Bioluminescence and other factoids about *Aequorea*, a hydromedusa

Aequorea victoria is a jellyfish in Puget Sound, Washington State, from which the luminescent protein *aequorin* and the fluorescent molecule GFP (green fluorescent protein) have been extracted, purified, and eventually cloned. These two products have proven useful and popular in various kinds of biomedical research in the 1990s and 2000s and their value is likely to increase in coming years; GFP is particularly easy to use and has wide-ranging value as a fluorescent marker-protein. Along with use of these products, comes a certain popular press explaining their source, with various factoids usually included about the jellyfish. Many inaccuracies have emerged from this press, and it might be difficult for someone to get the story right by reading what has been published. Because I study (and photograph) medusae, including *Aequorea victoria* in Friday Harbor, Washington, the source of most harvested specimens, I feel that I have become an unwilling but central player in this unintentional and seemingly never-ending misinformation campaign. Nearly every time that my photographs have been used by someone other than myself, the explanation of the photograph is somehow wrong.

If you use aequorin or GFP in your work, or you are in the process of writing something about *Aequorea* and its bioluminescence, this webpage has been written specifically for you. It has been stimulated by a long series of abuses of my (or other) photographs in the published media, but covers many other aspects of the biology of *Aequorea*. I would appreciate your citing this page as follows:
Mills, C.E. 1999-present. Bioluminescence of *Aequorea*, a hydromedusa. Electronic internet document available at http://faculty.washington.edu/cemills/Aequorea.html. Published by the author, web page established June 1999, last updated (see date at end of page).

Natural history of *Aequorea victoria* and related species

Tiny jellyfish, including *Aequorea,* are asexually budded off their hydroid colonies in early spring in the Puget Sound / Strait of Georgia region of Washington State and British Columbia. Like other hydromedusae, *Aequorea* medusae initially grow quite rapidly, putting most of their energy into somatic growth, resulting in increasing size. For *Aequorea victoria*, the rate of growth in size diminishes after they reach several cm in diameter, and most energy is then put into gamete production. Like other hydromedusae, *Aequorea* then free-spawn either eggs or sperm (each jellyfish is one sex or the other) into the sea daily, where the eggs are fertilized, develop into a swimming planula larva, and eventually settle and grow into a new hydroid colony. The hydroid of *Aequorea* has barely been studied and in fact has rarely been collected in the field - we know virtually nothing about its ecology other than the fact that it lives attached to the bottom or to hard substrates such as shells on or near the bottom. It is fairly easy to grow in culture in the laboratory.

Adult *Aequorea victoria* medusae in Puget Sound reach about 5 to 10 cm in diameter. They feed primarily on soft-bodied prey including other jellyfishes, ctenophores, and appendicularians. Most probably live 6 months or less in the field. The entire population of *Aequorea* medusae disappears (dies) by mid-autumn every year. Polyp colonies persist on the bottom and produce a new generation of medusae each spring, using specific, but undetermined, environmental cues for the fairly precise timing of this annual event.

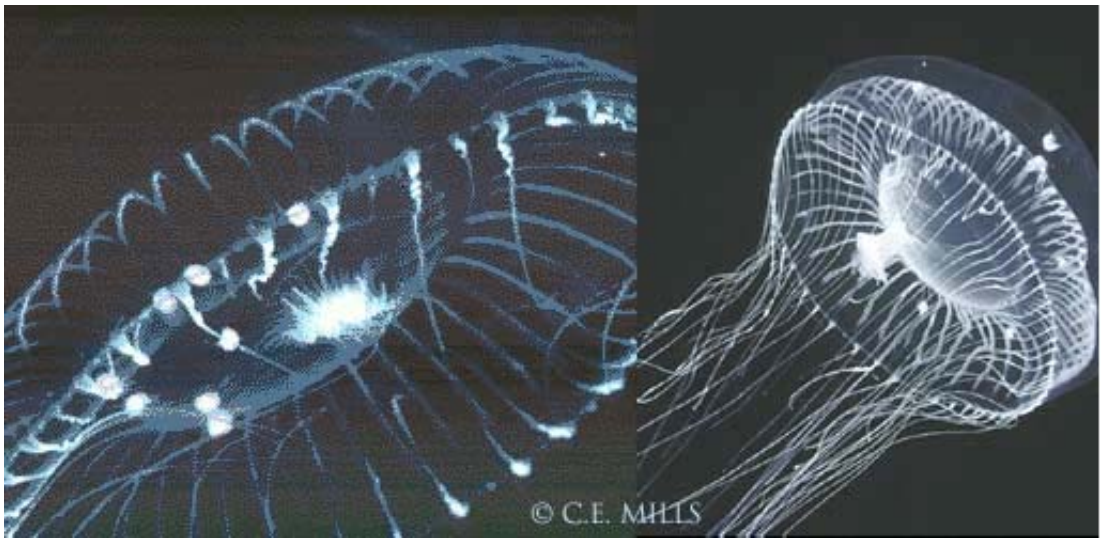
Aequorea medusae used to be enormously abundant during the summer in Friday Harbor, Washington, during at least the 1960s through the 1980s. Hundreds of thousands of medusae were harvested during that period by scientists interested in their luminescent properties. *Aequorea* medusae have become relatively scarce in the 1990s and 2000s and we do not know why - this change does not seem to be due to overcollection. In fact, numbers of all 75 or so species of hydromedusae are substantially down in the region now (thus exonerating the biologists who collected large numbers of only one of two of the species), but the decrease in *Aequorea* is especially

