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**Extended abstract**

# What can DNA in fish stomachs tell us about the Southern Ocean?

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Mesopelagic fish form an important link between zooplankton and higher trophic levels in Southern Ocean food webs, however, their diets are poorly known. Most of the dietary information available comes from morphological analysis of stomach contents (Hopkins and Torres, 1989; e.g. Gaskett et al., 2001; Pusch et al., 2004; Shreeve et al., 2009) and to a lesser extent fatty acid and stable isotopes. DNA sequencing could substantially improve our knowledge of mesopelagic fish diets, but has not previously been applied. We used high-throughput DNA sequencing (HTS) of the 18S ribosomal DNA and mitochondrial cytochrome oxidase I (COI) to characterise stomach contents of four myctophid and one bathylagid species collected at the southern extension of the Kerguelen Plateau (southern Kerguelen Axis), one of the most productive regions in the Indian sector of the Southern Ocean.

Diets of the four myctophid species were dominated by amphipods, euphausiids and copepods, whereas radiolarians and siphonophores contributed a much greater proportion of HTS reads for *Bathylagus* sp. (Figure 1a). Analysis of mitochondrial COI showed that all species preyed on bigeye krill (*Thysanoessa macrura*), but Antarctic krill (*Euphausia superba*) was only detected in the stomach contents of myctophids. Size-based shifts in diet were apparent, with larger individuals of both bathylagid and myctophid species more likely to consume euphausiids (Figure 2), but we found little evidence for regional differences in diet composition for each species over the survey area. The presence of DNA from coelenterates and other gelatinous prey in the stomach contents of all five species (Figures 1b and 1c), which are largely missed with morphological analysis, suggests the importance of these taxa in the diet of Southern Ocean mesopelagics has been underestimated to date. Our dual marker approach (18S and COI) combined the broad taxonomic coverage of 18S (e.g. to detect taxa such as radiolarians not amplified by COI) with the taxonomic resolution of COI (e.g. to distinguish different krill species). The COI marker also helped avoid misidentification of fish species. Indeed, the presence of four distinct COI operational taxonomic units assigned to *Bathylagus* sp. supports the presence of cryptic diversity in Southern Ocean specimens of this genus (Dettai et al., 2011).

The sensitivity of high-throughput sequencing raises the possibility of secondary predation influencing the results, namely detecting taxa consumed by dietary items of the focal species (e.g. Sakaguchi et al., 2017). Indeed, we detected protists like diatoms as prey items in 12.5% of myctophid stomachs that could represent secondary predation given how often these species prey on euphausiids and other taxa likely to consume diatoms. Applying high-throughput sequencing to individual diet items showed that, although secondary predation could potentially be detected in our fish diet samples, the relative abundance of these items would rarely be greater than 1%.

Our study highlights the utility and complementarity of DNA-based diet analysis to traditional morphology-based approaches for characterising trophic links in mesopelagic fish assemblages that are difficult to study. Critically, this method highlighted the importance and diversity of gelatinous prey items, consistent with DNA-based diet analyses of other Southern Ocean predators (Jarman et al., 2013; McInnes et al., 2017). The results of this study provide important information for developing models of Southern Ocean food webs and isotopic niche analyses for Southern Ocean mesopelagics.

