Interactive comment on **"Peru upwelling plankton respiration: calculations of carbon flux, nutrient retention efficiency and heterotrophic energy production"** by **T. T. Packard et al.**

RESPONSE TO ANONYMOUS REVIEWER #2:

Introduction:

1. *Comment #1, Page 16178, line 24-25: Although for insiders it might seem trivial, I suggest to add some citations here*.
   Authors' Response: This knowledge comes from integrating current understanding of biochemistry, microbiology, biological, chemical oceanography with our own measurements of the deep-metabolism.

   Proposed change in the manuscript: We have added citations here to include works by G A Riley, F A Richards, Nick Lane, Nelson and Cox (Lehninger Biochemistry), Madigan et al (Microbiology), as well as our own.

2. *Comment #2, Page 16178, line 26: Even in anoxic seawater it produces CO2 ... – by it do you mean respiration or the ETS?*
   Authors' Response: It refers to respiration. Respiration produces CO_2 via the Krebs Cycle and other enzymatic pathways. The respiratory ETS is driven by the reducing equivalents produced by the Krebs Cycle and other biochemical pathways and, in turn, drives the production of ATP as well as the consumption of a suitable electron acceptor (O_2, NO_3, NO_2, NO, N_2O, SO_4, Fe^{+3}, MnO_4, etc). The ETS is associated with all these types of respiration.

   Change in the manuscript: We have specifically stated, "*: Even in anoxic seawater respiration produces CO_2...*" Furthermore, we have quoted FA Richard's paper, Anoxic Basins and Fjords from 1965.

3. *Comment #3, Page 16180, line 5: The paper by Giering et al. is not unequivocally accepted in the community mainly because they were not measuring respiration but derive it from other parameters. Thus, I would not use this paper as the hook to introduce the concept of deriving the carbon flux from respiration measurements*.
   Authors' Response: The paper by Giering et al (2014) serves as a useful foil, but the concept of using respiration as a way to calculate carbon flux and the reverse goes back to Riley (1951), Richards (1957), and Redfield et al. (1963). We have assumed the validity of this concept since Packard et al (1971). Furthermore, it is intuitively clear from Suess (1980), Eppley and Peterson (1979), and the VERTEX paper by Martin et al (1987). In addition, it served as the basis of the papers by Steinberg et al. (2008), Burd et al (2010), and McDonnel et al. (2014).

   Proposed changes in the manuscript: We have added most of the above references to the Introduction to clarify the argument.

4. Comment 4, Page 16179, line 21: Is the review by Aristegui et al. 2009 not the better citation here?

Authors' Response: Laufkötter et al. (2013) is more recent, but we can add Aristegui et al. 2009.

Proposed changes in the manuscript: We have added a reference to both F D King's (1978) and J R Arístegui's (2009) research.

Comment # 5, *Page 16180, line 13: Is the abbreviation EMF really needed; it does not appear anywhere else*.

Authors' Response and proposed change in the manuscript: We spelled out "electromotive force".

Methods:

Comment #6, In general I suggest to extensively edit the methods for clarity.

Authors' Response and proposed changes in the manuscript: We have completely revised the methods and the tables that illustrate and tabulate the calculations. Table 2 explains in detail the calculation of carbon flux from ETS activity. The other tables are included to show, in detail, how we calculated our results.

Comment #7. The first paragraph (page 16181 – research site) was confusing to me as it was mainly some history of the site that to my mind is better explained in the introduction. I could not find a reason why the authors report C-line numbers and separate station numbers. I suggest to report either C-line or station numbers throughout the text. If either number is important for special purposes (that the authors do not mention) they are in table 1 anyway.

. Authors' response: We used the term "location" to designate the geographical positions of a sampling site. We used the ship's station numbering system to designate the data from a particular station cast. The C-line locations were occupied many times. In our revision we made this clearer. We changed the text from the 4th sentence on to read as follows.

