**PeerJ Submission Manuscript:** **#2015:02:4143:0:1:REVIEW**

**Title:** "Not all jellyfish are equal: isotopic evidence for inter- and intraspecific variation in jellyfish trophic ecology"

**by Nicholas E. C. Fleming, Chris Harrod, Jason Newton and Jonathan D.R. Houghton**

Firstly, we would like to thank the editor and each reviewer for their constructive comments. We were very happy to see that each reviewer was generally positive about our study and their comments have helped us tighten the manuscript considerably and increase its readability and impact. We hope that our responses are clear and reasonable. All line numbers in author’s responses refer to new line numbers in the revised manuscript. We have responded in red text throughout.

**Reply to reviewer 1 comments:**

The authors address an important topic. The general approach is robust and I think that the manuscript should be accepted after minor revision. I have one major concern regarding interpretation of baseline signatures that the authors should address carefully. Other comments are generally minor.

**Major comment:**

Paragraph starting line 246 - Baseline d15N values remained constant for the duration of the study but baselines were measured using filter feeding and grazing molluscs as surrogates for directly measuring isotopic signatures of phytoplankton and benthic algae. What is the turnover time of N in molluscs? The study only ran for 4 months and it is possible that the baseline may have shifted but slow turnover times in the molluscs may have prevented this being observed. If the jellies responded more quickly to a shift in the baseline than the molluscs then the pattern could still have reflected a change in the baseline. I think it would be useful to provide information on turnover times of N in molluscs and jellies to provide a more robust argument for the pattern being caused by a change in trophic status of the jellies.

**Authors’ response:** The reviewer raises a good point and we have added more information to the manuscript to deal with this.

Unfortunately, we do not have isotopic turnover data for all the taxa examined in our study. Isotopic turnover rates in the moon jellyfish (*Aurelia aurita*) were recently described by D’Ambra et al. (2014). They estimated a half-life for δ13C of 10.8 days and δ15N of 9.7 days. Dubois *et al*. showed experimentally that isotopic turnover times for *Mytilus* were very similar (δ13C = 9 days; δ15N = 14 days) (Dubois *et al*. 2007). As such, we feel that our use of Mytilus as a pelagic baseline indicator to be justified. Isotope turnover rates have not been estimated in *Littorina saxatilis*, but Woodland et al (2012) noted that gastropod grazers typically responded faster to changes in δ13C than filter feeders, but more slowly to changes in δ15N.

We have amended the methods section: lines 93-102: text now reads: *“Filter-feeding bivalves (Mytilus spp.) and grazing gastropods (Littorina saxatilis (Olivi)) were sampled over the study period from intertidal areas adjacent to the jellyfish sampling sites over the same period (Woodland et al. 2012). These species are long-lived, dominant and ubiquitous, providing a measure of isotopic baselines of the pelagic (bivalve) and benthic (gastropods) primary production pathways as suggested by Post (2002) and supported by others (e.g. Mallela & Harrod 2008; Richoux & Ndhlovu 2014). Furthermore, isotopic turnover rates (expressed as half-life) in the moon jellyfish (Aurelia aurita) recently described by D’Ambra et al. (2014) who estimated a half-life for δ13C (10.8 days) and δ15N (9.7 days) are similar to that of Mytilus (δ13C = 9 days; δ15N = 14 days) (Dubois et al. 2007), suggesting a similar ability to track temporal shifts in baseline isotope values.”*

**Minor comments:**

Abstract – how was the value of 1400 species of gelatinous zooplankton derived? I believe that there are more than 1000 species of hydromedusae alone.

**Authors’ response:** The reviewer was correct and this section of text has been amended. The new approximation is described from Condon et al., 2012 who cites Daly *et al.* (2007) and Mills, (2011). Text now reads: *“…Gelatinous zooplankton represent a polyphyletic assemblage spanning >2,000 species that inhabit coastal seas to the deep-ocean and employ a wide variety of foraging strategies…”*

In addition, Lines 377-380: text now reads: *“. In a broader context, as gelatinous zooplankton span > 2,000 species (Condon et al. 2012), occupying habitats ranging from the deep ocean through to shallow water near-shore environments, the inclusion of an ‘average’ jellyfish in such models is likely to underestimate the collective impact in terms of energy flow or consumption of prey (Pauly et al. 2009). . “*

Line 31 – size-based shifts in what? Presumably diet? Note that this sentence actually doesn’t make sense, so please rewrite.

