

REPLY COMMENT

## Use of mixing models for Humboldt squid diet analysis: Reply to Field et al. (2014)

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ABSTRACT: Field et al. (2014; Mar Ecol Prog Ser 500:281–285) comment on our application of a Bayesian isotope-mixing model (SIAR) to examine the relative contribution of prey from different regions to *Dosidicus gigas* diet, and point out that our model violated assumptions of *D. gigas* feeding. We agree in part with their position that the use of SIAR for assessing contributions of sources from different regions for an omnivorous species may be unreliable. However, the results from our study and from the prevailing literature and data indicate that the *D. gigas* we collected in the Northern California Current (NCC) isotopically matched the NCC baseline and were isotopically distinct from prey resources in the Southern California Current. Field et al.'s (2014) comments on the distribution and abundance of *D. gigas* in the NCC missed results from the primary literature which show that *D. gigas* and their purported prey are predominantly distributed along the shelf-slope waters of the NCC, well within the offshore extent of our study. The discrepancy of not finding myctophids as significant sources to *D. gigas* diet in our study may lie in the fact that our isotope values of myctophids came from adults only, and that smaller conspecifics with lower relative  $\delta^{15}\text{N}$  values would have shown a greater contribution from this trophic group. The conclusion we reached of lower trophic level feeding by *D. gigas* in our study relative to previous diet studies remains valid.

KEY WORDS: *Dosidicus gigas* · Stable isotopes · Trophic level · Source production

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### Introduction

Field et al. (2014) comment on the methodology and conclusions of our study on the contribution of prey groups to the diet of Humboldt squid *Dosidicus gigas* collected from the Northern California Current (NCC) (Miller et al. 2013). Our analyses incorporated

stable isotope data on N and C into a Bayesian isotope-mixing model (SIAR), examined the isotopic match of *D. gigas* to 4 regions along the California Current ecosystem, and estimated the relative contribution of prey groups to *D. gigas* diet. Field et al. (2014) state that our use of the migratory model was sensitive to applications of trophic enrichment factors

(TEFs) and re-ran the SIAR model with a modified TEF to increase the relative trophic level (TL) by 0.5. From this, Field et al. (2014) point out that the migratory model was sensitive to TEFs, and state that their results indicated that *D. gigas* from Miller et al. (2013) were not in isotopic equilibrium with the NCC system. Field et al. (2014) also comment on our SIAR trophic model results that did not find substantial contributions from trophic groups with mesopelagic species, which is in contrast to diet studies indicating otherwise. Here we address their comments on our paper and provide additional information that confirms our previous findings from Miller et al. (2013).

### Migratory model

Our use of the SIAR migratory model was based on assumptions that (1) *D. gigas* feed directly on primary consumers and (2) the prey availability and feeding behavior of *D. gigas* is the same from all regions. These assumptions were likely not met in our study, which negates the utility of the model in understanding relative equilibrium of any omnivorous species >2.0 TL across a large spatial scale. However, Field et al.'s (2014) modification and re-run of the SIAR migratory model using simulation data from our results, and their conclusion that *D. gigas* is an isotopic mixture of regional sources, is not correct. Field et al. (2014) focus on the assumption that *D. gigas* feed 1.0 TL above copepods by adjusting and running the SIAR model to show that having a model with *D. gigas* 1.5 TL above copepods from all regions shows greater contributions from the SCC. Their assumption of 1.5 above TL violates the second critical assumption. In our study, the only region that showed a 1.0 TL difference of *D. gigas* from copepods by using our literature-based TEF of 3.4‰ was the SCC nearshore, whereas the NCC showed relative trophic levels of 3.2 to 3.4 for nearshore and offshore systems, respectively. Moreover, a recent paper (Madigan et al. 2012) shows isotope values of *D. gigas* in the SCC to be 15.4‰ for  $\delta^{15}\text{N}$  and  $-18.4\text{‰}$  for  $\delta^{13}\text{C}$ , which are substantially higher than our values and those of Drazen et al. (2008). Thus, the conclusion that the output from Field et al.'s (2014) SIAR migratory model is more realistic compared to our results is unsupported, with NCC values of *D. gigas* trophically and isotopically consistent with being more in equilibrium with the NCC system.

