# DEVELOPMENT AND DEMONSTRATION OF A QUANTITATIVE PCR BASED METHOD TO ENUMERATE COPEPOD NAUPLII IN FIELD SAMPLES

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**Abstract**

Copepod nauplii are important members of the marine planktonic community, and they can be the most abundant component of the microzooplankton. Despite the importance of copepod early life history stages to food web dynamics and carbon flux in the sea, there is a paucity of information about their ecology due to challenges in identifying nauplii to species, and in sampling them quantitatively. I report here on the development and optimization of a new molecular method that uses quantitative PCR (qPCR) to identify and estimate the abundances of nauplii of a common coastal copepod, *Parvocalanus crassirostris*, in field samples. The following experiments were performed towards this goal: I surveyed the genetic diversity of copepods in the study region, optimized sample treatment for qPCR, developed a size fractionation protocol to separate life stages of the target species, quantified the mitochondrial cytochrome C oxidase subunit I (mtCOI) gene copies in each *P. crassirostris* life stage, tested the effect of food levels on mtCOI copy number in nauplii, and compared direct counts to qPCR estimates of the target species to validate the qPCR method. The number of mtCOI gene copies in each developmental stage of this species was found to increase by ~1.5 orders of magnitude from early nauplii to adult. Food level experiments suggested that mtCOI copy number may be influenced by feeding environment in late naupliar stages. In validation experiments, qPCR estimates were 68 to 130% of the number estimated from direct counts. Both methods had a coefficient of variation of approximately 16%, indicating similar precision across methods. As a field test of the method, daily samples were collected in southern Kane‘ohe Bay and used to quantify the density *P. crassirostris* nauplii over a 13-day period in the summer of 2011. The average density of *P. crassirostris* nauplii in developmental stages NII - NV was found to be $1.5\times10^{3}$ individuals $m^{-3}$ over the 13-day period. The qPCR-based method developed here will enable future studies on naupliar ecology in the field, including investigation of food web, population, and community dynamics.