**Authors’ response:** Accepted: this was an unfortunate typographical error, with the word “trophic” omitted from the text. Lines 35-37 now read: *“…Moreover, evidence of sized-based trophic shifts in the moon jellyfish Aurelia aurita (Linnaeus, 1758) (Fleming et al. 2011; Graham & Kroutil 2001) suggest …”*

Line 174 – delete the first sentence as there is no need to repeat information already provided in the methods.

**Authors’ response:** Line deleted as suggested.

I found the structure of the discussion a little convoluted. The authors first discuss the results of two-way ANOVAS comparing species and time (for months of June/July only) for isotopic centroids (Fig 2) and SEAB (Fig 3) They then discuss size/ontogenetic changes in isotopic centroids (Fig 4) and then switch back to comparing temporal variability in isotopic centroids and SEAB for individual species (thereby referring back to Figs 2 & 3 again). I think the results would flow better if they were rearranged such that temporal variability in centroids (both the two-way comparison between June/July and for individual species) were discussed first, then temporal variation in SEAB discussed second, and size differences in centroids discussed third. If they do this then the reader will be able to progress through each of the figures in a logical manner, instead of switching back and forth between them.

**Authors’ response:** A very fair point.We have rearranged the methods, results and discussion sections and hope this will make the story clearer.

Paragraph starting line 254 - It would be useful to see how the temporal changes in isotopic centroids/isotopic niche width reflected size/ontogenetic differences through time.

**Authors’ response:** On each sampling occasion a range of sizes of each species were collected from what was available in the Lough (i.e. there are all sizes of jellies in all months). As such, it was difficult to include size as a consistent factor in analysis (see figure below). We have also added text to the discussion Lines 3406-410: text now reads: *“When considered as a whole, the δ15N values of the scyphozoan jellyfish community in Strangford Lough increased as the season progressed (Fig. 2), even though baseline levels remained constant. This increase in δ15N was unlikely to be a result of a general increase in size of jellyfish over time, as a range of sizes of each species were collected and analysed each month (see Appendix S1). .”*



Figure Response 1 – Variation in log mass of jellyfish captured over the study period

Fig 2 – it would be useful to indicate on the figures which species and months differed so that the reader doesn’t need to keep referring to the text to interpret patterns.

**Authors’ response:** We appreciate that the use of multiple comparisons can be confusing. However, responding to the reviewer’s suggestion is difficult in our case as our statistical comparisons are multivariate (i.e. have compared δ15N-δ13C centroids) whilst the data shown in the figure are univariate. As such, we prefer not to complicate the figure or text by adding univariate comparisons.

Lines 273 – I found the first paragraph of the discussion quite odd and, for the most part, irrelevant. Why is it necessary to discuss the value we assign to jellies, potential expansion of jelly populations or the potential causes of jellyfish blooms? This information doesn’t really seem relevant to the topic of trophic diversity of jellies (sorry but I’m really tired of reading these types of statements – let’s keep to the topic!!). I recommend starting the discussion at line 283.

**Authors’ response:** We have removed the information that does not specifically deal with aspects relating to the trophic ecology of jellyfish. We have amended the text and Lines 324-332: now read: **“***Pauly et al. (2009) described jellyfish as arguably the most important predators in the sea. There is little ambiguity in this statement which, in part, prompted the present study. There is no doubt that the potential expansion of jellyfish in highly depleted oceans is a matter of grave concern (Lynam et al. 2006; Purcell et al. 2007), and an underlying knowledge of how jellyfish function within marine systems is required, so that long-standing trends in populations and communities can be teased apart from shifts in ecosystem structure. Stable isotope analysis offers a powerful biochemical approach to the estimation of trophic and dietary composition of individuals through to communities (Bearhop et al. 2004; Bolnick et al. 2003) and the results presented here support the idea that jellyfish play a more complex trophic role than once envisaged.*

Line 290 – “inferring” is used incorrectly here.

