

COMMENT

Stable isotope analysis of Humboldt squid prey: Comment on Miller et al. (2013)

John C. Field^{1,*}, Steven Y. Litvin², Aaron Carlisle², Julia S. Stewart^{2,4},
William F. Gilly², Rocio I. Ruiz-Cooley³

¹Fisheries Ecology Division, SWFSC/NMFS/NOAA, 110 Shaffer Rd, Santa Cruz, California 95060, USA

²Hopkins Marine Station of Stanford University, 120 Oceanview Dr, Pacific Grove, California 93950, USA

³Ocean Sciences Department, University of California Santa Cruz, 1156 High Street, Santa Cruz, California 95064, USA

⁴Present address: National Center for Ecological Analysis and Synthesis, Santa Barbara, California 93101, USA

ABSTRACT: Miller et al. (2013; Mar Ecol Prog Ser 477:123–134) used bulk stable isotope analysis (SIA) and a Bayesian isotope-mixing model (SIAR) to assess the diet of the Humboldt squid *Dosidicus gigas* in the Northern California Current. Their conclusions starkly contrast decades of food habits and other research on this organism. The methods they employed are sensitive to a range of assumptions and parameter estimates, and the trophic model they used is confounded by the interactive effects of movement and diet on *D. gigas* isotopic values. This is compounded by the sensitivity of the migratory model to critical assumptions of the trophic level of *D. gigas* (specifically the assumption that large *D. gigas* are approximately 1 trophic level above copepods), which are inconsistent with the literature. Therefore, Miller et al.'s (2013) conclusions regarding the contribution of specific prey to *D. gigas* in the Northern California Current are unsupported.

KEY WORDS: Humboldt squid · *Dosidicus gigas* · Stable isotope analysis · Bayesian mixing models · California Current

Resale or republication not permitted without written consent of the publisher

Introduction

Miller et al. (2013) assessed the diet of the Humboldt squid *Dosidicus gigas* in the Northern California Current (NCC) using bulk stable isotope analysis (SIA) and a Bayesian isotope-mixing model (SIAR, Parnell et al. 2010). SIA and Bayesian mixing models are powerful complementary approaches to stomach content analysis for determining diet. However, these methods are sensitive to a range of assumptions and parameter estimates, including the assumption of isotopic equilibrium with prey resources, trophic enrichment factors (TEFs, $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$), adequate isotopic characterization of prey resources and

their variance, the geometry of prey resource isotopic values, and isotopic turnover rates (Martínez del Río et al. 2009, Parnell et al. 2010, Boecklen et al. 2011, Bond & Diamond 2011, Layman et al. 2012, Ruiz-Cooley & Gerrodette 2012, Smith et al. 2013). Critical assumptions of both the migratory and trophic model in Miller et al. (2013) are inconsistent with the literature. For instance, the trophic model is confounded by the interactive effects of movement and diet on *D. gigas* isotopic values, and therefore, the conclusions regarding the contribution of specific prey to *D. gigas* in the NCC are unsupported.

The conclusions reached by Miller et al. (2013) relied on a sequential approach to developing an

understanding of *D. gigas* feeding habits. This included (1) a migratory model to examine the residency of *D. gigas* across 4 regions of the California Current and (2) a trophic model to examine the relative importance of prey resources in the diet of *D. gigas* within the NCC. These modeling approaches have different inherent *a priori* assumptions regarding *D. gigas* being in isotopic equilibrium with their diets at a given time and within a given region. For the migratory approach, there is no assumption of equilibrium; rather, the expectation is that *D. gigas* feeding across regions with isotopically distinct food webs would have isotopic signatures that are intermediate to those food webs, reflecting residency patterns and tissue turnover times (in the case of metabolically active tissues). Thus, mixing model results from the comparison of *D. gigas* isotopic signatures to their potential prey across regions (or sources that reflect production at the base of food webs within each region) are a relative measure of *D. gigas* residency among those locales (Hansson et al. 1997, Hobson 1999, Graham et al. 2010, Carlisle et al. 2012). Conversely, the trophic mixing model implicitly assumes that *D. gigas* is in isotopic equilibrium with prey resources within the NCC over the temporal and spatial scale of the model (Martinez del Rio & Wolf 2005).