"*In addition, between 10 and 24 September, productivity stations, that focused on the biological, nutrient chemistry, and biochemical properties at depths where the light was 100, 50, 30, 15, 5, 1, and 0.1% of the surface incident radiation (light-depths), were made at C-Line positions Packard and Jones (1976). These productivity stations were not made in order along the C-line section, hence the irregularity of the their numerical sequence in tables 3-7. In addition, some locations along the C-Line were occupied several times. For this reason, as well as to coordinate the results presented here with the results of other CUEA reports (Brink et al., 1981), both the C-Line location and the station number are given through the paper."*

As we said above, this numbering system is useful in cross-referencing results from the CUEA-JOINT-II cruises to the Peruvian upwelling. Many

of these results have already been published in books and journal articles by J. Walsh, R. Smith, J. Huyer, J. Allan, T. Whitledge, L. A. Codispoti, G. Friederich, some are in the reference list here (Richards, 1981;; MacIssacs et al., 1985), but more are forthcoming because of the extensive amount of data still unpublished. If we were to change our system of numbering the stations and the locations at this date, we fear confusion would reign in the future.

Comment #8 . At the end of the methods section (page 16185) there is a paragraph on 'ocean setting'where some of the abbreviations in the first paragraph (e.g. CUEA) are explained. I suggest that in the methods the authors purely introduce their research site (stations, coordinates, etc.) leaving aside all the history of the upwelling area. The ocean settings paragraph should go into the results to my mind. The question that arose from this is whether this paper represents a reanalysis of data. If so, it should be stated more explicitly.

Authors' Response: The original data in this paper has never been published except as a CUEA technical data Report (Packard and Jones, 1978). All the calculations of respiration, carbon flux, Nutrient Retention Efficiency, and Heterotrophic Energy Production are new.

Proposed changes in the manuscript: We have eliminated the "Ocean Setting" section, but moved the modified text to the first paragraph of the RESULTS section. That was a good change.

Comment #9. *Also I miss at what depth ETS measurements have been taken and how. The sampling depth can be derived from tables but I think it should be stated more explicitly in the text*.

Authors' Response and proposed changes to the manuscript:
The depths are given in Table #3. We have stated in the text that ETS activity was determined down to 2000m. This information is in the Methods/Sampling section.

Comment #10. Page 16182, line 18-20: Why have two methods been used? What is the difference between them and how was ETS measured. I suggest a brief explanation of the method(s) here.

Authors' Response: Because phytoplankton dominate the microplankton in the euphotic zone and because the Kenner and Ahmed (1975) ETS method was developed for phytoplankton, this method was used for euphotic zone samples. Because the Packard et al, (1971) ETS assay was a more universal ETS assay, designed to detect ETS activity in prokaryotes as well as autotrophic and heterotrophic eukaryotes, it was used in the deep-waters below the euphotic zone. The signal from the Packard et al (1971) assay is about a third (1/3.35) of the signal from the Kenner and Ahmed assay and since the Kenner and Ahmed (1975) assay was related to the Bryan et al. (1976) Winkler-based respiration technique (Packard and Williams, 1981; Packard and Christensen, 2004),

the deep ETS measurements are multiplied by 3.35. (Christensen and Packard (1980) explain, in detail, the differences between the assays.) Once equivalent, both ETS measurements can be converted to respiration using the R/ETS ratio of 0.26 as explained in Packard and Christensen (2004) and used successfully in Packard and Codispoti (2007).

Proposed change in the manuscript: Since all this is published in the peer-reviewed literature elsewhere we have tried to be succinct by not repeating it. However, we will be happy to include the above text in the methods section of the manuscript.