**Authors’ response:** Section rewritten and text no linger used.

Line 302 – please provide a reference to support the claim that the cnidomes vary among individuals.

**Authors’ response:** This section has been deleted and the text amended. Lines 360-364: Text now reads: *“A. aurita have a much reduced capture surface (shorter tentacles) compared with the Cyanea spp. Heeger & Möller (1987) found that the majority of prey capture by A. aurita in Kiel Harbour, N Germany, occurred on the tentacles as opposed to the subumbrellar surface, so this reduced capture area may account for the low trophic position and narrowest niche width of this species in the present study.”*

Line 302 – 305 – how does a reduced cnidome and reduced capture surface relate to Aurelia having a low trophic position? Further discussion of how the cnidome of Aurelia relates to the types of prey it captures is required.

**Authors’ response:** Text amended. Lines 366-371: Text now reads: *“Although they differ in terms of maximum individual size, the congenerics C. lamarckii and C. capillata have similarities in both nematocyst complement (Ostman & Hydman 1997; Shostak 1995) and morphology (Holst & Laakmann 2013). Previous studies have reported predation of C. capillata on A. aurita medusae, therefore it is possible that the differences observed with A. aurita may be a symptom of intra-guild predation by the larger C. capillata (e.g. Hansson 1997; Purcell 2003; Titelman et al. 2007).”*

Line 307 – “…yet differed here with regard to their del13C and del15N values and niche widths.” but the centroids and isotopic niche width of both Cyanea species overlapped during June and July (see lines 187-188 and 195-197) so this interpretation seems only partially supported by the data. My feeling is that the authors need to provide a more nuanced discussion about differences in isotopic signatures and niche widths given that differences among species were observed among some times but not others.

**Authors’ response:** Agreed – we have tightened this section up – see lines 341-348.

Line 376 – “in” missing between “variation” and “body” also “dives” should be “drives”

**Authors’ response:** We have modified the text: line 450-451: Text now reads: *“…an inference that variation in body size in some way drives variation in the trophic ecology…”*

**Reply to reviewer 2 comments:**

Overall, I thought this paper did a good job describing inferred changes in jellyfish diet over a few month period at a specific site. The statistical methods seemed robust and suitable for the expressed difficulties obtaining even sample sizes at all times considered. It did support the authors’ point that their trophic role should be considered more specifically than an aggregate functional role, but I thought that significance could be explained better.

Introduction: You often mention the importance of elaborating models that include the jellyfish as one functional group, and in lines 38-41 mention that 100 models were considered, only 23% of which included jellyfish as a distinct functional group. Did any of these focus on your area of study? If so, how might your findings affect the overall model outcome? If better parameterization of these models is an argument for the significance of the study and your study is focused on a specific site, then the link needs to be better expressed.

**Authors’ response:** This is a good point, but the paper as it stands highlights the fact that there are relatively few models that include jellyfish at more than two trophic levels. Therefore the main point of this paper is to provide information showing that all jellyfish are not equal and therefore cannot be included in a model as a single functional group regardless of which area a model focuses on.

How widespread are the three jellyfish species sampled? Have any other isotope values for these or similar species been reported? Is there any background data on what their diets consist of specifically?

**Authors’ response:** These three jellyfish species are found (often in large aggregations) across the NE Atlantic. *Aurelia aurita* is a ubiquitous species found in Northern boreal to tropical waters (Russell, 1970; Lucas, 2001). *Cyanea capillata* (L.) have a circumpolar distribution that overlap in northern boreal waters (Russell, 1970). *C. lamarckii* has been recorded from Norway, Iceland and the Faroes to the Bay of Biscay (Russell, 1970).

To our knowledge this is one of the most comprehensive SIA studies conducted specifically on jellyfish and represents a useful contribution (e.g. to ecologists, ecosystem and fisheries modellers and ecosystem managers). The study has in part been prompted by the lack of suitable data on the trophic ecology of jellyfish and is a solid first step to addressing these issues.

