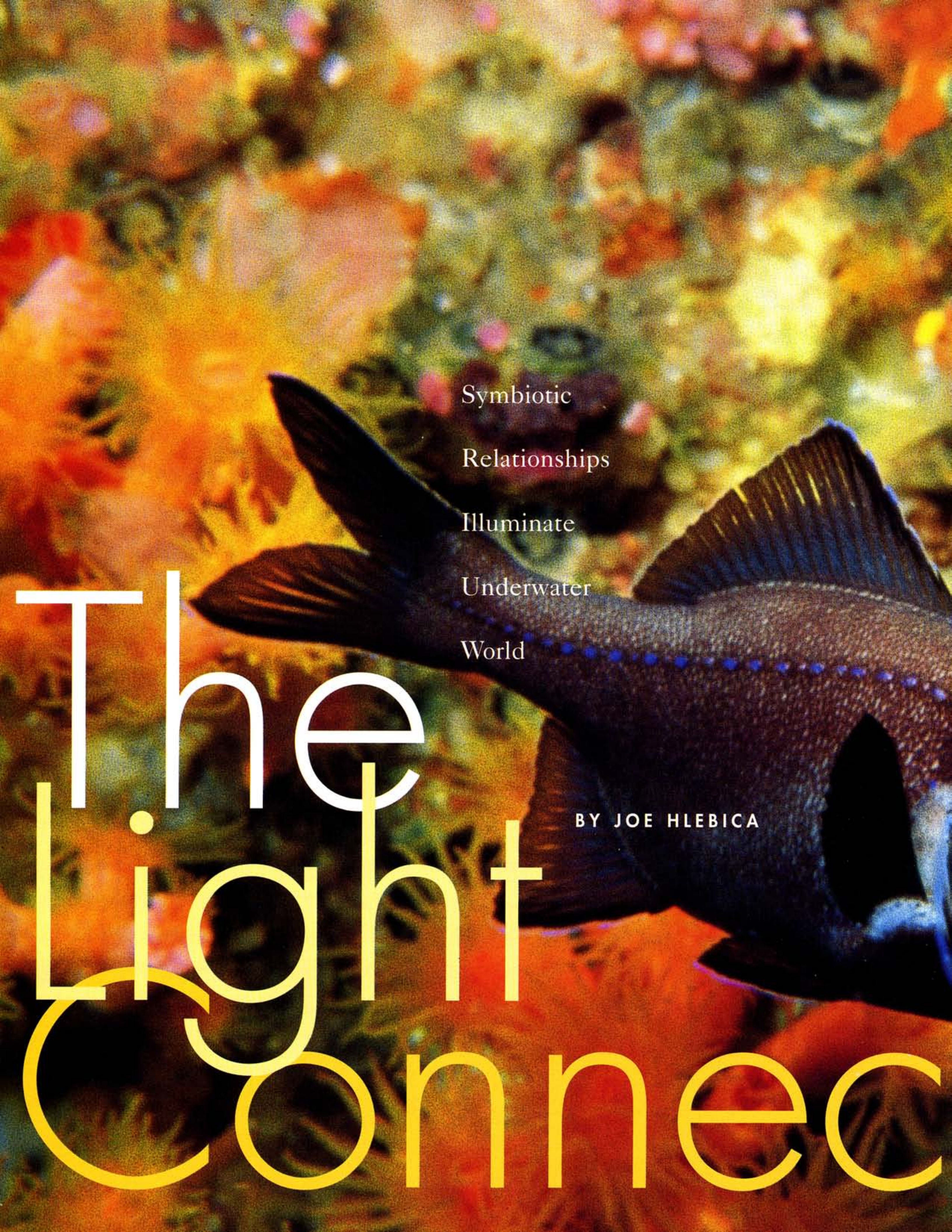


The Light Connec

Symbiotic Relationships Illuminate Underwater World

BY JOE HLEBICA

The chamber resembles something from a spy thriller. A warning sign next to the door reads "Authorized Entry Only—Light Sensitive Fish," referring to the inhabitants of this inner sanctum of the Scripps Experimental Aquarium.

To enter, a visitor must don a camper's headlamp equipped with a red lens to filter the light. As his eyes grow accustomed to the dark, the visitor can begin to detect the darting movements of small, flashing green lights. What look like the eyes of tiny demons are actually the light organs of harmless fishes. These unusual appendages bear cultures of glowing bacteria that produce one of nature's most curious spectacles—bioluminescence.