Comment 11, Page 16182, line 25: How was RN_2 calculated? I suggest to briefly show the calculation here.
Authors Response: Starting on line 27 (page 16182 and on to the top of page 16183) the rate of nitrate respiration is explained. The conversion factor, 105 mol e^- per mol N_2, is the equivalent of Codispoti and Packard's (1980) factor, 2.4 microL O_2 $L^{-1}h^{-1}/(gN_2\ m^{-3}\ yr^{-1})$. Its calculation is as follows:

2.4 micro LO_2 $L^{-1}h^{-1}/(gN_2\ m^{-3}\ yr^{-1})$
2.4 milli L O_2 $m^{-3}h^{-1}/(gN_2\ m^{-3}\ yr^{-1})$
2.4 milli L O_2 $h^{-1}/(gN_2\ yr^{-1})$
21.02 L O_2 $yr^{-1}/(gN_2\ yr^{-1})$
21.02 L O_2 $/(gN_2)$
0.938 mol $O_2/\ (gN_2)$
3.754 mol $e^-/\ ((1/28)N_2)$
105.1 mol e^- per mol (N_2)

Changes in the manuscript: We have inserted between the two sentences on line 26 : "In Table 2, column 2, nanoeq min^{-1} L^{-1} multiplied by 60 is equivalent to micro mol e^- h^{-1} m^{-3}. Then, dividing this by 105 mol e^- per mol N_2 yields R_{N2} in units of micromole N_2 h^{-1} m^{-3}. The conversion factor, 105 mol e^- per mol N_2, is the equivalent of the Codispoti and Packard (1980) factor, 2.4 microL O_2 $L^{-1}h^{-1}/(gN_2\ m^{-3}\ yr^{-1})$."
(This section was again rewritten in April."

12. Page 16183, line 1: Please explain the numbers in the equation. Do not just refer to another paper.

Authors' Response: RCO_2 = [106/60 mol C $(mol\ N_2)^{-1}$ x ETS activity (mol e^- h^{-1} m^{-3})]/[105 mol e^- $(mol\ N_2)^{-1}$]. This equation can be read as: $RCO_2 = K_1$ x ETS activity $/K_2$. K_1 = 106/60 mol C $(mol\ N_2)^{-1}$ and represents the Redfield ratio for the carbon (CO_2) produced during denitrification (Gruber and Sarmiento et al. (1997)). ETS activity is in units of mol e^- h^{-1} m^{-3}. K_2 = 105 mol e^- $(mol\ N_2)^{-1}$ and represents the ratio, R_{N_2}/ETS. It is calculated from the ratio, 2.4 microL O_2 $L^{-1}h^{-1}/(gN\ m^{-3}\ yr^{-1})$ given in Codispoti and Packard (1980). When inverted and converted to equivalent molar, volume, and time units it gives the mols of electrons needed to produce 1 mol of molecular nitrogen (N_2). In this conversion of 2.4 microL O_2 $L^{-1}h^{-1}/(gN\ m^{-3}\ yr^{-1})$ to 105 mol e^- $(mol\ N_2)^{-1}$

the volume and time units cancel out, the value, molecular oxygen, has been represented by its electron equivalents, and the nitrogen is expressed as molecular nitrogen (N_2) produced in denitrification. Hence, 105 represents the mols of electrons needed to produce 1 mol of N_2 starting with NO_3.

Manuscript change:
This now section 2.3 and it has been completely rewritten in response to all three reviewers..

Comment 13, *Page 16183, line 5: Similar to referee #1 the normalization step was not clear to me. What is it good for?*

Authors' Response: We feel that our reply to Reviewer was correct. This normalization technique was used by John Martin's group in VERTEX sediment trap paper (Martin et al. (1987). We used it in our Gulf of Maine carbon-flux-from-respiration paper (Packard and Christensen, 2004). It is critical, mathematically, as Daniel Bourgault from UQAM, Québec has pointed out (personal communication), because $R_z = R_O\ z^b$ is dimensionally unbalanced. The right-hand side of the equation has units of nmol $CO_2\ min^{-1}\ L^{-1}\ m^b$, while the left-hand side of the equation has units of nmol $CO_2\ min^{-1}\ L^{-1}$. Only if depth is normalized ($R_z = R_t\ (z/z_t)^b$) does the equation achieve balance with units of nmol $CO_2\ min^{-1}\ L^{-1}$.

Authors' Changes in the manuscript: We feel that no change is needed, but would be happy to add the above information if requested.

