Empirical equations and image analyses for estimating zooplankton biomass in the Southern Ocean

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Zooplankton plays important roles as secondary producers of marine food webs and drivers of biological carbon pump. The Antarctic krill (*Euphausia superba*), known as a key player in such ecological roles in the Southern Ocean, have been centered in various ecological studies. However, recent studies have shown the heterogeneous distribution of this species, and the importance of the food web structure centered on other zooplankton species is increasing. This food web structure has not been studied as much as the krill-dependent food web. Therefore, the players that take the place of krill in this food web structure have rarely been quantitatively evaluated. These players include diverse taxonomic groups such as copepods, amphipods, ostracods, molluscs, pteropods, and salps. Although these groups are diverse in size, feeding, and body structure, they have often been evaluated as a single group. This prevent us to quantitative understand of the ecosystem structures and the energy flow in the Southern Ocean. One of the major reasons why zooplankton community structure with biomass of each taxon have not yet been accumulated is that analysis requires a great deal of time and labor. This is because manual analysis has been the main method used in the past, either by directly measuring weight or by measuring body length and converting it to weight. To solve this problem, a method has been developed to automatically measure surface area from image data of a sample and obtain quantitative data from the relationship between surface area and weight, although there are only two studies condusted around the Antarctic Peninsula.

Zooplankton samples were collected off Vincennes Bay in the Southern Ocean by using ORI net, MOHT net during TV *Umitaka maru* cruise in January 2019. The samples were frozen by liquid nitrogen immediately after collection and stored at <-60°C until analyses. The frozen samples were thawed, photographed, dried, and weighted on land laboratory. Body length and width data were measured using the image processing software ImageJ (National Institute of Health), and surface area was calculated by assuming an ellipse with these values as its diameter. After then, each zooplankton specimen was dried at 60°C for 24 hours for measuring dry weight using a microbalance. Totally, 408 specimens (covers 26 taxonomic groups) were analyzed. For many taxa, relationship between surface area and dry mass showed better correlations than those of length-dry mass relationship. Crustaceans showed similar regression equations for the relationship between surface area and weight regardless of taxonomic group, while the equations of two copepods, *Calanoides acutus* and *Rhincalanus gigas*, which known as dominant suspension feeder, were different from those of other crustaceans. The regression equations of the other taxa were completely different from those of crustaceans. In addition, we examined the best method for the area detection using the ImageJ. The binarization methods "RenyiEntropy" and "Default" recorded high detection rates for many taxa. However, both methods have their strengths and weaknesses, and some individuals were not recognized at all. We will continue to discuss which method to use and how to use it in the binarization process.

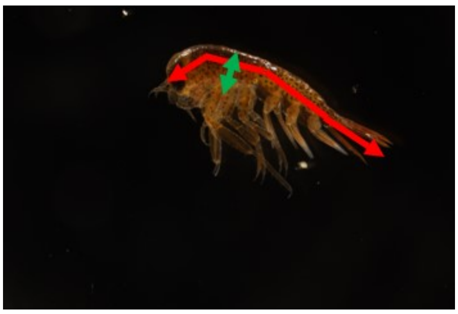

Figure 1. Lengths measured for area calculation. Length and height.

Figure 2. Image after binarization using the "Default" binarization method