15}\text{N}$ values of zooplankton?

References Capone, D.G., Ferrier, M.D., Carpenter, E.J., 1994. Amino acid cycling in colonies of the planktonic marine cyanobacterium *Trichodesmium thiebautii*. *Appl. Environ. Microbiol.* 60, 3989–3995. McCutchan Jr., J.H., Lewis Jr., W.M., Kendall, C., McGrath, C.C., 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102, 378–390. Moisander, P.H., Beinart, R.A., Hewson, I., White, A.E., Johnson, K.S., Carlson, C.A., Montoya, J.P., Zehr, J.P., 2010. Unicellular Cyanobacterial Distributions Broaden the Oceanic N₂ Fixation Domain. *Science* 327, 1512–1514. doi:10.1126/science.1185468 Montoya, J.P., Carpenter, E.J., Capone, D.G., 2002. Nitrogen fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic. *Limnol. Oceanogr.* 47, 1617–1628. Ploug, H., Adam, B., Musat, N., Kalvelage, T., Lavik, G., Wolf-Gladrow, D., Kuypers, M.M.M., 2011. Carbon, nitrogen and O₂ fluxes associated with the cyanobacterium *Nodularia spumigena* in the Baltic Sea. *Isme J.* 5, 1549–1558. doi:10.1038/ismej.2011.20 Rolff, C., 2000. Seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of size-fractionated plankton at a coastal station in the northern Baltic proper. *Mar. Ecol. Prog. Ser.* 203, 47–65. Sellner, K.G., 1997. Physiology, ecology, and toxic properties of marine cyanobacteria blooms. *Limnol. Oceanogr.* 45, 1089–1104.

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