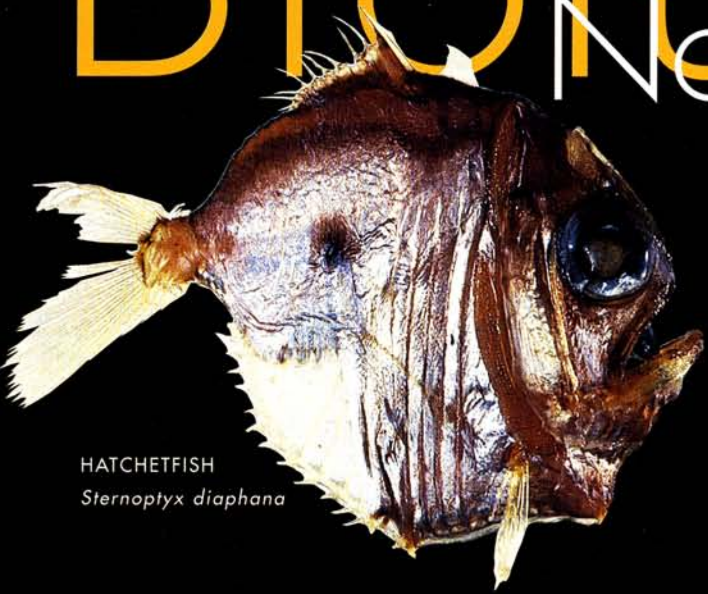
The designer of this habitat is Margo Haygood, Scripps associate professor of marine biology and a specialist in the symbiotic relationships of bioluminescent bacteria and their host fishes.

While there are many types of symbiosis, in which different organisms live in a close relationship usually beneficial to both, Haygood has long been interested in obligate symbiosis. This

In this remarkable photograph taken in Borneo, a flashlight fish of the species *Photoblepharon palpebratus* has been captured in a rare moment of exposure to broad daylight. Flashlight fishes typically inhabit the dark recesses of reefs and only emerge at night to feed. Note the distinctive light organ beneath the eye, inside of which light-producing bacteria live in a special relationship with the host fish.

Biolumine

No Stranger to



HATCHETFISH
Sternoptyx diaphana

SLACKJAW FISH
Malacosteus niger

Bioluminescence, light given off by living organisms, may seem remarkable and unique, but it is more the rule than the exception among certain sea creatures.

In the deep sea where little or no sunlight penetrates, a variety of fishes live out their lives dependent, in large part, upon bioluminescence. Among these fishes, light organs have evolved to serve a number of purposes.

Anglerfishes, a group studied by Margo Haygood, have glowing appendages attached to their heads. These attachments act as luminous lures, drawing prey into unseen, gaping mouths.

"This organ, which glows from the bioluminescent bacteria it contains, is analogous to the fleshy, wormlike lures of certain shallow-water fishes," explains H. J. Walker of the Marine Vertebrates Collection at Scripps.

Another species, the hatchetfish, is decorated with a dazzling array of specialized light organs called photophores. These are believed to act as camouflage in mid-water depths where some sunlight penetrates.

"Why would a fish light itself up like a sign on Broadway if it wanted to hide from predators?" muses Walker's associate, Cynthia Klepadlo. "In order to blend an otherwise black silhouette into the background of scattered light," she points out.

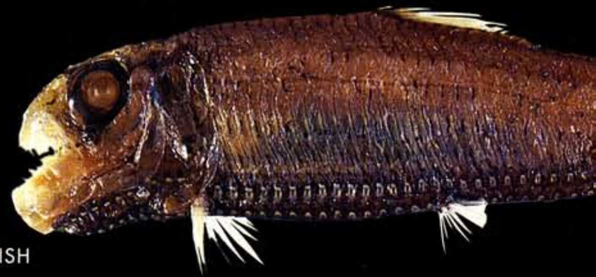
Fishes apparently also use bioluminescence to communicate. Walker explains that, like fireflies, some fishes may use their light organs to send signals to potential mates and rivals. According to Klepadlo, in a few species multicolored light

describes a situation in which the symbiotic organisms—in her studies, certain bacteria—are apparently unable to establish themselves outside the host.

"The mystery of what makes the bacteria so tightly adapted to their host and the nature of the relationship between host and symbiont are irresistible to a microbiologist like me. It's a fascinating problem, but a very difficult one to study.

"With the bioluminescent fish facility, we can sample bacteria from the light organs of their fish hosts without killing the fish, and bring the bacteria into the laboratory for study," explains Haygood. "I based the habitat design on my experience with these fish in the field, and everything else I have learned that would make them happy."

