# The uROV PICASSO, the Visual Plankton Recorder, and other attempts to image plankton

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*Abstract-* **A recently developed untethered but remotely operated survey platform, the PICASSO system, is described. This vehicle was designed specifically for surveys of macro- and megazooplankton and marine particulates (maximum depth 1000 m), to link information on gelatinous zooplankton diversity, behaviour and community structure with their function as packagers and producers of marine snow. In addition, an autonomous Visual Plankton Recorder, which is also deployable on the PICASSO vehicle, has been used to investigate particle profiles and plankton distribution vs. depth. Some results from these two systems from eastern Antarctica, the Coral Sea in Australia, and off Japan are introduced. Other techniques for imaging plankton in three dimensions are also introduced.**

## I. INTRODUCTION

The vast majority of attempts to image the deep ocean have focused on the ocean floor. However, in terms of living space or habitat, the sea floor comprises less than 0.001% of the volume inhabited by deep-sea organisms. The midwater pelagic realm is by far the largest and also the least explored biome on this planet. Imaging this three-dimensional space is not without its challenges. The lack of a reflective surface such as provided by the sea floor, and the high concentrations of particulate matter or marine snow make effective lighting difficult. Unlike the mostly two dimensional nature of the scenes to be recorded on the sea floor, plankton and the minefield of suspended matter scattered through their environment present a real challenge for focusing. A variety of techniques have been used with varying levels of success. These include shadowgraph illumination and line scan camera systems such as the In Situ Ichthyoplankton Imaging System (ISIIS) [1], Submersible Digital Holographic Particle Imaging Systems (LISST-HOLO, Sequoia Scientific) [2], and systems using darkfield illumination with highly sensitive black and white digital cameras such as the Underwater Vision Profiler 5 (UVP5, Hydroptic) [3], to name just a few. In order to obtain information on the distribution and composition of both the marine snow or particulate fraction and the living plankton fraction, where large gelatinous megaplankton can reach lengths of 30 m or more [4], a variety of different imaging techniques operating at different scales are needed. The present paper introduces the uROV PICASSO-1, an imaging platform designed for surveying the macro- and megaplanktonic size fraction (>20 mm), and the Autonomous Visual Plankton Recorder (AVPR) designed for imaging the mesoplanktonic (0.2-20 mm) size fraction. It also discusses techniques for imaging plankton in the laboratory.

*The untethered Remotely-Operated Vehicle PICASSO-1*

PICASSO-1 is a relatively small (2.7 m long, 230 kg weight in air) underwater robot that was designed specifically for in situ surveys of deep-sea plankton [5]. With conventional ROVs the biggest obstacle to successful biological surveys in the ocean's midwater zone is the problem of tether drag. Organisms of sizes sometimes as small as 5 mm must be observed while both they and the observation platform are moving in 3D space. With a tethered vehicle, unless both it and the tether are neutrally buoyant and operations are run without the tether being taut, normally considered a dangerous mode of operation, then the longest successful in focus observation is of the order of seconds. Due to the combination of a thin optical fibre cable (Φ0.9 mm single mode nylon-coated fibre, Fujikura Ltd.) for communications, lithium ion batteries (2kWh) and a broadcast-quality high definition television camera (HDC-X300K, Sony), PICASSO-1 has been able to track a 10 mm diameter comb jelly (*Bathyctena* sp.) in focus for 5 minutes 15 seconds at a depth of 660 m (Fig. 1), and film a 22 mm-long seahorse on a gorgonian at 102 m depth on a vertical reef wall for several minutes [6].

Forward-mounted NTSC cameras (WAT-240 Vivid (G-2.5), Watec) allow size measurements to be made of organisms within the overlapping field of view of the cameras for which a disparity map has been prepared. The disparity map is constructed by imaging a calibration board with the two forward-mounted NTSC cameras prior to a dive (Fig. 2). An attempt was made to compare the accuracy of the disparity map and resulting size measurements by attaching the calibration board to a stainless steel frame that was lowered by winch from the mother ship (Fig. 3). PICASSO-1 followed the wire cable to the frame to just below the thermocline and the NTSC feed from the cameras was captured via two NTSC-USB converters (PC-SDVD/U2G, Buffalo) input into a Dell Precision M6300 notebook computer with the AVSCalib_USBNTSC software installed. Unfortunately, high concentrations of marine snow interfered with the recognition routine of the software and in situ calibration proved challenging. Using the in air calibration data, size accuracy was measured to be 2.7±2.1% when measuring a 10mm-long object at a distance of 1 m from the camera lenses.

In order to acquire image data at a variety of scales simultaneously, the PICASSO-1 employs a nested field of view approach. A fixed focus, wide angle, rear-mounted NTSC camera captures images of a large volume fore and to the sides of the PICASSO-1 vehicle (Fig. 4). Three NTSC cameras with overlapping fields of view allow size measurements to be made and capture images of smaller volumes forwards of the vehicle. The HDTV camera, which has zoom and focus capability, captures high resolution video images of an even smaller volume within the volume imaged by the front-mounted NTSC cameras (Fig. 4). Illumination is by HID lamps (three custom 30 watt lamps diverted from car use) and/or two handmade 20 watt LED array lights. Two of the HID lights are positioned below and forward of the HDTV camera on a stainless steel bracket and angled such that the beams of light illuminate targets approximately 50cm from the HDTV camera lens at an angle of about 45 degrees. This ensures that out-of-focus marine snow particles occurring closer to the camera lens are not illuminated. The single forward-pointing HID light is used when searching for targets and video quality is not paramount.

Figure 1. *Bathyctena* sp. imaged during PICASSO Dive 39 in Sagami Bay (35˚09.9'N 139˚23.1'E) on 5 February 2010.