Field et al. (2014) assert that *D. gigas* from our study were recent arrivals to the area (possibly from significantly offshore regions) and therefore came

from outside our sampling area. They noted that *D. gigas* in the NCC can be found as far as 300 km offshore; however Chesney et al. (2013) and Field et al. (2013) observed highest densities of *D. gigas* occurring off the shelf-slope waters of the NCC, well within the area of our study. Moreover, Field et al. (2014) argue that *D. gigas* follow known patterns in abundance of mesopelagic species with the assumption that mesopelagics are largely distributed offshore of our study area, when in fact highest abundances of myctophids have been observed closer to the shelf-slope waters of the NCC (Pearcy 1976, Brodeur et al. 2003). Therefore, while *D. gigas* can travel great distances and occur far offshore, the main distribution of *D. gigas* and their known prey was well within the offshore range of our study. In addition, Field et al. (2014) comment that the low catch of *D. gigas* across months and years indicates they are rare, but they failed to note that *D. gigas* do not show strong schooling behavior in the NCC (Stewart et al. 2013), and they provide no explanation as to how rarity would have any bearing on their residency or future abundance. As for the arrival of *D. gigas* into the NCC, our results only extend to the time of our study. It is likely that some individuals were recent arrivals given the variation in *D. gigas*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , but the fact that most were isotopically matched to the NCC baseline and the disparity between *D. gigas* from the SCC (Madigan et al. 2012) and the NCC (our study) indicate that most had been feeding and growing within the NCC for at least 2 to 3 mo.

### Trophic model

Field et al. (2014) are correct in noting that in our stable isotope analysis we were unable to capture and measure all of the contributions of prey species to *D. gigas* diet. That is precisely why we applied isotopically based trophic groups so the SIAR model could contend with the multiple sources of prey and their variability. Our results indicated that *D. gigas* diet had the highest contributions from groups that included euphausiids, juvenile fish, and ichthyoplankton, but very low contributions from mesopelagic species. The  $\delta^{15}\text{N}$  values of myctophid species relative to *D. gigas* from slope waters offshore of the NCC from our study (Miller et al. 2013), from Drazen et al. (2008) taken ~220 km west of Point Conception, California, and values from the SCC ecosystem by Madigan et al. (2012) are all too  $^{15}\text{N}$ -enriched to be significant isotopic contributions to *D. gigas* values

from these respective regions. Although Madigan et al. (2012) did find mesopelagic species (myctophids) contributing 63% to a trophic group that included *D. gigas*, their results were likely biased for two reasons. First, they ignored assumptions as to whether their trophic groups were at isotopic equilibrium, and second, they combined  $^{15}\text{N}$ -depleted, large, migratory, pelagic species with species that had coastally-enriched  $\delta^{15}\text{N}$  values into the same trophic group. The resulting trophic groups were confounded by pelagic signatures of top TL species that artificially truncated the food web, and therefore masked useful measures of source contribution to any specific species and the trophic structure of the SCC food web. The discrepancy between diet and isotope results from our study with respect to contributions of myctophids is more likely an artifact of not capturing the ontogenetic range of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in myctophids, which have previously shown strong shifts in  $\delta^{15}\text{N}$  with body size (Cherel et al. 2010). We mostly analyzed myctophids between 6 and 8 cm length for stable isotopes, which represents the upper range of the sizes estimated to be consumed by *D. gigas* in Field et al. (2013, their Appendix 1). As was done for juvenile and adult rockfish and hake in our study, the inclusion of smaller myctophid conspecifics (which have lower  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values) in our analysis would have likely resulted in a greater contribution of myctophid prey to *D. gigas*.

