

# **Characterization of Fluorescence in the Marine Environment**

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## **LONG-TERM GOALS**

This is an exploration project aimed at documenting fluorescence of seafloor marine organisms. We wish to determine the nature and distribution, both geographic and taxonomic, of the effect. The data gained has potential application to mapping and assessment of the sea floor.

## **OBJECTIVES**

The objectives of the FY02 effort were to conduct fieldwork at several locations; refine techniques for photographing and videotaping fluorescence; make laboratory measurements of fluorescence properties; create a database of observations, images, and measurements; and prepare for Year 2 efforts by selecting sites for additional fieldwork and planning deep-sea work.

## **APPROACH**

This is a field-oriented project, supplemented by laboratory measurement and documentation. We are diving at a variety of locations, using several different imaging/exploration techniques to locate instances of seafloor fluorescence. We are concentrating on effects not previously observed or documented by concentrating on habitats that have not been explored much or at all, and on taxonomic groups that have not been examined in detail. We are also examining structures such as old shipwrecks to follow up on an early report (Woodbridge, 1959) of anomalous fluorescence associated with an old wooden wreck site.

When possible we collect specimens for laboratory measurement of fluorescence excitation/emission properties. We are organizing our findings in a master database that includes comparative white-light and fluorescence imagery and spectral measurements when these are available.

We are experimenting with new techniques for imaging fluorescence. This includes the use of new underwater lights, using the lights in association with several different video camera systems to test performance, and experimenting with underwater digital fluorescence photography.

We are developing a new algorithm for interpretation of fluorescence images. This is to be applied to several different means of collecting fluorescence imagery (e.g., photography, video, laser line scan) and is intended to produce a robust approach to the interpretation that is relatively immune to variations in sensor-to-subject distance and in water optical properties.

The project is a direct collaboration with Dr. Michael Lesser, University of New Hampshire, who is funded under a separate award. Dr. Lesser is collaborating in the fieldwork and performing chemical

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analysis of some of the fluorescing pigments. In addition, we are forming new collaborations as the opportunity arises. These can arise when we discover a novel fluorescence in the field and contact researchers with long-term interests in those specific organisms. An example is a collaboration with several researchers with interests in mantis shrimp (see Transitions, below). Not only are our findings of interest to them, but they are also sending us new specimens, thus expanding our sampling capability beyond what we could achieve on our own.

## **WORK COMPLETED**

We conducted fieldwork at locations in Hawaii, Australia, the Bahamas, and New England. The effort ranged from a few dives-of-opportunity at some locations to a concerted multi-week effort at others. Our efforts focused on 1) exploring for fluorescence and 2) experimenting with several new viewing/imaging approaches. Two of the dive sites were old shipwrecks - one a Revolutionary War era ship in Maine, and the other a canal barge in Lake Champlain, Vermont.

We compared the performance of several combinations of underwater lights and video cameras. The experimentation with underwater digital photography was very successful and demonstrated that high-quality fluorescence imaging could be carried out in the presence of moderate levels of ambient light. Because of this finding we conducted a focused modeling effort using Hydrolight to better understand the potentials and limitations of this approach.

We have formulated plans for fieldwork in the second year of the project. This will include a 2-week trip to the Pacific Northwest, where very few fluorescence dives have ever been made. We applied for and received spots in the 2003 schedule for two deep-water fluorescence exploration missions using submersibles: to the deep walls in the Bahamas using the Johnson Sea-Link submersible (Harbor Branch Oceanographic Institution), and to hydrothermal vents and nearby abyssal plains off the Pacific Northwest using Alvin (Woods Hole Oceanographic Institution).

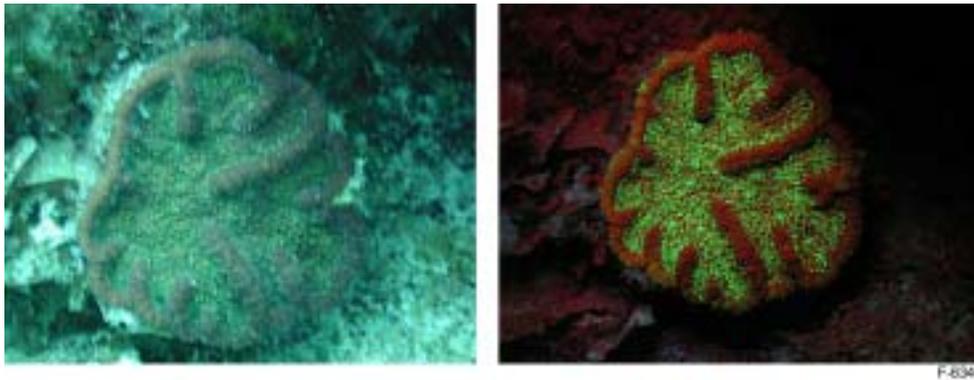
## **RESULTS**

In the course of our initial field trips we have discovered novel instances of fluorescence in several species each of: nudibranchs, polychaete worms, crustaceans (crabs and shrimp), stomatopods (mantis shrimp), molluscs, and fish. Most of these observations are supported by one or more of: still photography, videography, and measurement of spectral characteristics. Observations are added as they are acquired to a master database that records a wide variety of information, including taxonomic identification, date and location of sample, source and method of observation, and a verbal description of fluorescence characteristics. When the data are available, links are provided to white-light and fluorescence images of the subject and to spectral measurements. An example of the imagery and of the fluorescence spectral characterization for the operculum of a tulip snail (Bahamas) is shown in Figure 1.