The only case where identical *D. gigas* isotopic data can be used in both approaches is when the migratory model indicates that *D. gigas* tissues reflect residency within (i.e. are in isotopic equilibrium with) the region of interest for which potential prey resources are included within the trophic model. This is not the case in the migratory model of Miller et al. (2013), where southern California Current (SCC) end-members contribute considerably (>25%) to the *D. gigas* isotope values (see Fig. 3 in Miller et al. 2013), indicating that *D. gigas* are not in isotopic equilibrium with prey resources within the NCC. Nevertheless, the trophic model only includes isotope data for prey from the NCC, violating a basic assumption of this approach. Although Miller et al. (2013) claim that the contribution of NCC end members (74%) is sufficient to utilize the trophic model to estimate the contribution of NCC prey to the *D. gigas* they collected, they provide no evidence or reference to support their view that this level of disequilibrium with the NCC did not significantly bias their results.

Furthermore, in their trophic model, Miller et al. (2013) only include isotopic data for prey from near-shore areas (less than 70 km offshore) to isotopically characterize prey across the entire NCC, despite the fact that the distribution of squid is known to extend

at least 300 km offshore (Field et al. 2013, Ruiz-Cooley et al. 2013). Due to the strong isotopic gradient between inshore and offshore areas within the NCC (Fig. 3 in Miller et al. 2008; as well as Olson et al. 2010, Ruiz-Cooley & Gerrodette 2012), it would have been important to account for these gradients in the absence of information on isotopic values of potential offshore prey. In particular, the relative effect of foraging in regions isotopically distinct from the coastal NCC—as opposed to prey preferences within the NCC—on *D. gigas* isotopic values needed to be considered, and the inshore–offshore gradient in prey isotopic values within the NCC should have been fully characterized. In the absence of these analyses, Miller et al.'s (2013) conclusions on the contribution of specific prey to *D. gigas* in the NCC are doubtful. Moreover, recent analysis has demonstrated that squid inhabiting the NCC could have originated from multiple regions of Baja California, the Northeastern tropical Pacific and even far offshore waters of the California Current (Ruiz-Cooley et al. 2013), further complicating the assumptions of the migratory model.

Sensitivity of the migratory model

While the migratory model as presented by Miller et al. (2013) demonstrates that *D. gigas* are not in isotopic equilibrium with NCC prey resources, another key issue is that the *a priori* assumption that large (~60 cm) *D. gigas* feed at a low trophic level likely inflated the contribution of NCC end-members to *D. gigas* isotope values. While not explicitly stated in the methods, the choice of copepods as baselines for migratory end-members and a single TEF in the migratory model *a priori* presumes that the isotopic signatures of *D. gigas* are a result of feeding exclusively on copepods (or other organisms at the same trophic level as copepods) across their ontogeny and in all regions. As discussed comprehensively in the next paragraph, this assumption is incorrect. While copepods can indicate regional differences in isotopic baselines, the assumed trophic level (TL) difference between a consumer and the species chosen as the baseline will greatly affect mixing model results (Graham et al. 2010, Bond & Diamond 2011).

Decades of research have demonstrated that Humboldt squid are opportunistic and voracious predators, preying primarily on mesopelagic fishes (particularly in oceanic habitats) throughout their distribution (Nigmatullin et al. 2001, Markaida et al. 2008, Rosas-Luis et al. 2011, Field et al. 2007, 2013,

and references therein). In a comparison among a number of these food habits studies between the Gulf of California and the central California Current (essentially between the 'northern' and 'southern' regions of Miller et al. 2013), mesopelagic fishes were present in 80% of stomachs in both regions, accounting for approximately 80% of the larger (fish and cephalopod) prey items in the Gulf of California and 50% in the California Current (Stewart et al. 2013). Although krill and other macrozooplankton are occasional prey, and in fact their contribution is potentially underestimated due to differences in digestibility and other factors, these studies demonstrate that prey items at a considerably higher trophic level than copepods represent a substantial fraction of the diet of *D. gigas*.