Discussion: As mentioned regarding the introduction, how applicable are these results to a more broad understanding of jellyfish trophic roles?

**Authors’ response:** We feel thatthis study provides strong evidence that jellyfish cannot be considered as a single functional group and aims to inform ecologists, modellers and provide data for online repositories.

Pauly (2009) says “*Consistency among models in how detailed they are with respect to ‘jellyfish’ is an important consideration when attempting to evaluate and compare them. We are mindful that the overwhelming majority of models do not include a jellyfish compartment, and those that do tend to collapse all things considered gelatinous into a single functional ‘jellyfish’ group. With few exceptions, such as the Chesapeake Bay (Baird & Ulanowicz, 1989; Walters et al., 2005) and the eastern Bering Sea (Trites et al., 1999; NRC, 2003) models, a single jellyfish group poorly represents the true diversity of taxa and trophic interactions involving jellyfish.”* *We suspect the low number of models incorporating jellyfish, far from reflecting the true importance of jellyfish in marine ecosystems, in fact highlights the limited knowledge many fisheries scientists or marine biologists have of jellyfish.”*

Can you mention any models that might specifically benefit from these findings and how?

**Authors’ response:** Although the data generated here is not suitable for input into models at present, this study is designed to be a primer for more investigation into trophic complexities in jellyfish. As they are increasingly prevalent in some regions they must be included with more detail into ecosystem and fisheries models in those systems. This paper indicates that including jellyfish as a homogenous group will not give accurate model output and more research is needed.

Methods: Lines 85-89: why were these two species alone chosen to assess changes in baseline 15N? Additionally, you assess changes in isotopic niche space over time and attribute these changes to variation in food resources. Why then are baseline 13C values not reported to account for the changes within the same potential food resources?

**Authors’ response:** The two species were chosen as they fulfil the requirements of baseline indicators as suggested by Post (2002). They are functionally associated with consumption of pelagic- (bivlaves) or benthic-derived algae, they are long-lived and widely distributed.

With regard to the reviewer’s second question, they have made an important point that we had missed. We have added more text regarding our selection of these indicators and the implications of the results associated with them. We have also included sections in the results and discussion focussing on variation in baseline isotope values. Overall, these did not differ by month, but by functional group (as expected) meaning that the differences in isotope values over time in the jellyfish likely reflected changes in diet rather than changes at the base of the foodweb. Isotopic niche width values are calculated without reference to baseline isotopic values, but with this observation we can be confident that the temporal shifts seen at the intraspecific and community level represent real changes in isotopic niche width.

The key section where text has been modified in response to this (and also in response to Reviewer 1) include can be found between lines 93-102: text now reads: *“Filter-feeding bivalves (Mytilus spp.) and grazing gastropods (Littorina saxatilis (Olivi)) were sampled over the study period from intertidal areas adjacent to the jellyfish sampling sites over the same period (Woodland et al. 2012). These species are long-lived, dominant and ubiquitous, providing a measure of isotopic baselines of the pelagic (bivalve) and benthic (gastropods) primary production pathways as suggested by Post (2002) and supported by others (e.g. Mallela & Harrod 2008; Richoux & Ndhlovu 2014). Furthermore, isotopic turnover rates (expressed as half-life) in the moon jellyfish (Aurelia aurita) recently described by D’Ambra et al. (2014) who estimated a half-life for δ13C (10.8 days) and δ15N (9.7 days) are similar to that of Mytilus (δ13C = 9 days; δ15N = 14 days) (Dubois et al. 2007), suggesting a similar ability to track temporal shifts in baseline isotope values “*

Results: Figure 2 seems to suggest that all species considered had a shift in isotopic signature between May and June in both 13C and 15N – do you infer a reason for this?