This paper is based on Clarke et al. (2018): DNA-based diet analysis of mesopelagic fish from the southern Kerguelen Axis. *Deep-Sea Res. II*.
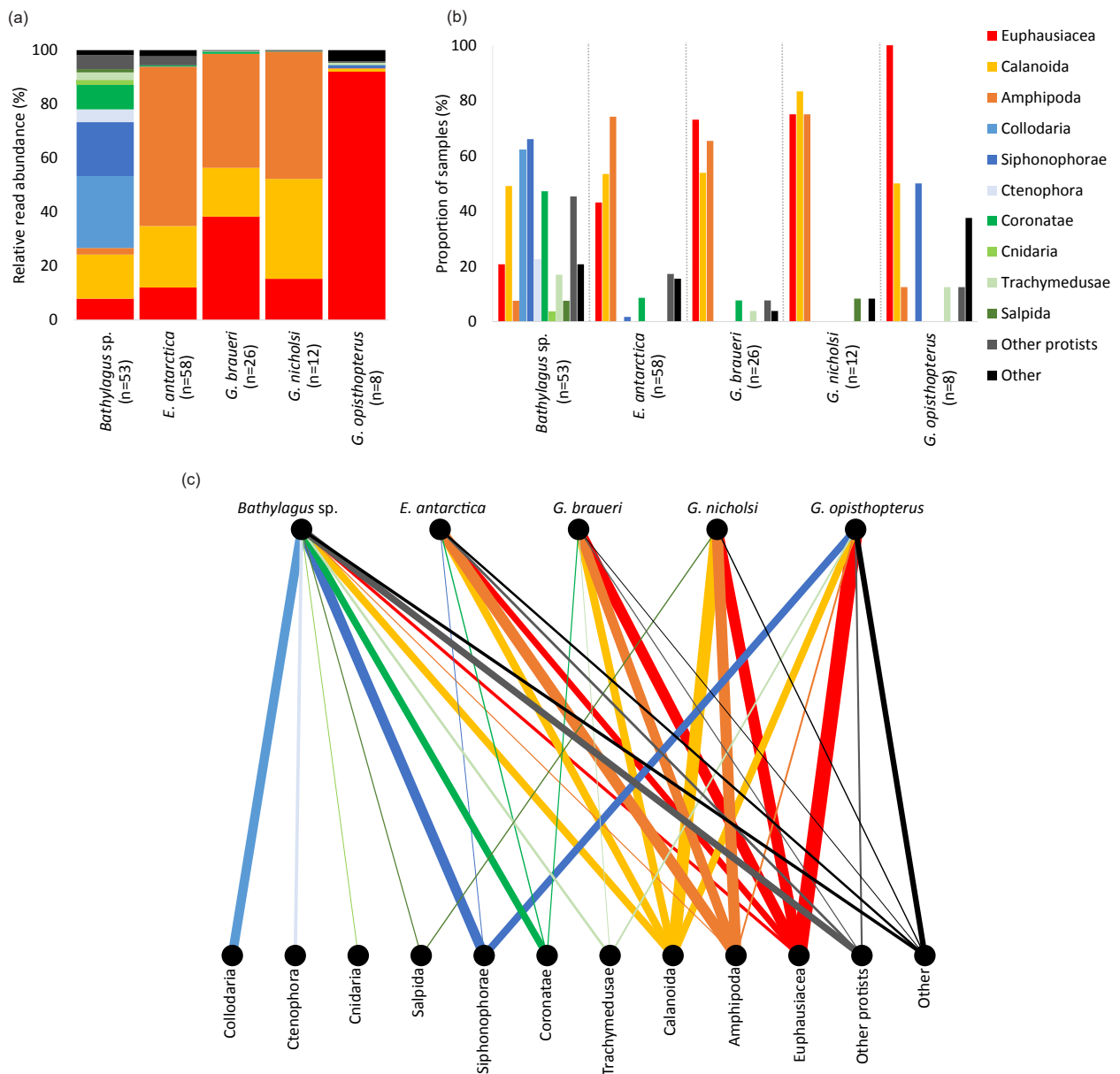
Figure 1: Prey items (classified to order) recovered in each fish species. (a) Mean relative read abundance and (b) frequency of occurrence. (c) Representation of prey preferences using DNA-based diet analysis. The width of each link is proportional to the frequency of prey item occurrence in each fish species. Prey items were deemed present in a sample if they comprised >1% total reads obtained. Prey items that represented >50% of the reads in any sample are shown, remaining taxa are pooled as 'Other protists' or 'Other'. Reproduced from Clarke et al. (2018).
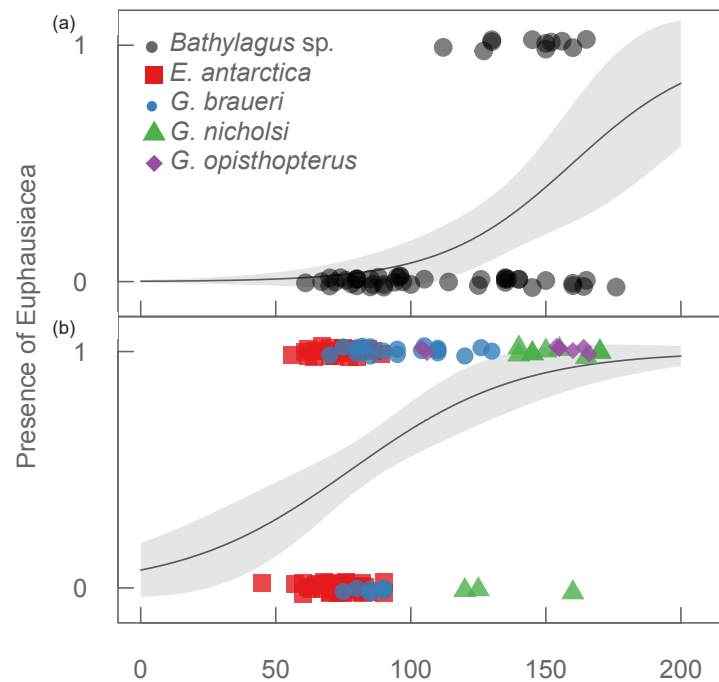
Figure 2:   Relationship between fish size and detection of Euphausiacea
(>1% read abundance) in stomach contents for (a) *Bathylagus* sp. and (b)
myctophids. Fitted curves are logistic regressions for *Bathylagus* sp. and all
myctophids, respectively. Reproduced from Clarke et al. (2018).