Moreover, the habitat affinities of *D. gigas*, which overlap strongly with the range of mesopelagic fishes, provide additional strong evidence of the tight interactions among these species (Stewart et al. 2013). Contrary to the suggestion of Miller et al. (2013) that the discrepancy in food source could have been a consequence of 'net-feeding' in trawl-caught squid, <30 of the over 2000 squid stomachs analyzed in the studies summarized by Stewart et al. (2013) were trawl-caught. Instead, nearly all were caught using vertical jigging gear that is widely acknowledged to be the least likely method to induce bias in empirical food habits studies of cephalopods.

Finally, in the California Current, the diet composition of Humboldt squid varies substantially across space, particularly between inshore and offshore domains, as well as across seasons, with greater predation on larger prey at larger *D. gigas* sizes (Field et al. 2013). SIA has also revealed such ontogenetic changes in the trophic position of *D. gigas* in various ecosystems from the Eastern Pacific, and these patterns differ from system to system (Ruiz-Cooley et al. 2010). As a consequence of this wide body of literature and empirical results, an assumption of feeding at >1 TL above copepods in the migratory model would better reflect what is known about the feeding habits of Humboldt squid—particularly for the larger (mean \pm SD: 59 \pm 5.6 cm) *D. gigas* that Miller et al. (2013) collected in the NCC.

We simulated the effect of the assumed trophic level of *D. gigas* on Miller et al.'s (2013) migratory mixing model results by randomly selecting 76 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from the empirical distribution of *D. gigas* reported in Table 1 of Miller et al. (2013). We then ran SIAR using the regional copepod end-member values reported in Miller et al. (2013; see their 'Methods: Data analysis' section), the simulated *D.*

gigas data, and mean (\pm 1 SD) $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ of 3.4 ± 1.0 and 0.8 ± 1.3 ‰, respectively. In the methods section, Miller et al. (2013) state that they used $\Delta\delta^{13}\text{C}$ of 0.4 ± 1.3 ‰ in their migratory mixing models but the legends of both their Figs. 2 & 3 state that 0.8‰ were used to generate the results. Because the results graphically depicted in Miller et al.'s (2013) Fig. 3 seem to reflect those given in the written results (their 'Results: Migratory end-members' section), we used mean $\Delta\delta^{13}\text{C}$ of 0.8‰ in our simulation. When we assume 1 TL between *D. gigas* and copepods in all regions, our results are similar, though not identical, to Miller et al.'s (2013), with a median contribution of 36% for NCC offshore, 26% NCC nearshore, 34% SCC nearshore and 4% SCC offshore (regions as defined in Miller et al. 2013).

However, if we modify Miller et al.'s (2013) TEF assumptions, such that *D. gigas* arriving in NCC feed just 0.5 TL higher, i.e. 1.5 TL above copepods (adjusted TEF with propagated error: $\Delta\delta^{15}\text{N}$ and $\Delta\delta^{13}\text{C}$ of 5.1 ± 1.1 and 1.2 ± 1.5 ‰, respectively), the results change substantially: the contribution of the offshore SCC region rises to 27%, and the SCC overall to 45%, with the 2 NCC regions contributing evenly to *D. gigas* isotope values. Under this latter scenario, *D. gigas* is clearly not in equilibrium with any particular region, and this in turn precludes limiting a trophic mixing model analysis of prey contributing to *D. gigas* diet to only those prey from NCC regions. Assuming a TL of 1.5 above copepods for *D. gigas* in both the SCC and NCC would further increase the estimated contribution of the SCC region (50% total), particularly SCC offshore (43%), to *D. gigas* isotope values.

In either of our scenarios (TL of 1.5 for *D. gigas* in the NCC only or SCC and NCC), the results indicate that the isotopic values of *D. gigas* collected by Miller et al. (2013) in the NCC are likely and to a great extent the result of foraging in other isotopically distinct regions, rather than solely due to prey preference within the NCC. This strengthens our assertion that the conclusions of Miller et al. (2013) on the contribution of specific prey to *D. gigas* in the NCC are unsupported. While the seasonal and spatial variability in the relative contribution of prey to *D. gigas* may not be well known due to the squids' opportunistic behavior, the assumption that *D. gigas* feed exclusively 1 TL above copepods is unreasonable; an assumed TL >1 for the migratory model is more appropriate. At a minimum, Miller et al. (2013) should have undertaken the same level of sensitivity analysis on the effect of TL in the migratory model as they did for TEF in the trophic approach (their Fig. 6).

