Microscopic and DNA-based diet analyses of larval Antarctic myctophid fish *Electrona antarctica* in the Southern Ocean

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Myctophid fishes are the most abundant mesopelagic fish in the Southern Ocean; their estimated biomass in those waters is 130×10^6 t. Myctophids form a trophic link in the Southern Ocean between the zooplankton and higher predators. Of the 35 species of myctophid in the Southern Ocean, *Electrona antarctica* is the most abundant. The larval stages of *E. antarctica* are categorized into planktonic (< 12–13 mm body length [BL]) and nektonic (between 12–13 and 19–21 mm BL) phases. Transformation in this species occurs between 19 and 21 mm BL. Adult and juvenile *E. antarctica* feed on zooplankton, and it has been suggested that larval *E. antarctica* are also primarily zooplanktivorous. However, 83% of the guts of larvae examined in the Scotia Sea were empty. Gut content analysis using light microscope may cause an underestimation of digestible and visually unrecognizable food items. To diminish these biases, this study analysed diet of larval *E. antarctica* by a metagenomic analysis to detect dominant eukaryotic taxa in gut contents.

A multidisciplinary research cruise was conducted off Wilkes Land (East Antarctica) during January 2018 and 2019. Fish larvae were sampled by oblique tow of a ring net (mouth diameter: 1.60 m, mesh size: 500 μm) from ca. 200 m depth to the sea surface and Matsuda-Oozeki-Hu Trawl (MOHT, mouth area: 5 m^2, mesh size: 1.92 mm) equipped with five sets of opening/closing net. Nets of MOHT were opened and closed at 400, 200, 100, 50 and 0 m depth. Samples were preserved in 90% ethanol. We randomly selected 33 larvae and extracted DNA of their gut contents using 5% *Chelex* 100 resin. Gut contents were identified and counted under a light microscope before DNA extraction. Eukaryotic universal primers 1389F and 1510R were used to amplify the 18S rRNA V9 region. We designed a blocking primer intended to bind to the *E. antarctica* sequence amplified by the universal primers. A modified PCR protocol were applied in this study.

We observed a low feeding incidence; prey items were found from 20% of planktonic and 38% of nektonic larvae in microscopic observations. The frequency of zooplankton was 10% and 15% in the planktonic and nektonic phase, respectively (Fig. 1). Diatoms were detected from all larvae in both planktonic and nektonic phases by metagenomic analysis. Dinoflagellates and haptophytes were detected at higher frequencies of 42% and 21% in planktonic and nektonic phases, respectively. Pteropods and copepods occurred in higher frequency in zooplankton taxa. Pteropods were detected from 95% and 62% of the planktonic and nektonic larvae, respectively. Copepods were detected from 65% and 46% of the planktonic and nektonic larvae, respectively.

The previous study, which concluded that larval *E. antarctica* is primarily zooplanktivorous, also observed a low frequency of zooplankton (9%, n = 332). Results of these microscopic analyses indicate that larval *E. antarctica* do not preferentially feed on zooplankton. Meanwhile, the metagenomic analysis found out high frequency of copepods and pteropods in gut contents. Diatoms, dinoflagellates and haptophytes were also frequently detected. Herbivorous copepods primarily feed on diatoms, and pteropods filter variety of phytoplankton using mucus webs. We therefore suggest that faecal pellets of herbivorous zooplankton such as copepods and pteropods are essential food items for *E. antarctica* throughout larval stages.

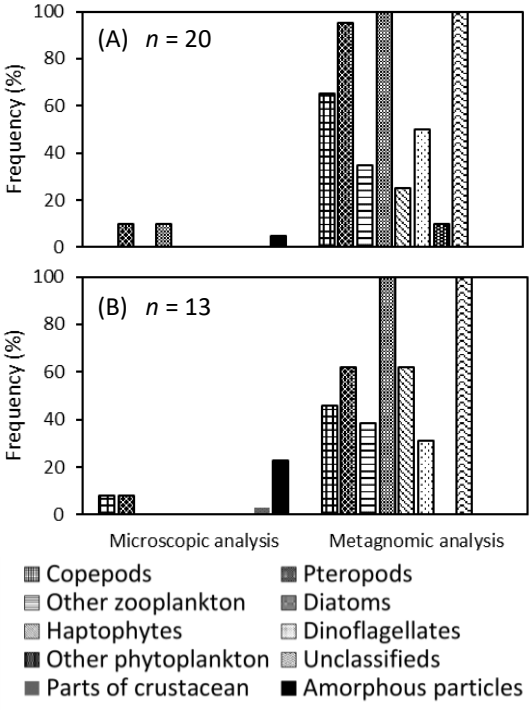

Fig 1. Frequency occurrence of gut contents found by microscopic and metagenomic analysis from larval *Electrona antarctica* in the (A) planktonic (<12mm body length) and (B) nektonic phase (>12 mm body length); Parts of crustacean and Amorphous particles were visually identified only at microscopic analysis.