According to Haygood, flashlight fish, named for the flashing light organs beneath their eyes, are both nocturnal and light sensitive. Normally, they remain inside reef structures or in deep water during the day or in moonlight hours, and come out to feed only under the darkest conditions. Thus, they never encounter any significant amount of light in nature. In designing her facility, Haygood has provided the fish with an environ-



LIGHTFISH
Ichthyoccus irregularis

organs on certain parts of the body probably function to identify members of the species.

Though a relatively shallow-water group, the flashlight fishes studied by Haygood have light organs similar in function to those of some deep-sea animals. Haygood believes they use their light organs to find the plankton upon which they feed. It also has been suggested that by turning off their lights and changing direction, flashlight fishes can surprise or elude predators.

The role of light organs in hunting has been highly adapted in certain deep-sea species. "Evolution is an arms race," quips Scripps ichthyologist Richard Rosenblatt, a leading authority on deep-sea fishes. Illustrating his point is a sinister-looking fish with light organs that are the equivalent of tiny sniper scopes. "Since red light is absorbed by water in relatively shallow depths, most deep-sea fishes cannot see red. These fish are unique because they not only see but produce red light."

According to Rosenblatt, the red light produced by their photophores reflects off their prey. The hunter can see it, but the hunted cannot. This gives the "invisible" sniper a distinct advantage when hunting in the dark.

Flashlight fish are currently on display at the Birch Aquarium at Scripps in a special exhibit that reproduces the natural habitat of bioluminescent fishes.

DRAGONFISH
Echiostomma barbatum

ment similar to their natural one, allowing them to avoid the stress of exposure to too much light.

Haygood's interest in bioluminescent bacteria was awakened by a summer course in microbial ecology she took as a visiting undergraduate at Woods Hole Oceanographic Institution. She recalls, "The experience changed my life. I realized then that microorganisms make the world go 'round."

The physiology and symbiotic associations of microorganisms inspired her to attempt to isolate bacteria from the light organs of deep-sea anglerfish. This ambitious effort started her on the road to examining the bacteria of pineconefish and flashlight fish, two groups of temperate-tropical reef fishes that she has studied for more than ten years.

Her interest in pineconefish, named for the distinctive pattern of their scales, began while she was a graduate student at Scripps. A colleague urged her to go to Japan, where the natural habitat of these fish is found.

"I wanted to study the process through which pineconefish get their bacteria by raising larval young and challenging them with various bacterial strains. I wanted to find out how they obtain a specific

A pineconefish of the species *Monocentris japonica,* one species of bioluminescent fish studied by Margo Haygood.
FACING PAGE Haygood examines pineconefish specimens in the Bioluminescent Fish Facility within the Scripps Experimental Aquarium.

bacterial species and how they maintain it as a pure culture. That was pretty ambitious because no one had ever bred pineconefish in captivity."

Haygood succeeded in fertilizing eggs and raising the larvae to an age sufficient to determine that the young do not acquire the symbiotic bacteria from their parents—as she believes may be the case involving obligate symbionts. Instead, they must acquire them from the environment. Revealing this relationship, an example of facultative symbiosis, was an important breakthrough.

Back at Scripps, Haygood began working on the physiology of the pineconefish's bacteria in an attempt to understand the mechanism of their relationship with the host.

"I was interested in the fact that bacteria in the fish's light organs grow very slowly. They have to, or the fish would be wasting a tremendous amount of energy to support fast-growing bacteria. The question was: 'How can the fish slow the growth of the bacteria and still have them make as much light as possible?'"

She concluded from her experiments that iron regulates the synthesis of the luminescent system in the bacteria, and that they produce the most light per cell under low-iron conditions. By maintaining a low-iron physiology, therefore, the host fish gets the most light for the least energy investment. The fish, which live their entire lives in almost total darkness, may benefit in several ways from the light produced by the bioluminescent bacteria. These include enhanced vision, prey attraction, communication with others of their species, and the ability to confuse predators.

As to how these symbiotic relationships developed, Haygood theorizes that some bacteria evolved from a free-living existence to obligate symbiosis in a gradual process not unlike domestication. Bacteria are continuously passed out into the environment and then taken back in by the fish. A cycle develops, during which the bacteria become ever more dependent for survival. Haygood describes it as a sort of "corralling" of the bacteria by the host. During the process, the bacteria come to depend upon their host fishes for a safe environment in which to thrive. They eventually lose the ability to cope with the challenges of an autonomous life.