Timing of sample collection

Regardless of the degree of contribution of SCC prey to *D. gigas* (~25 to 50%), additional evidence suggests that squid used in the Miller et al. (2013) study were not in the NCC long enough to reach isotopic equilibrium. Miller et al. (2013) collected samples in July, August and September in Oregon, and they cite Litz et al. (2011) as evidence that *D. gigas* arrived in Oregon sufficiently early to be in isotopic equilibrium with regional prey resources. Litz et al. (2011) report that in over 900 trawls over a 6 yr period (May to November, 2004 to 2009), 2612 squid were encountered in 60 different trawls. However, only 3 of the trawls encountering *D. gigas* (<5% of the total) and 15 of the individual squid (0.5%) occurred before August, despite proportionately greater sampling effort in the May–July period. These data demonstrate an extreme rarity of *D. gigas* in this region prior to August, particularly in nearshore waters. Humboldt squid is a highly mobile predator, capable of horizontal migrations of ~40 km d⁻¹ (Gilly et al. 2006, Stewart et al. 2012). Their virtual absence in the May–July period contradicts the assertion of Miller et al. (2013) that *D. gigas* collected in August and September were all likely resident in the NCC long enough, at least 100 d according to Miller et al. (2013), to approach (~80%), but not reach, isotopic equilibrium with regional prey resources.

Conclusions

Mixing models are sensitive to a wide range of assumptions and parameter estimates (Martínez del Rio et al. 2009, Boecklen et al. 2011, Bond & Diamond 2011, Layman et al. 2012, Smith et al. 2013), and Parnell et al. (2010) explicitly caution that ‘SIAR will always attempt to fit a model’ (p. 5). Miller et al.’s (2013) results do not demonstrate that the *D. gigas* collected were in equilibrium with prey resources of the NCC, under any assumption of trophic level, rendering any assessment of prey contribution to *D. gigas* in the NCC using the approach of Miller et al. (2013) unsupported. No single method is without limitations, and there are rarely sufficient data available in any study to rigorously address every uncertainty. We suggest that additional sampling of squid late into the fall and winter, perhaps combined with retrospective isotopic analysis via sequential sampling of *D. gigas* gladii, could help resolve the timing of migration and residency patterns in order to evaluate if isotopic equilibrium has been reached with prey

resources (Ruiz-Cooley et al. 2010, Lorrain et al. 2011, Argüelles et al. 2012).

LITERATURE CITED

- Argüelles J, Lorrain A, Cherel Y, Graco M and others (2012) Tracking habitat and resource use for the jumbo squid *D. gigas*: a stable isotope analysis in the Northern Humboldt Current System. *Mar Biol* 159:2105–2116
- Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. *Annu Rev Ecol Evol Syst* 42:411–440
- Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol Appl* 21:1017–1023
- Carlisle AB, Kim SL, Semmens BX, Madigan DJ and others (2012) Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). *PLoS ONE* 7: e30492
- Field JC, Baltz K, Phillips AJ, Walker WA (2007) Range expansion and trophic interactions of the Humboldt squid, *Dosidicus gigas*, in the California Current. *Calif Coop Ocean Fish Invest Rep* 48:131–146
- Field JC, Elliger C, Baltz K, Gillespie G and others (2013) Foraging ecology and movement patterns of the Humboldt squid in the California Current. *Deep-Sea Res II* 95:37–51
- Gilly WF, Markaida U, Baxter CH, Block BA and others (2006) Vertical and horizontal migrations by the Humboldt squid *Dosidicus gigas* revealed by electronic tagging. *Mar Ecol Prog Ser* 324:1–17
- Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: West JB, Bowen GJ, Dawson TA, Tu KP (eds) *Isoscapes: understanding movement, pattern and process on earth through isotope mapping*. Springer, Dordrecht, p 299–318
- Hansson S, Hobbie JE, Elmgren R, Larsson U, Fry B, Johansson S (1997) The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. *Ecology* 78: 2249–2257
- Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120:314–326
- Layman CA, Araujo MS, Boucek R, Hammerschlag Peyer CM and others (2012) Applying stable isotopes to examine food web structure: an overview of analytical tools. *Biol Rev Camb Philos Soc* 87:545–562
- Litz MNC, Phillips AJ, Brodeur RD, Emmett RL (2011) Seasonal occurrences of Humboldt squid (*Dosidicus gigas*) in the northern California Current system. *Calif Coop Ocean Fish Invest Rep* 52:97–108
- Lorrain A, Argüelles J, Alegre A, Bertrand A and others (2011) Sequential isotopic signature along gladius highlights contrasted individual foraging strategies of jumbo squid (*Dosidicus gigas*). *PLoS ONE* 6(7):e22194
- Markaida U, Gilly W, Salinas-Zavala C, Rosas-Luis R, Booth A (2008) Food and feeding of jumbo squid *Dosidicus gigas* in the central Gulf of California during 2005–2007. *Calif Coop Ocean Fish Invest Rep* 49:90–103
- Martínez del Rio C, Wolf BO (2005) Mass balance models for animal isotopic ecology. In: Starck MA, Wang T (eds) *Physiological and ecological adaptations to feeding in*

