The activities for our portion of the collaborative project with Dr. Johnsen primarily involved our cruise off the coast of San Diego in Fall of 2010 (9/24/10 – 10/4/10) and a second cruise in the Northwest Atlantic in Fall 2011. We made further measurements on a recent cruise (ONR funded) in the Hawaiian Islands. In months prior to the cruises we refined the calibrated imaging methods, developed a method for doing spectroscopy through a dissecting scope, and developed a method for examining the tissue of cephalopods for the fluorescence associated with NADH. All three methods were used on the cruises with continual modification and varying degrees of success. The species examined were Cephalopods (*Chiroteuthis sp.*, *Galiteuthis sp.* (Fig. 1), *Dosidicus gigas* and *Grimaulditeuthis sp.*, the pelagic octopus *Japatella diaphana*), Crustaceans (*Pleuroncodes*), Gastropods (Pteropods and Heteropods), and leptocephalus eel larvae. The results from these cruises have allowed us to develope methods that should be quite robust and accurate for regular use in future research. Additionally, we measured ventilation and heart rates, stroke volume and oxygen binding in diverse zooplankton using video image analysis.

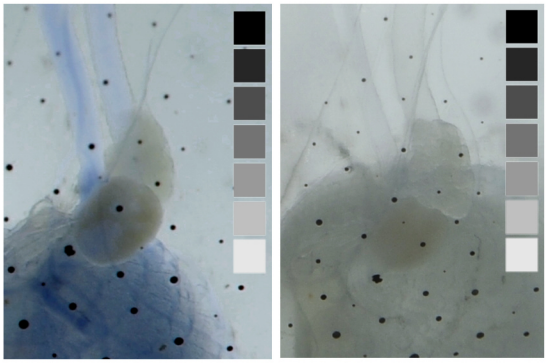

Figure 1. Galiteuthis respiratory system in oxygenated (left) and deoxygenated (right) seawater.

The calibrated imaging methods were described in the previous annual report and will not be described in detail here. The main adaptations from the previous description were to work in both transmissive and reflective modes. The original plan was to work entirely in reflective mode, using the onboard flash of the Nikon D700 as the illumination source. While this was a compact and convenient geometry, it had several disadvantages. First, because the animals were in relatively rectangular acrylic chambers (used to control the oxygenation of the water), the light from the flash was scattered and occluded in complex ways (Figure 2: left panel). A second issue was that the irradiance at the reflectance standard (the grey squares) was slightly different from that at the animal itself, because they were different distances from the camera. This led us to work in transmissive mode.

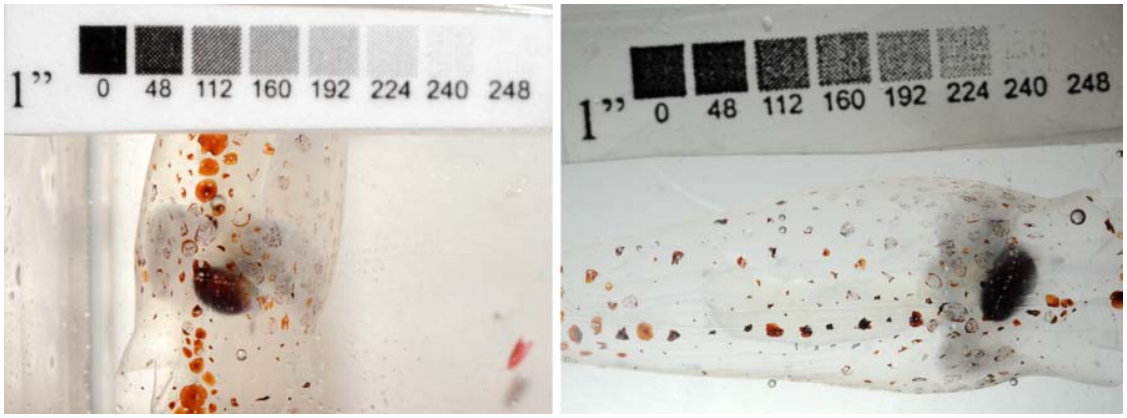

Figure 2: The squid *Chiroteuthis sp.* illuminated in reflective (left) and transmissive (right) modes.

