Feeding and food limitation in copepod nauplii, the neglected life stage
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Project summary (from final report):

Laboratory experiments

We focused most of our laboratory effort on answering question 1 for copepod species readily available near the Romberg Tiburon Center, focusing on two species with very different feeding modes as adults. *Pseudodiaptomus marinus* feeds by creating a feeding current; *Oithona davisae* is an ambush predator that focuses on small motile prey such as flagellates and ciliates. The thrust of the experimental work has been with autofluorescing cells so that we can use fluorescence techniques to detect feeding. We conducted experiments to determine feeding capability (i.e., whether the adults or nauplii feed on a particular species of phytoplankton) and feeding rate (consumption relative to body carbon mass by adults and nauplii).

To determine feeding capability, copepods were held in filtered surface water for 3 hours to clear their guts, then placed in containers of the selected phytoplankton species and allowed to feed for 3060 minutes. Then 12 copepods of each stage were examined under an epifluorescence microscope and the degree of gut fullness as indicated by chlorophyll fluorescence in each copepod was given a score. The scores were then averaged to determine an overall index of feeding on the particular algal species. We conducted these experiments on both species with 14 species of phytoplankton differing in size, motility, presence of spines, and (apparently, judging from our results) taste.

Feeding rate for some of the phytoplankton was determined through a novel adaptation of the gutfluorescence method that uses a microplate reader to allow for measurements on individuals or small groups of copepods. Again, copepods were starved and then allowed to feed for 3060 minutes. Individual adults (*Pseudodiaptomus marinus*) or groups of 5 adults (*Oithona davisae*) or groups of nauplii of about the same carbon mass were frozen on dry ice and stored at 80˚. Chlorophyll was extracted in ethanol and a subsample of the extract was read along with blanks and pure-chlorophyll standards on a Tecan Infinite 200 microplate reader with 430 nm excitation and 630 nm emission filters. Samples were acidified and read again to allow for calculation of phaeopigments.

All samples from an experiment were read at the same time along with blanks and standards. This eliminated any problem with instrument drift, thereby reducing the nuisance variance in analyses of chlorophyll content between microplate cells, e.g., in comparisons of nauplii with adults and comparisons among algal species.

Results have been presented in a Master's thesis at San Francisco State University (Vogt 2013), and in a paper in Limnology and Oceanography (Vogt et al. 2014). Briefly, we found that nauplii of both species were capable of feeding on most of the same prey as conspecific adults, with a somewhat smaller upper size limit than for adults. The lower size limit for *P. marinus* was smaller for adults than for nauplii, probably because of the elaborate suspensionfeeding apparatus of the adult that is absent from the nauplii. Both stages of *O. davisae* fed on a variety of prey, including nonmotile prey such as diatoms. This was a surprise, because the prevailing opinion about *Oithona* spp. is that they feed only on motile organisms. We assume that the

feeding observed was a result of bloomlevel food density which increased the encounter rate above what would be observed in a more dilute feeding environment.

Feeding rates were measured on three prey species: the cryptomonad *Rhodomonas salina*, 30 the prasinophyte *Tetraselmis suecica*, and the diatom *Thalassiosira pseudonana*. Although both life stages of *P. marinus* fed at similar rates on all three phytoplankton, *O. davisae* did not feed on *R. salina*, and only nauplii fed on *T. pseudonana*. The lack of feeding on *Rhodomonas* by this copepod has been reported before, despite the use of this phytoplankton as the sole food in long-term cultures of other copepods.

We have broadened the scope of these experiments to include ciliates using Cell Tracker Green fluorescent dye, and a newly acquired Olympus model IX81 epifluorescence microscope acquired under NSFFSML grant 1227231. In collaboration with an REU student we conducted preliminary experiments to verify staining, ensure normal behavior by stained ciliates, and detect ingestion by both adults and nauplii of *P. marinus*. This was followed up with another REU student who conducted feeding experiments and confirmed that nauplii are equally capable of feeding on ciliates as adults, but only if the ciliates are not too large.