Comment: 14. Page 16183, line 6: Please define R_m, z_m and b.

Authors' Response: R_m is the respiration at its maximum in the water column. m_m is the depth of the R_m, and b is the maximum curvature of the respiration-depth profile, exponent on (z/z_t) in the power function. This information will be added to the paper on *page 16183, line 6.*

Change in the manuscript: We have changed the Modelling section of page 16183 to read: "To generate R models as depth functions, the ETS-based R was plotted against depths (z) normalized by the depth of the R maximum (z_m), as we did in Packard and Christensen (2004). From these plots, power functions of the form, $R = R_m\ (z/z_m)^b$ were fitted to the data using Sigma Plot (version 12.5) according to Charland (2002). Note that R_m is the depth of the respiration maximum and b, the exponent, is always negative. The exponent, b, represents the maximum curvature of the respiration-versus-depth profile. Note that R in the Ez of these sections is based directly on the ETS measurements while the R in the aphotic zone below is based on the R models in Table 4."

Comment 15, Page 16183, line 8-10: Why was R calculated differently in the Ez and in the aphotic zone?
Author's Response: We could have made the section entirely from the respiration models, but since we had relatively dense direct

measurements for the euphotic zone (Table 3) we opted to use them. The section in Fig. 2a will not change much either way.

We propose to leave the Fig. 2a as it is.

Results:

RESULTS-DISCUSSION Comment: As is, the results are more a mix of results and discussion. I think the data would lend itself to be it that way. Thus, I suggest to combine the results and discussion.

Authors' Response and proposed change: Two reviewers felt the paper was well written. We have kept the Results and Discussion separate and folded the Ocean Setting section into the Results. However we examined both sections, made modifications, eliminated duplication, and did move some text up to the methods

Comment 16*, Page 16185, line 23: I think RO_2 appears for the first time here! Please explicitly define in the methods section.*
Authors' response and proposed change: RO_2 is the respiratory oxygen consumption. The definition has been added to the manuscript. See the last sentence of the Introduction.

Comment 17, Page 16186, line 12: Table S3 should Table 3?
Authors' response and proposed change: Yes! It is now corrected.

Comment 18, Page 16186, line 16-20: This part should definitely go into the methods section.
Authors' response and proposed change: Yes! It is now corrected.

Discussion:

Comment 19, *Page 16189, line 8-9: Why should HEP reflect RCO_2? Please explain more explicitly*.

Authors' response: R_{CO2} has been calculated from ETS activity, an R/ETS ratio, and a Redfield ratio. The major purpose of respiration is to make ATP. All facets of respiration, including *RCO_2*, are related to ATP production. Thus any calculation of the rate of ATP production (HEP) will be related to any measure of respiration.

Proposed change in the manuscript: We have replaced the sentence on line 8-9 that reads, "As expected, it reflects the **R**CO_2 section." with the sentence, "Because a major purpose of all forms of respiration is to make ATP, HEP should reflect RCO2 in any section or profile. The similarity of the R_{CO2} pattern in Fig.2a and the HEP pattern in Fig. 2d shows that it does."

Comment 20, *Page 16190, line 5-15: What is the main message in this paragraph? I suggest to bring forward your proposed importance of measuring/estimating HEP*.

Authors' response: This paragraph was a discussion of the background

and interpretation of HEP. We sensed its weakness and revised it. Now the paragraph discusses HEP's background, one of its characteristics, and future measurements to be made with it.