She also believes that light-organ symbiosis arose from enteric (intestinal) symbiosis, involving bacteria in the alimentary tracts of host fishes. Strong support for this hypothesis is derived from the fact that some marine enteric bacteria are bioluminescent, and many of the light organs of fishes develop from the guts.

In testing her theory, Haygood has devoted much time and effort to acquiring samples of the various flashlight fish symbionts. The laboratory process entails sequencing ribosomal RNA genes, "the gold standard," in her words, for looking at evolutionary relationships among bacteria.

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Pier Project Aims for Cancer Cure

An experiment currently assembled on Scripps Pier holds promise in one of scientists' toughest battles against human illness. Results of this investigation may point the way toward a cure for certain cancers.

At this experimental station, Margo Haygood and colleagues are successfully culturing *Bugula neritina,* common encrusting organisms known as bryozoans. These colonial marine animals are abundant along the seashore and sometimes form crinkled, flat sheets or seaweedlike branching colonies on pier pilings, rocks, and other substrates. Bryozoans are the source of a promising new anticancer drug called bryostatin-1, now in Phase II human clinical trials being conducted by the National Cancer Institute. *B. neritina* is widely distributed in waters around the world, though only a few populations produce the drug, and these occur mainly along the coast of southern California.

Collaborating with Haygood in the experiment is the San Diego-based biotech and aquaculture firm CalBio-Marine. The firm was co-founded by Dominick Mendola, a former development engineer at Scripps, and Janice Thompson, a Scripps graduate. According to Mendola, "The oceans are where the next wave of new drugs to combat cancer and other debilitating disease will be discovered."

The experiment on Scripps Pier has been proceeding for about two years, and the colonies of *B. neritina* are thriving. The growing organisms are transferred from the pier to the ocean by divers and are raised on a structure located in 50 feet (15.2 m) of water.

Haygood's mission is to conduct basic research on *B. neritina,* and the support provided by both CalBio-Marine and the California Sea Grant College System has been welcome.

The research team's goal is to find out why *B. neritina* populations vary in bryostatin content. According to one hypothesis, the chemical may come from a microbe inhabiting *B. neritina* symbiotically.

Seana Davidson, a Haygood graduate student and research diver, has conducted a study to identify the bacterial symbiont found in the bryozoan's larvae. She now is attempting to eliminate the known symbionts and then directly test whether the colonies can still make bryostatin.

"Our main goal is to characterize symbionts in order to establish responsibility for bryostatin. I'm looking for genes that might be involved in its production," Davidson explains.

Haygood elaborates, "We are trying to obtain gene sequences and other information to allow us to isolate microorganisms or clones that can be used to develop an economical way to produce bryostatin."

Graduate student Seana Davidson (top and right) collects specimens for study during a dive near Scripps submarine canyon. (far right) A platform designed to support colonies of bryozoans is lowered from Scripps Pier in preparation for deployment.

"Combining the capabilities of different organisms for practical purposes is the biotechnological equivalent of the evolution of symbiosis."

"What we've found is that each flashlight fish species hosts unique bacteria. They aren't shared from species to species."

While flashlight fish symbionts are unique and obligate, those of pineconefish, passing freely through the water from host to young, are not. Haygood wants to understand why. Comparisons between the genetic structures of the different bacterial strains may tell her. The greatest mystery is how bacteria move from one generation of flashlight fish to the next. How do they survive until the young pick them up?

"We don't know very much about the physical state of those bacteria, or whether they have to be picked up by the young fish a very short time after they leave an adult."

Haygood speculates that the young flashlight fish may actually get bacteria from the surface of the adult's light organ. But she doesn't yet know how the bacteria then populate the light organ of their new host.

As Haygood searches for clues that will help solve this mystery, other questions arise: "I'm fascinated by the role of symbiosis in evolution. How does a host turn bacteria into something that serves the host's purpose? How are the bacteria controlled to the point that they do exactly what is needed by the host?"

According to Haygood, many symbiotic bacteria are highly efficient in carrying out the process on which their symbiosis is based. If researchers can discover the physiologic and genetic means by which integration and control are achieved in these relationships, the results may provide tools for manipulating existing associations in nature. Practical applications might include the development of new compounds to prevent the establishment of fouling organisms on ship hulls and the creation of new drugs and enzymes.

In Haygood's words, "Combining the capabilities of different organisms for practical purposes is the biotechnological equivalent of the evolution of symbiosis."