Our stable isotope results indicate that *D. gigas* feeds at a lower TL in the food web relative to that depicted based on diet studies. We suggest that even large *D. gigas* can feed on macroplankton such as euphausiids to a greater extent than revealed by diet analysis. This is consistent with stable isotope analyses of *D. gigas* from the Humboldt Current region, which suggested that *D. gigas* shows much plasticity in its diet and that even large individuals can consume euphausiids or other lower trophic level organisms (Lorrain et al. 2011, Argüelles et al. 2012). Euphausiids in upwelling areas are omnivorous, feeding on both phytoplankton and zooplankton (Gibbons et al. 1991, Dilling et al. 1998, Nakagawa et al. 2003), and occupy a similar TL to copepods based on their  $\delta^{15}\text{N}$  values (Miller et al. 2010). Although Field et al. (2013) list euphausiids in the diet of California Current *D. gigas* (13.9% by occurrence), they could not calculate the relative index of importance to the diet since they were not able to obtain specific abundances of prey.

There are several errors in Field et al.'s (2014) arguments that require clarification. The authors state there is a 'strong isotopic gradient' in the NCC;

however, this was only observed for  $\delta^{13}\text{C}$  and not  $\delta^{15}\text{N}$  in Miller et al. (2008), and a second cited paper (Olson et al. 2010) only covered the SCC system with isotopic gradients across ~1000 km from shore. In another instance, Ruiz-Cooley et al. (2010) are cited regarding trophic shifts in *D. gigas*, but these authors only examined relative ontogenetic shifts in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  along the gladius, the values of which are a function of geographic variation in isotopic baselines, trophic enrichment, and shifts in the TEF with ontogenetic shifts in diet; Ruiz-Cooley et al. (2010) did not account for these factors and therefore did not measure true trophic shifts. Field et al. (2014) also asserted that we noted specific prey in our analyses and results; however, our study was limited to prey groups that were applied to the model. Our results were based on generalities in the relative trophic position of prey groups, and we used available samples and published data to obtain a general view of how *D. gigas* may fit into the NCC food web. This limitation was clearly noted in our study.

## Conclusions

Given the assumptions and limitations in the SIAR migratory model, we concur with Field et al. (2014) that the migratory model is incapable of accurately assessing relative equilibrium of *D. gigas* across the California Current. However, Field et al.'s subsequent use of the model to indicate mixed sources of *D. gigas* is similarly invalid. The disparity between the isotopic values of *D. gigas* from the SCC (Madigan et al. 2012) and our study, the relative trophic position of *D. gigas* that is consistent with feeding between TL 3.2 and 3.4, and the  $\delta^{13}\text{C}$  match of *D. gigas* to offshore NCC organisms indicate that most of the animals from our study were in isotopic equilibrium, allowing for assessment of their prey sources. Field et al. (2014) cited papers in support of their assertion that the *D. gigas* in Miller et al. (2013) were recent migrants to the area and from far offshore (300 km); however, these papers contradict the prevailing literature from the region, which shows that *D. gigas* are predominantly distributed along the shelf-slope waters (e.g. Field et al. 2013, Stewart et al. 2013) and that this distribution overlaps with distributions of myctophids and their other prey. Moreover, Field et al. (2014) missed isotope values of *D. gigas* from the SCC (Madigan et al. 2012), and they did not address the match of our  $\delta^{13}\text{C}$  values of *D. gigas* to the NCC system relative to the SCC, which is more trophically conserved and a better indicator

of source production. This supports our original findings that the primary prey groups contributing as sources to *D. gigas* diet are shelf-slope species from the NCC system. Our finding that myctophids were not contributing significantly to *D. gigas* as a prey source was likely an artifact since we only analyzed adults (no data from other life history stages were available). Myctophids show strong size-dependent increases in  $\delta^{15}\text{N}$  with size (Cherel et al. 2010) and the inclusion of smaller conspecifics with lower relative  $\delta^{15}\text{N}$  values would have likely resulted in them being isotopically grouped with other offshore juvenile pelagic fishes. The conclusions of our study, namely that the specimens of *D. gigas* we examined from the Northern California Current, likely fed at a lower trophic level relative to what has been reported from diet studies, would not likely have changed.

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