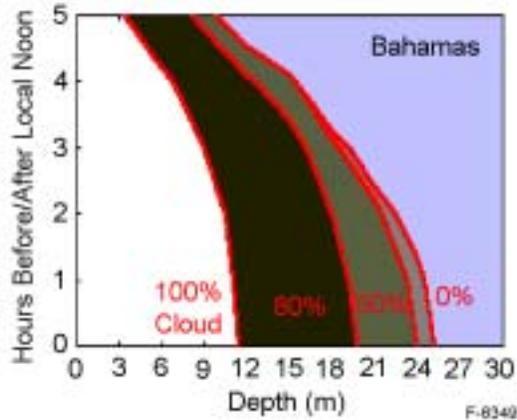
**Figure 1. White light (A) and fluorescence (B) photographs of the operculum of a tulip snail collected in the Bahamas. The sample appears brown under white light illumination and fluoresces with an intense greenish-yellow color over the entire surface. The spectral characterization (C) reveals the presence of two primary fluorescing substances, with excitation/emission peaks at approximately 370/460 nm and 480/540 nm.**

Through trial and error we learned that with proper control of the settings of the digital camera we were able to take high-quality fluorescence images in the presence of moderate levels of ambient light. This enabled us to take photographs that assisted in fluorescence exploration at depths as shallow as 3 m at midday, as long as the subject was in shadow. Figure 2 shows ambient-light and induced fluorescence photographs taken at 1130 at a depth of 9 m on a cloudy day in the Bahamas.



**Figure 2. Ambient light (left) and fluorescence (right) photographs of a coral on a reef wall at a depth of 9 m, Lee Stocking Island, Bahamas. Both images were made at approximately 1130. The fluorescence image clearly shows the weak red fluorescence of chlorophyll in algae and zooxanthellae, with no evidence of ambient light influence on the image.**

The Hydrolight modeling led to a better understanding of the potentials for applying this technique. Taking into consideration the combined effects of depth, time of day, cloud cover, and the fluorescence barrier filter we determined the approximate range of conditions under which daytime fluorescence photography could be carried out successfully (Figure 3). This work will be presented as a poster at the Ocean Optics XVI conference in November 2002 (Mazel (2002b)).



**Figure 3. Contour plots illustrating the combinations of conditions (depth and hours before/after local noon) that provide the reduction in ambient light level of six f-stops (factor of 64 reduction) needed for fluorescence photography with our digital imaging system, with cloud covers of 0, 50, 80 and 100%, for the Bahamas. For 0% cover the practical depth of approximately 24 m changes little for two hours on either side of local noon, then decreases to approximately 9 m at +/- five hours from noon. For 100% cover, the practical depth starts at 12 m at noon and shallows to almost 3 m at +/- five hours.**

## IMPACT/APPLICATIONS

The discovery of intense fluorescence in a wider array of reef organisms has implications for our interpretation of fluorescence imagery. In an effort funded as part of the Coastal Benthic Optical Properties (CoBOP) program (Mazel, 2002a) we demonstrated the potential to use multi-wavelength fluorescence imagery generated by the Fluorescence Imaging Laser Line Scanner (FILLS) to perform automated classification of coral reef surfaces (Mazel et al., 2003). In that work we interpreted all brightly fluorescing pixels as being associated with corals, even for very small targets of 1 – 5 pixels. We now know that small brightly fluorescing features can arise from polychaete worms, fish, or other sources. This will not have a large impact on estimates of percent cover, but could be very important in regard to estimation of numbers of juvenile (i.e., small) corals.

The demonstration of the ability to do fluorescence photography in the daytime will be of great value in this project, and may open the technique up for more widespread application. There are difficulties and dangers associated with night boating and diving operations that are a barrier to the use of any technique that must be carried out in darkness. An application of fluorescence that is of great current interest is in location of juvenile corals, and the ability to conduct some or all of the work in daytime would be very useful.

The novel observations of fluorescence in several taxonomic groups is generating interest among researchers who specialize in those organisms. They were unaware that fluorescence occurred and are now intrigued by the possible role that fluorescence coloration may play in the animals' behaviors. This is leading directly to new collaborations (see Transitions, below).

## **TRANSITIONS**

Our discovery of fluorescence in stomatopods (mantis shrimp) made during our field effort in the Bahamas has led to a direct collaboration with leading researchers in stomatopod vision and biology (Dr. Roy Caldwell, University of California, Berkeley; Dr. Tom Cronin, University of Maryland Baltimore County; and Dr. Justin Marshall, University of Queensland, Australia). They have been sending stomatopod samples to Physical Sciences Inc. for measurement of the fluorescence of various body parts. This not only contributes to the new line of research on the stomatopods, but also to the database being assembled by this project.

In addition we are now collaborating with Dr. Neils Lindquist, University of North Carolina, on the fluorescence of isopods, and with the NOAA Center for Coastal Fisheries and Marine Habitats on application of fluorescence to research on coral recruitment.

## **RELATED PROJECTS**

There is overlap between this effort and my continuing analysis of the fluorescence data collected as part of the CoBOP research program (Mazel, 2002a).

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