Molecular identification of prey

Progress on molecular identification of the carnivorous copepod *Tortanus dextrilobatus* and its prey has been significant and we detected differences between prey of adults and nauplii. *T. dextrilobatus* nauplii were reared and their feeding observed in the laboratory. Adults and nauplii were obtained from plankton samples, which were also used to determine abundance of potential prey. Microzooplankton samples were also analyzed from all but one of the sample sites.

In order to block amplification of the predator DNA, we designed a stop oligonucleotide that blocks *Tortanus dextrilobatus* DNA from amplifying during PCR while allowing universal primers to amplify prey. T. dextrilobatus DNA is blocked when present at concentrations 104 times greater than prey DNA. In a preliminary trial on an adult simultaneously fed three prey items in the lab, the three prey types were recovered using this method. The stop oligonucleotide contains seven locked nucleic acids to increase its melting temperature relative to that of the universal PCR oligonucleotides (70oC vs 61oC) so that the blocker remains bound to T. dextrilobatus DNA through subsequent PCR cycles. The stop oligonucleotide has a C3 Spacer that prevents DNA polymerase from extending the strand.

We used a single set of universal primers to amplify a 350 basepair piece of the 18S gene. Nauplii were pooled into groups of 100 and adults were analyzed individually. DNA was amplified using PCR with the blocking and universal primers, then cloned to isolate different types of DNA from the mixed PCR product. Then 4060 clones were sequenced for each sample. The sequences were identified using NCBI's BLAST (Basic Local Alignment Search Tool) or by comparing to a library of sequences we have built for local species. Taxonomic or clade identity assignments were made after comparison of two algorithms that evaluate similarities among the query and a set of related species.

The key result is that we have detected substantial signals of copepod prey (Acartia sp., Oithona davisae, Pseudodiaptomus marinus) in samples of adults, but only a small amount of DNA of small copepods (Oithona, Limnoithona) in nauplii. Most of the clones from nauplii have contained DNA of tintinnid and strombidiid ciliates and dinoflagellates. Both adults and nauplii

have had signals from a variety of taxa not likely to constitute prey, e.g., ctenophores, diatoms, fungi, and parasitic ciliates.

The method was presented in a paper in the Journal of Plankton Research (Craig et al. 2013). This paper describes the methods and the results based on seven samples of nauplii and adults. Subsequent work examined more samples for a second paper focusing on explicit comparisons within a series of 8 tows of adult and larval diets and prey from the same tows, and using the newly developed methods. This paper is nearing completion for submission. Clear differences were seen between the relative abundance of some taxa in the field sample and predator guts. For example, copepod species dominance varied sharply in prey field vs. adult gut abundance. And, invertebrate larvae (e.g., polychaetes and bivalves) that were occasionally abundant in prey field sampling were disproportionately abundant in both adult and nauplius diets in some cases.

This work has been conducted largely by graduate students Robert Vogt and Carrie Craig and research technician Toni Ignoffo. Both students have benefited from mentoring by Kimmerer and Cohen, particularly by discussions and experiments run jointly between the two laboratories. A highschool intern, Annie de Lancie, learned culture techniques and experimental design with Vogt in 2012. During summer of 2013 an REU student, Claire Hoffmann, participated with Ignoffo in the development of methods for applying the gut fluorescence approach to fluorescentlystained ciliates. Another REU student, Kelly Flanders, worked with Ignoffo to apply the method to ingestion of ciliates by P. marinus.

Carrie Craig has provided mentoring to summer and academic year REU students, STAR teacher interns, and high school students in basic zooplankton field sampling at RTC. In an REU supplement to the project, 2 interns, Catie Alves from Connecticut College and Erica Perry from SFSU have been mentored principally by Carrie Craig, as well as others including Dr. Vanessa MillerSims, a parttime postdoc on the project. Erica Perry received additional REU funding for the academic year from a supplement to the SFSU/RTC REU site program. The supplements written by Cohen were aimed at gathering data on genetic variability among approximately 10 species of invasive copepods found in SF Bay, building on previous work in this project and others. The REU students collected nearly 25 sequences each from the candidate species which span nearly a complete range of genetic diversity using haplotypes of CO1 sequence obtained on the FSML funded DNA analyzer at RTC.