**Authors’ response:** We address this in the manuscript see Lines 411-419 *“. In terms of isotopic niche width, there was an interesting dissimilarity between the start of the season (May) and the following months (June, July and August), suggesting a shift to a broader dietary niche in the latter months (Fig. 3). This increased resource utilisation is consistent with previous studies that suggested jellyfish dietary niches are extremely broad, with species operating as generalists (Dawson & Martin 2001; Ishii & Båmstedt 1998; Schneider & Behrends 1998) feeding opportunistically across a range of plankton (Båmstedt et al. 1997; Titelman et al. 2007). Therefore, our data suggest that a different and possibly constrained resource pool is being exploited at the beginning of the ‘jellyfish season’. “*

**Reply to reviewer 3 (Tjasa Kogovsek) comments:**

I have really enjoyed reading the Fleming et al. manuscript in which the authors investigated the trophic ecology of three co-occurring jellyfish. Stable isotopes (δ13C and δ15N) were used to address the following three points: 1) to investigate the inter-specific differences in trophic ecology, 2) to detect the possible size-base shifts in trophic ecology and 3) whether the jellyfish tropic position and isotopic niche remain constant over time. In accordance to the results they concluded that the three selected species occupy different trophic position. Further, the isotopic niche width combined for all three species was not constant over the investigated period; in fact, the isotopic niche width increased throughout the season, reflecting temporal shifts in trophic position and seasonal succession in the investigated species.

The manuscript is very well structured, the figures and tables being very informative. I particularly like the detailed description of the methodology and data analysis. The relevant results to address the initial hypotheses are described and very well discussed. However, there are few comments I would like the authors to address, in particular I am concerned about the methodology used for drying the samples and how this might have affected the stable isotope composition of gelatinous tissues. Once the authors discuss the above mentioned issue, I believe the manuscript will be acceptable for publishing.

Specific comments:

Line 42: The reference Graham & Kroutil, 2001 report on prey types only for A. aurita and intra-specific differences are not mentioned. Please, remove or refer to it when writing about the ontogenetic shifts in jellyfish diet.

**Authors’ response:** This has been amended. Lines 46-48: text now reads:*“…seasonal or ontogenetic shifts in diet (Graham & Kroutil 2001; Fleming et al. 2011), intra-specific differences in prey types (Fancett 1988) and intra-guild predation (Bayha et al. 2012; Robison 2004; Titelman et al. 2007) are sometimes…”*

Line 51: I think a better reference would be Purcell 2009 instead of Purcell 1992. (Please see below for the complete reference).

**Authors’ response:** Agreed: text has been amended. Line 54: text now reads:*“…and methodological limitations (Purcell 2009).”*

References have been updated and now include: *“*Purcell, JE. 2009. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. *Hydrobiologia* 616(1): 23-50.

Material & Methods: As already mentioned above, I am concerned that the stable isotope composition of the samples might have been affected during the process of sample preparation. The samples were oven dried at 60°C. This is a standardised drying method commonly used for drying zooplankton. However, a recent work (even cited by the authors (Kogovšek et al. 2014)) revealed that exposing gelatinous tissues to 60°C may affect stable isotope composition of the jellyfish. This change was not uniform among the species; it seems that the change in δ15N was more pronounced in the species that are more protein rich compared to the protein depleted one. In the current study, the protein rich Cyanea tissue may therefore be more affected when exposed to the drying temperature than Aurelia aurita and thus affecting the δ15N composition in a non equal manner. I would like the authors to comment this in the discussion section of the manuscript.

**Authors’ response:** This is a valid point and we checked variation in C:N ratios (admittedly after drying) in the three species (as an estimate of relative protein content). In Kogovšek et al. (2014), you showed that C:N ratios were not affected by drying method, so we feel that it was unlikely that there were large biochemical differences between the species prior to drying.

We have added a small section to the methods addressing this. Lines 123-126 text reads: *“Recently evidence has emerged that air-drying gelatinous tissue can increase δ15N in more proteinaceous species (Kogovšek et al. 2014). C:N ratios of the three species were compared and found not to differ (F2, 120 = 1.48, P = 0.232), suggesting that any effect of air-drying would be consistent across species.”*

Discussion: (line 314, 315): From the current text it is not clear whether the differences in δ15N are due to direct effect (differences in size of nematocysts and toxins may result in different quality and quantity of proteins) or indirect, as the different pray captured and consumed.