noticeable since they are relatively large and used to be so plentiful, dominating the summer surface plankton. Knowledge about the ecology of the benthic polyps now seems even more important for understanding the present trend. I have witnessed an enormous change from the late 1970s to the present, particularly in numbers of *Aequorea*, and have a 30-year data set, yet to be fully analyzed, that shows these vast jellyfish declines in Friday Harbor.

Aequorea medusae are found from the Bering Sea to southern California in the northeast Pacific (I do not know the distribution in the northwest Pacific; similar medusae also occur in the North Atlantic). Several names have been applied to the northeast Pacific jellyfish, of which *Aequorea victoria* is only one, and perhaps not even the "best" one. Other names used in the literature for these animals include *Aequorea aequorea*, *Aequorea forskalea* and *Aequorea coerulescens* - see below for a more in-depth discussion of the species name. Some specimens in Alaska get much larger, commonly to 20 cm diameter. It is not known if they are the same as or a different species than the more southerly populations. Sorting out the names and life cycles of these temperate *Aequorea* species has not been an easy task, or it would have been done long ago - cloning the protein aequorin from the jellyfish was perhaps a much more straightforward proposition at the time it was accomplished. A genetic study of the different *Aequorea*s may now yield the associations without the arduous life cycle work; collections over the entire geographic range would still be useful.

Photographs of *Aequorea* - the problem

The photographs below are typical of those usually published. **They DO NOT show bioluminescence or fluorescence**. These images show light reflected off various morphological features of the animal. I took both of these photographs using an overhead flash in an aquarium. The same visual image is seen in sunlight or by just shining any white light on the jellyfish, but a flash was used to speed up the process and stop the action. Similar images can be produced in the wild, using sunlight as the illlumination source.



Two views of the hydromedusa *Aequorea victoria* from Friday Harbor, Washington, copyright Claudia E. Mills 1999.

All medusae in the genus *Aequorea* are virtually colorless in life. Photographs made with color film sometimes take on a bluish tinge (see above left) which seems to have led to some of the problem in understanding what the images represent. The small bright, roundish blobs (about 10) in the above photographs are symbiotic amphipods living on or in the medusae. The bright raggedy thing in the center of each jellyfish is its highly-expandable mouth.

Aequorea bioluminescence

Aequorea medusae bioluminesce only around the margin; such luminescence does not show in the above images - click here to see (2nd image on the left) Dr. Osamu Shimomura's image of *Aequorea* bioluminescence - that green ring is IT, all the rest of the jellyfish is "unlit." Dr. Shimomura is the scientist who began studying bioluminescence of *Aequorea* in 1961 at the Friday Harbor Laboratories, ultimately leading to the use of two bioluminescent proteins from this jellyfish in all sorts of biological research (see some particularly interesting examples here). Bioluminescence of *Aequorea,* as in most species of jellyfish, does not look like a soft overall glow, but occurs only at the rim of the bell (the localization of bioluminescence in jellyfish appears to be genus- or species-specific), and would appear as a string of nearly-microscopic fusiform green lights, given the right

viewing conditions.

The luminescent light produced by *Aequorea* is actually bluish in color, attributable to a molecule known as aequorin, but in a living jellyfish it is emitted via a coupled molecule known as GFP, or green fluorescent protein, which causes the emitted light to appear green to us. Click here to read about the history of GFP or here for an in-depth introduction by Nikon to fluorescent proteins including specific information about these jellyfish proteins.

Research on *Aequorea*'s green fluorescent protein by Drs. Osamu Shimomura, Martin Chalfie and Roger Tsien was awarded the 2008 Nobel Prize in Chemistry. Interviews about their research with Shimomura, Chalfie and Tsien in 2008 after receiving the Nobel Prize are posted online along with text transcripts. Another interview in 2002 with Martin Chalfie, whose work was seminal in the use of GFP for studies of gene expression, also offers some interesting insights on how the initial discoveries were made. Lectures by all three scientists about their research can also be viewed on the Nobel Prize website.