- vertebrates. Science Publishers, Enfield, NH, p 141–174
- Martínez del Río C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biol Rev Camb Philos Soc* 84:91–111
- Miller TW, Brodeur RD, Rau GH (2008) Carbon stable isotopes reveal relative contribution of shelf-slope production to the northern California Current pelagic community. *Limnol Oceanogr* 53:1493–1503
- Miller TW, Bosley KL, Shibata J, Brodeur RD, Omori K, Emmett R (2013) Contribution of prey to Humboldt squid *Dosidicus gigas* in the northern California Current, revealed by stable isotope analyses. *Mar Ecol Prog Ser* 477:123–134
- Nigmatullin CM, Nesis KN, Arkhipkin AI (2001) A review of the biology of the jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae). *Fish Res* 54:9–19
- Olson RJ, Popp BN, Graham BS, López-Ibarra GA and others (2010) Food-web inferences of stable isotope spatial patterns in copepods and yellowfin tuna in the pelagic eastern Pacific Ocean. *Prog Oceanogr* 86:124–138
- Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5:e9672
- Rosas-Luis R, Tafur-Jimenez R, Alegre-Norza AR, Castillo-Valderrama PR, Cornejo-Urbina RM, Salinas-Zavala CA, Sánchez P (2011) Trophic relationships between the jumbo squid (*Dosidicus gigas*) and the lightfish (*Vinciguerria lucetia*) in the Humboldt Current System off Peru. *Sci Mar* 75:549–557
- Ruiz-Cooley RI, Gerrodette T (2012) Tracking large-scale latitudinal patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ along the E Pacific using epi-mesopelagic squid as indicators. *Ecosphere* 3: art63, doi:10.1890/ES12-00094.1
- Ruiz-Cooley RI, Villa EC, Gould WR (2010) Ontogenetic variation of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ recorded in the gladius of the jumbo squid *Dosidicus gigas*: geographic differences. *Mar Ecol Prog Ser* 399:187–198
- Ruiz-Cooley RI, Ballance LT, McCarthy MD (2013) Range expansion of the jumbo squid in the NE Pacific: $\delta^{15}\text{N}$ decrypts multiple origins, migration and habitat use. *PLoS ONE* 8:e59651
- Smith JA, Mazumder D, Suthers IM, Taylor MD (2013) To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods Ecol Evol* 4: 612–618
- Stewart JS, Hazen EL, Foley DG, Bograd SJ, Gilly WF (2012) Marine predator migration during range expansion: Humboldt squid *Dosidicus gigas* in the northern California Current System. *Mar Ecol Prog Ser* 471:135–150
- Stewart JS, Field JC, Markaida U, Gilly WF (2013) Behavioral ecology of jumbo squid (*Dosidicus gigas*) in relation to oxygen minimum zones. *Deep-Sea Res II* 95:197–208

Editorial responsibility: Christine Paetzold, Oldendorf/Luhe, Germany

Submitted: August 22, 2013; Accepted: February 7, 2014
Proofs received from author(s): February 15, 2014