Proposed changes in the paragraph on *page 16190, line 5-15:* We have replaced the previous text to read, "HEP, as ATP generation in the ocean water column, could have been calculated from RO2 since 1943, the time the Nobelist, Severo Ochoa first established the connection between ATP production and R (Ochoa, 1943). However, until Fig. 2d, calculations of biological energy production, including HEP, in the ocean have not been made (Karl, 2014). Now the time is more propitious to make such calculations with recent research (Lane, 2002, 2005, 2009; Wilson et al., 2012; Chen and Strous, 2013) documenting the ubiquity of respiratory ETS in the biosphere, how it relates to RO2 , to all other ocean respiratory processes, and to HEP as ATP production. As we have seen above, HEP and RCO2 in the Peru upwelling system have similar time and space distributions (Figs. 2a and d). The small difference in the ATP/2e− relationships between oxidative phosphorylation and the rate of electron transfer in aerobic metabolism and denitrification has minimal impact. In aerobic metabolism the ATP/2e− ratio is 2.5; in denitrifying microbes ATP/2e− is 1.0 (van Loosdrecht et al., 1997; Smolders et al., 1994). At the rate anammox research is progressing (Dalsgaard et al., 2012), its relative contribution will soon be known, too. In any case, less ATP should be produced in anoxic waters resulting in a lower HEP. It will be interesting in the future to look for this difference by comparing HEP offshore sections made through oxic and anoxic sectors of upwelling systems."

Comment 21. *Page 16190, line 5-15: What would be the implications if the ratio of ATP/2e- is different?*

Authors' response: The difference will be slight a slight shift in the magnitude of the HEP. It would be interesting in the future to compare HEP sections through oxic and anoxic parts of an upwelling system.

The proposed changes are given in the response to comment #20.

Comment 22. Page 16190, line 25-27: Please be more explicit when stating that HEP should be a small fraction of the solar energy input. Why should this be the case?
Authors' response: On page 16188, lines 16-18 we state; "This average HEP is only 0.7 % of the average solar radiation (13.5 ± 4.0 MJ d^{-1} m^{-2}) at the C-Line sea surface between Sept 12-24 during the JASON-76 cruise (Packard and Jones, 1976)." However, to be sure, we researched the literature and found that according to Lewis and Crabtree (2005), solar radiation illuminates the Earth with about 120,000 terawatts of energy per year. Of this amount photosynthesis fixes only about 100 terawatts per year, a small proportion. This is 0.08% of the solar input. Over the year and over geological time respiration almost balances photosynthesis. Thus HEP would be expected to be about 0.08% of the

incident radiation at any time on the sea surface, a small proportion of the solar input. From direct measurements we calculate 0.7%, but still a small proportion of the solar input.

We propose to leave the text as it is considering our statement on page 16188, lines 16-18.

Tables and Figures:

Comment 23. *Table 1: I don't understand why the dates are so arbitrary and do not correspond to the stations.*

Authors response: During the cruise the ship made 3 round-trip transects, but on some transects only hydrographic data was taken, on others biology and biochemistry data were included. Table 1 was constructed from the station data that included ETS measurements. It was organized in the offshore direction. We have revised the section on sampling to clarify the use of dates, sampling station numbers, and C-Line positions.

Comment 24. *Table 1: Is surface respiration the average over Ez?*
Authors' response: No. The respiration is for the sea-surface. The sample was taken at 0.5 m depth (below the surface). We feel it is clear in the caption of Table 1.

Comment 25. *Table 2: As for referee #,1 describe the calculation of potential R from ETS activity.*

Authors' response: This is explained in the caption of Table 2 and also in the text (Methods,/ETS). We measure tetrazolium reduction to its formazan in the ETS assay. Two electrons are required for each molecule of tetrazolium to be reduced. After a few minutes in a spectrophotometer we know how many moles of formazan are produced (Beer-Lambert Law) and hence the "moles" of electrons flowing during this time through the ETS. The potential respiration is just the application of the logic that if 4 electrons ($4e^-$) are needed to reduce molecular oxygen (O_2) to water ($2H_2O$) and $2e^-$ are required for each molecule of tetrazolium to be reduced, then 2 moles of formazan produced is equivalent to 1 mole of oxygen reduced to water. Thus we have potential respiration from measured tetrazolium reduction. This has been explained in Packard et al. (1983), Packard (1985a and b), Packard and Christensen (2004) and in Packard and Codispoti (2008). We tried to make it clear here also.