In this mode, we illuminated the chamber from below via a custom-built "soft box" that sent diffused light from an external, coupled flash through the animal up toward the camera (the reflectance standards were replaced with transmission standards; Figure 2, right). The lighting was far more even and controllable in this mode. However, because the light now passed through the gills only once (instead of twice in reflective mode), their coloration was less pronounced and the differences between oxygenated and deoxygenated blood were difficult to ascertain. We calibrated and analyzed all the images taken, but were in general relatively unsatisfied with the results. A central difficulty for the entire cruise was that we were unable to capture large specimens of any of the cranchid squids, in particular large specimens of *Galiteuthis*, which formed the preliminary data for our original proposal. Because the intensity of light absorption is exponentially related to the size of the tissue, smaller animals led to smaller changes in coloration. We are currently discussing how to address this, perhaps by using the MBARI ROVs to collect larger specimens. However, we may abandon the calibrated imaging approach in favor of a spectroscopic approach that we developed, which we now describe.

The scope/spectroscopy methods were an adaptation of a fiber-optic-based microspectrophotometer developed in the Johnsen lab for another project. In brief, a fiber-optic cable is fitted to one of the two ocular ports of a dissecting scope. The image is viewed through the other ocular and the magnification is adjusted until the region of interest (in our case the gills of mesopelagic squid and leptocephalus eel larvae) filled the field of view. Because the ocular is removed from the port that contains the fiber, the fiber optic cable sees a completely defocused image that contains information about the color (in our case the oxygenation of the blood). The fiber optic cable is then couple to a highly-sensitive, low-noise spectrometer (QE65000, Ocean Optics Inc.) that records its spectrum. This method was quite successful in recording the absorbance spectra of oxygenated and deoxygenated blood in leptocephalus eel larvae (e.g. figure 2). We plan to further develop this method for a cruise in Monterey Bay in collaboration with Steve Haddock at MBARI by adding a UV-visible light source (the source used in Fall 2010 only had visible light) and by altering the optics somewhat to improve the capture of the spectra.

The last optical method we explored on the Fall 2010 cruise was to test various squid for fluorescence caused by NADH.

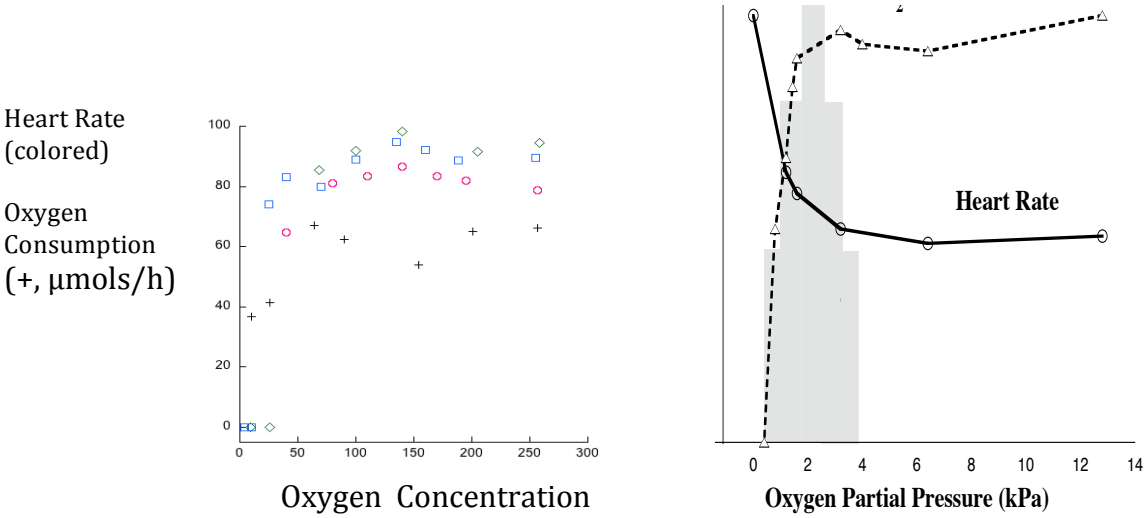

Figure 3. Heart Rate of heteropod molluscs (left) and cranchiid squids (right) measured using digital video analysis through transparent tissues. For comparison the oxygen consumption rate of both groups is presented. The critical oxygen levels are similar using both measures.

We used a relatively narrow band UV illumination source couple with a number of highly selective filters used in series. We plan to use a UV laser of the right wavelength as the illumination source for the Fall 2011 cruise and the QE65000 spectrometer as the detector. The laser has extremely high out-of-band rejection and the spectrometer (unlike a camera system) will clearly be able to separate light leakage from actual fluorescence. The results of this project have also been presented in a number of scientific and public talks, described elsewhere in this report.