**Authors’ response:** This is an interesting point and something we aim to investigate in the future. This particular part of the text has been amended as there is very little information on the ‘cnidome’ and the effects of different toxin and nematocyst compliment with regard to isotopic values. We have therefore amended the text and it now reads: lines 360-364: *“A. aurita have a much reduced capture surface (shorter tentacles) compared with the Cyanea spp. Heeger & Möller (1987) found that the majority of prey capture by A. aurita in Kiel Harbour, N Germany, occurred on the tentacles as opposed to the subumbrellar surface, so this reduced capture area may account for the low trophic position and narrowest niche width of this species in the present study.”*

Some previous studies report on predation of Cyanea on Aurelia medusae (for example Hansson 1997, Purcell 2003). I miss this point in the discussion when addressing inter-specific interactions.

**Authors’ response:** The text has been amended to include this important reference. Lines 366-371: Text now reads: *“Although they differ in terms of maximum individual size, the congenerics C. lamarckii and C. capillata have similarities in both nematocyst complement (Ostman & Hydman 1997; Shostak 1995) and morphology (Holst & Laakmann 2013). Previous studies have reported predation of C. capillata on A. aurita medusae, therefore it is possible that the differences observed with A. aurita may be a symptom of intra-guild predation by the larger C. capillata (e.g. Hansson 1997; Purcell 2003; Titelman et al. 2007).”*

Further, the turnover rates of the isotopes in the jellyfish tissues are unknown. However, we can assume that the turnover rates (bulk or tissue specific) would be affected by the temperature and/or the food quality, as for example was previously observed in fish. In the current study, the samples from May were significantly different from other months for all species. May this be in part a consequence of the lower temperature in May compared to other months? Or maybe the spring zooplankton bloom? I think it would be useful to include this information in the discussion too.

**Authors’ response:** This is an interesting point and we have addressed the issue of turnover times in the materials and methods, lines 98-102: text now reads: *“Furthermore, isotopic turnover rates (expressed as half-life) in the moon jellyfish (Aurelia aurita) recently described by D’Ambra et al. (2014) who estimated a half-life for δ13C (10.8 days) and δ15N (9.7 days) are similar to that of Mytilus (δ13C = 9 days; δ15N = 14 days) (Dubois et al. 2007), suggesting a similar ability to track temporal shifts in baseline isotope values.”*

With regard to temperature we have added to the discussion. Line 419-424: text now reads *“There are of course environmental factors such as temperature which could have an effect on N metabolism & excretion in jellyfish (Morand et al. 1987; Nemazie et al. 1993) and temperature can have a significant effect on isotopic turnover times in a range of taxa (see Thomas & Crowther 2015). The temperature increase in Strangford Lough over the course of the study was modest (from 8.7 – 14.2°C) but cannot be discounted as a possible influence on isotopic variation over time.”*

The point about the zooplankton bloom is correct but we feel this has been covered implicitly in the discussion lines 411-419: *“In terms of isotopic niche width, there was an interesting dissimilarity between the start of the season (May) and the following months (June, July and August), suggesting a shift to a broader dietary niche in the latter months (Fig. 3). This increased resource utilisation is consistent with previous studies that suggested jellyfish dietary niches are extremely broad, with species operating as generalists (Dawson & Martin 2001; Ishii & Båmstedt 1998; Schneider & Behrends 1998) feeding opportunistically across a range of plankton (Båmstedt et al. 1997; Titelman et al. 2007). Therefore, our data suggest that a different and possibly constrained resource pool is being exploited at the beginning of the ‘jellyfish season’. ”*

There are some small typing mistakes throughout the text.

**Authors’ response:** These have been amended.

References:   
Hansson, L. J. (1997). "Capture and digestion of the scyphozoan jellyfish Aurelia aurita by Cyanea capillata and prey response to predator contact." Journal of Plankton Research 19(2): 195-208.  
Purcell, J. E. (2003). "Predation on zooplankton by large jellyfish, Aurelia labiata, Cyanea capillata and Aequorea aequorea, in Prince William Sound, Alaska." Marine Ecology Progress Series 246: 137-152.  
Purcell, J. E. (2009). "Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research." Hydrobiologia 616(1): 23-50.

**Authors’ response:** These have been all been added to the references and added to the new text within the manuscript.