Comment 26. *Figure 3a Panel 2: Sort the bars according to depth from left to right, i.e epipelagic upper meso, lower meso etc*.

Authors' response: We have made the changes to the figure (lower part of Fig 3a) as the reviewer requests.

Comment 27. *Figure 3a Caption: I suggest to write out the 'C' to*

carbon.

Authors' response: Good! We have done this here and throughout the paper. (This helps to avoid confusion with the C-Line stations, etc.).

REFERENCES (These were not originally included in the manuscript, but now some of them are included):

Arístegui, J., Gasol, J.M., Duarte, C.M., Herndl, G.J., Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. 54, 1501–1529, 2009.

Blasco, D., J.J. MacIsaac, T.T. Packard and R.C. Dugdale. Relationship between nitrate reductase and nitrate uptake in phytoplankton in the Peru upwelling region. Limnology and Oceanography, 29 (2): 275-286 (1984).

Dugdale, R.C., F.P. Wilkerson, R.T. Barber, D.B. Blasco and T.T.Packard. Changes in nutrients, pH, light penetration and heat budget due to vertical movements of photosynthetic organisms in Peru coastal waters. Oceanologica Acta, special volume #6:103-108 (1987).

Dugdale, R.C., J.J. Goering, R.T. Barber, R.L. Smith and T.T. Packard. Denitrification and hydrogen sulfide in Peru upwelling during 1976. Deep-Sea Research, 24: 601-608 (1977).

Eppley, R.W., Peterson, B.J., 1979. Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282, 677–680.

Lewis, N. S. & Crabtree, G. (eds) Basic Research Needs for Solar Energy Utilization. (Report of the Basic Energy Sciences Workshop on Solar Energy Utilization, US Department of Energy, Washington DC, 2005); ,http://www.er.doe.gov/bes/reports/abstracts.html#SEU. (18–21 April, 2005).

Lane, N. Oxygen: The Molecule that made the World. OUP, Oxford, pp. 384, 2002. (ISBN 978-0198607830)

Lane, N. Power, Sex, Suicide: Mitochondria and the Meaning of Life. OUP, Oxford, pp. 368, 2005. (ISBN 978-0199205646)

Lane, N. Life Ascending: The Ten Great Inventions of Evolution. WW Norton/Profile, pp 352, 2009. (ISBN 978-1861978486)

McDonnell, A. M. P., Boyd, P.W., Buesseler, K. O. Effects of sinking velocities and microbial respiration rates on the attenuation of particulate carbon fluxes through the mesopelagic zone. Global Biogeochemical Cycles, 15 Feb. 2015. (DOI: 10.1002/2014GB004935).

MacIsaac, J.J., R.C. Dugdale, R.T. Barber, D. Blasco and T.T. Packard. Primary production cycle in an upwelling center. Deep-Sea Research, 32(5): 503-529 (1985).

Packard, T. and Jones, V.: Biochemistry and ecology of the Peru Current: the JASON expedition to the Peru upwelling system, September 1976, CUEA Tech Rep, Bigelow Laboratory, Boothbay Harbor, ME, USA, 46, 129, 1978.

Redfield, A. C., Ketchum, B. H., Richards, F. A. The influence of organisms on the composition of seawater. Pp. 26-77. In Hill, N. M. (Ed), The Seas vol II, Interscience New York, 1963.

Richards, F A. Oxygen in the Ocean. Pp 185-238 (Chapter 9). In Hedgepeth, J W. (Ed). Treatise on Marine Ecology and Paleoecology. Geol Soc America 67 (1), 1957.

Richards, F.A. (ed.), 1981. Coastal upwelling. Washington, DC: American Geophysical Union. Pp. 529.

Riley, G A. Oxygen, phosphate, nitrate in the Atlantic Ocean. Bull. Bingham Oceanogr. Collection. 13, 1-169, 1951.

Steinberg, D.K., Van Mooy, B.A.S., Buesseler, K.O., Boyd, P.W., Kobari, T., Karl, D.M., 2008. Microbial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. Limnology and Oceanography 53, 1327-1338.