It is not well understood how and why jellyfish use their bioluminescent capabilities, or what biological function this serves. Jellyfish **do not** flash at each other in the dark, nor do they glow continuously. Whereas scientists who study jellyfish bioluminescence can easily stimulate medusae and thereby see their bioluminescent response, this is rarely seen in undisturbed animals. I have spent many hours in the dark watching medusae contained in a 2 m tall by 1 meter wide cylindrical aquarium and remember only one occasion when I saw what seemed to be "auto-bioluminescence," that is, light emission that was not stimulated by a human. It is possible that the medusa in this case (*Mitrocoma cellularia*, not even *Aequorea victoria*) had hit something, perhaps another medusa or the wall of the aquarium, but I could not discern the precise cause of this rare display. To see some beautiful images of stimulated-jellyfish (and other marine animals) bioluminescence by Dr. Edith Widder, click here.

If you live near or visit Puget Sound, the Strait of Georgia, Southeast

Alaska, or even Prince William Sound, you may see *Aequorea* medusae at the surface of the sea in the summer. If you pick up one of these medusae at night (they do not sting) and shake it gently in your hand in the dark, you will see the marginal ring of green bioluminescence. The glow of light lasts a few seconds. Some luminescent particles may stick to your hand after you put the animal back in the water.

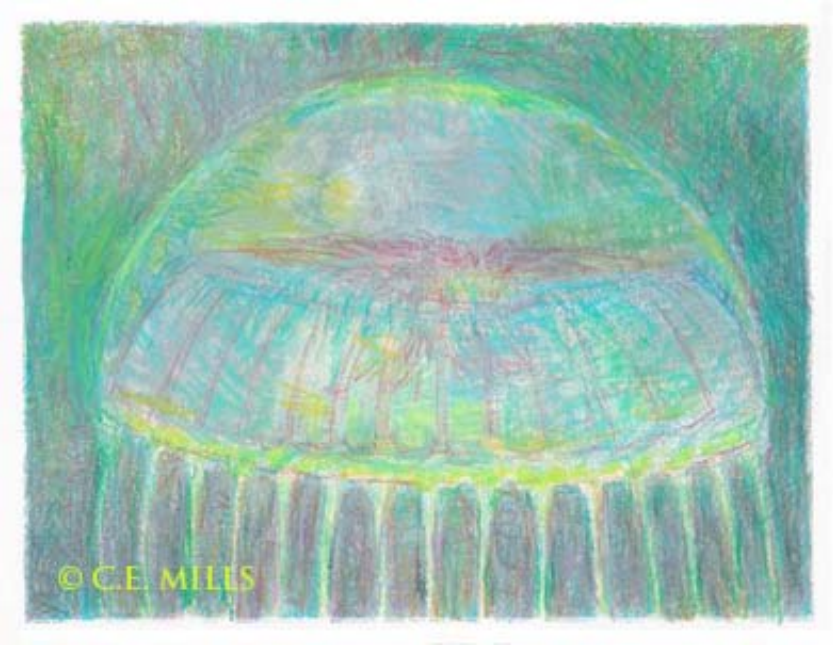
There is some nice music representing the protein structure of aequorin, composed by biologist Dr. Mary Anne Clark (yes, you are right, the jellyfish pictured there is not really *Aequorea*, but listen to the music anyway).

Choice and use of species name for *Aequorea*

This is a big problem and has led to the use of at least three different species names (*Aequorea aequorea*, *A. forskalea*, and *A. victoria*) in the modern literature for what is very likely all the same animal in the NE Pacific (I am not even discussing whether the Pacific and Atlantic material is the same right now). A fourth name, *Aequorea coerulescens*, is being used in Japan, but not on the American side of the North Pacific. In fact, even more names were available, but have not been used in the scientific literature by experimentalists, so will not be included in the present discussion. *Aequorea aequorea* (Forskal, 1775) was originally applied to specimens in the Mediterranean and then in the North Atlantic. These were also at times called *A. forskalea*, but Henry Bigelow, in 1913 stated about *Aequorea aequorea* "This species has universally been called *forskalea*, following Peron and Lesueur (1809), but this name was expressly given by them to the *Medusa aequorea* of Forskal (1775), and all modern authors are agreed that the animal in question is the same that Forskal described" - this statement means that Bigelow felt that *A. aequorea* is the correct scientific name for everything also called *A. forskalea* in the literature. F. S. Russell, another highly reputable jellyfish systematist, in 1953 chose to accept only *A. forskalea* as the name for all of these medusae, stating that the species name *aequorea* was preoccupied by an unidentifiable species.