## References

Clarke, L.J., R. Trebilco, A. Walters, A.M. Polanowski and B.E. Deagle. 2018. DNA-based diet analysis of mesopelagic fish from the southern Kerguelen Axis. *Deep-Sea Res. II*, doi: 10.1016/j.dsr2.2018.09.001.

Dettai, A., A.C. Lautredou, C. Bonillo, E. Goimbault, F. Busson, R. Causse, A. Couloux, C. Cruaud, G. Duhamel, G. Denys, M. Hautecoeur, S. Iglesias, P. Koubbi, G. Lecointre, M. Moteki, P. Pruvost, S. Tercerie and C. Ozouf. 2011. The actinopterygian diversity of the CEAMARC cruises: Barcoding and molecular taxonomy as a multi-level tool for new findings. *Deep-Sea Res. II*, 58 (1–2): 250–263, doi: 10.1016/j.dsr2.2010.05.021.

Gaskett, A.C., C. Bulman, X. He and S.D. Goldsworthy. 2001. Diet composition and guild structure of mesopelagic and bathypelagic fishes near Macquarie Island, Australia. *New Zeal. J. Mar. Fresh.*, 35 (3): 469–476, doi: 10.1080/00288330.2001.9517016.

Hopkins, T.L. and J.J. Torres. 1989. Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. *Deep-Sea Res.*, 36 (4): 543–560.

Jarman, S.N., J.C. McInnes, C. Faux, A.M. Polanowski, J. Marthick, B.E. Deagle, C. Southwell and L. Emmerson. 2013. Adélie penguin population diet monitoring by analysis of food DNA in scats. *PLoS One*, 8 (12): e82227, doi: 10.1371/journal.pone.0082227.

McInnes, J.C., R. Alderman, M.-A. Lea, B. Raymond, B.E. Deagle, R.A. Phillips, A. Stanworth, D.R. Thompson, P. Catry, H. Weimerskirch, C.G. Suazo, M. Gras and S.N. Jarman. 2017. High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. *Mol. Ecol.*, 18: 4831–4845, doi: 10.1111/mec.14245.

Pusch, C., P.A. Hulley and K.H. Kock. 2004. Community structure and feeding ecology of mesopelagic fishes in the slope waters of King George Island (South Shetland Islands, Antarctica). *Deep-Sea Res. I*, 51 (11): 1685–1708, doi: 10.1016/j.dsr.2004.06.008.

Sakaguchi, S.O., S. Shimamura, Y. Shimizu, G. Ogawa, Y. Yamada, K. Shimizu, H. Kasai, H. Kitazato, Y. Fujiwara, K. Fujikura and K. Takishita. 2017. Comparison of morphological and DNA-based techniques for stomach content analyses in juvenile chum salmon Oncorhynchus keta: a case study on diet richness of juvenile fishes. *Fisheries Sci.*, 83 (1): 47–56, doi: 10.1007/s12562-016-1040-6.

Shreeve, R.S., M.A. Collins, G.A. Tarling, C.E. Main, P. Ward and N.M. Johnston. 2009. Feeding ecology of myctophid fishes in the northern Scotia Sea. *Mar. Ecol. Prog. Ser.*, 386: 221–236, doi: 10.3354/meps08064.