Figure 2. Shipboard calibration for construction of the disparity map.

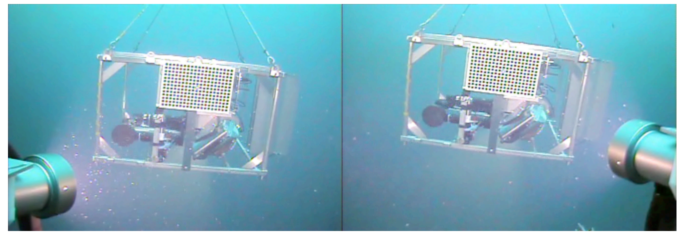

Figure 3. In situ calibration attempt.

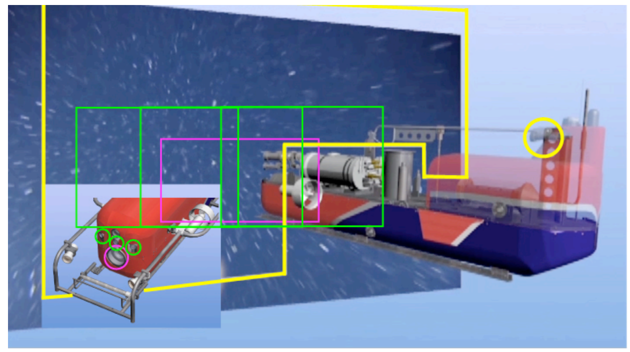

Figure 4. Fields of view for the cameras on PICASSO-1.

*The Autonomous Visual Plankton Recorder (AVPR)*

The AVPR is a digital camera in a pressure housing rated to 1000 m depth and which records colour images of 1028X1024 pixels, compressed and stored on a hard drive as JPEG2000 files at a rate of 15 Hz. Darkfield illumination is provided by a ring strobe (Perkin-Elmer FX4400, 1 joule/flash) and photographs are linked to CTD data to give environmental information on depth, water temperature and salinity for the plankton in each photo (Fig. 5). The field of view is usually set to 43 mm square during deployment resulting in an imaged volume of 255 ml when region of interest (roi) extractions are done with a focus value of 40 sobels, or 208 ml when roi extractions are done using a sobel value of 50. Full frames were subject to particle analyses using the software package Image-Pro Plus 6.3J (Version 6.3.1.535, Media Cybernetics, Inc.) using a macro routine developed in-house. The volume subjected to particle analyses was the entire volume illuminated by the strobe and was 518 ml. Particles positioned close to the camera were out of focus and appeared much larger than their actual size as inferred by the size calibration done at the lighting and focus "sweetspot" where the field of view was 43X43 mm. These particles would hide other particles behind them that were further from the camera, resulting in an underestimate of true particle number per litre.

The large number of photographs taken at each depth allowed such "noise" in the data values to be filtered out and plots of particle number vs depth were able to be obtained. Binning of particles per 5 m depth stratum allowed visualization of increasing particle size vs depth, presumably due to particle scavenging where sticky particles collect other particles as they sink through the water column (Fig. 6).

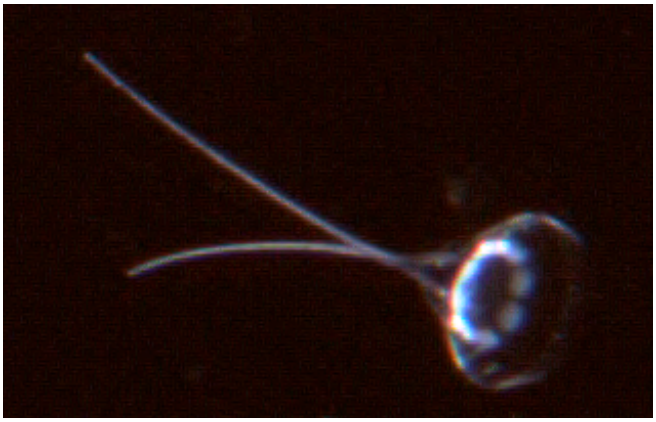

Figure 5. The narcomedusa *Solmundella bitentaculta*, photographed by the AVPR at 103.4 m depth at 03:48:22 on 19 July 2012 off Osprey Reef in the Coral Sea.

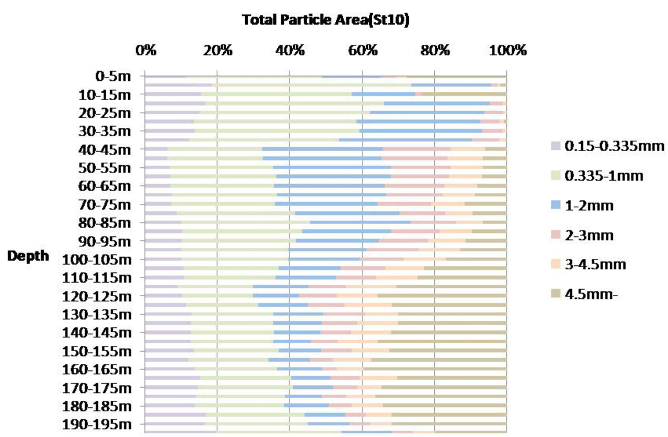


Figure 6. Particle size vs depth at the continental shelf break in East Antarctica at 66˚S 143˚E (st. 10) on 11 February 2008.

*Three-dimensional imaging of plankton*

Certain groups of plankton known as the gelatinous plankton are extremely fragile. For example, ctenophores have bodies composed of 98% water and many species are unable to be preserved in chemical fixatives such as formalin or ethanol. Experiments were carried out on imaging such animals using a combination of half mirrors (MRH001T30, Acry-ya) and light field cameras (11 megaray Red Hot, Lytro; R1-C-USB2, Raytrix).

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