Bigelow, already familiar with the North Atlantic fauna, in 1913 carefully

compared *Aequorea* specimens in Alaska with those in Friday Harbor, and arrived at using two variety names in order to differentiate the very large Alaskan *Aequorea aequorea var. albida* from the smaller Alaskan and Friday Harbor specimens *Aequorea aequorea var. aequorea*. (Russell did not look at Pacific material.) No one since Bigelow's time has published a comparison of Alaskan and Washington *Aequorea*. My own incomplete study indicates that medusae being called *Aequorea coerulescens* (Brandt, 1838) in Japan look like those designated *A. aequorea var. albida* by Bigelow. The type locality for *Aequorea coerulescens* is the open Pacific, about half-way between California and Hawaii. I have collected these plate-sized *Aequorea*s on rare occasions in the open ocean off central California and in Friday Harbor. In Alaska, small specimens of *Aequorea*, which are the size of Friday Harbor *A. aequorea var. aequorea* (Bigelow's distinction) all have that morphological look, making it very uncertain if the big ones are another species or their morphology changes (gaining LOTS of radial canals) with size.



Crayon drawing of *Aequorea victoria*, from memory, not from life (it's art, not biology).
Claudia Mills 2007, Friday Harbor.

Arai and Brinckmann-Voss in 1980 reexamined *Aequorea* from southern British Columbia, comparing it with specimens and drawings of European material. They found several characters which they felt were unique to the NE Pacific material and which warranted assigning it the name *Aequorea victoria* (Murbach & Shearer, 1902), although they chose not to acknowledge the older name *Aequorea flava* (A. Agassiz, 1865), for medusae described and illustrated from the Gulf of Georgia, which must be the same as those commonly collected in nearby Friday Harbor. *Aequorea coerulescens* is

the other possibly good older name, having been applied to specimens from San Diego and Unalaska Island in the Aleutians, as well as Japan.

It is particularly difficult to assign species names to *Aequorea* based on morphological characters, which are quite plastic in this group. Species are distinguished morphologically by a combination of the numbers of tentacles, radial canals and marginal statocysts, but at least tentacles and radial canals, and probably statocysts, are added as the medusa grows. A molecular project aimed at discovering relationships between the common temperate *Aequorea*s in the North Atlantic and North Pacific would seem both timely and important at this point. Dr. Mike Dawson, now of the University of California at Merced, has begun a project comparing genetic material in *Aequorea* medusae collected from around the world. He should eventually be able to shed some light on the systematics of these medusae. (Dr. Dawson is doing a similar project on the (unrelated) moon jellies in the genus *Aurelia*.) When the species are finally sorted out, nomenclatural rules determine that the names given to each animal will be the oldest (first) name applied to each species.

In summary, for those of you reading the scientific literature, there is **one** common *Aequorea* in Washington and southern British Columbia waters, no matter what species names have been applied to it in the literature. This "Friday Harbor *Aequorea*" may or may not turn out to be the same species as is found in New England and Europe, or further south into California. The much larger, dinner-plate sized *Aequorea* common in Alaska and Japan (and on rare occasions seen in Friday Harbor) may or may not be the same species, but until recently this glorious animal had not been used by experimentalists; it has only recently appeared in the aequorin/GFP literature, as *A. coerulescens* (sometimes misspelled).

The press-misinformation problem (and my unfortunate part in it)

One purpose of this page is to try to correct the many inaccuracies and myths about jellyfish luminescence and fluorescence. Many species of

hydromedusae, siphonophores and scyphomedusae (and ctenophores, but they are another phylum) are bioluminescent. Many others are not bioluminescent. Such bioluminescence is **never an all-over glow,** but is typically localized in a genus- or species-specific pattern (see links above). Some cnidarians emit their luminescence through a green fluorescent protein (GFP) that causes the luminescence to appear green (rather than blue).

Green fluorescent protein has recently become an extremely useful tool in molecular techniques, and the biologists who use it and publicists who write about them like to show pictures of the jellyfish that makes this protein. Without understanding the process or the animals, some scientists and most editors blindly settle on a published image that, together with its caption, is inaccurate and/or misleading. Classic examples of this misguided digital alteration, where some artist false-colors a photograph of living, colorless and transparent *Aequorea* jellyfish, so that they appear to be "bioluminescent" green, can be see here (Evrogen), or here (Haseloff lab, Cambridge), or here (AMS Bio). In fact, only a "dotted" marginal ring (or go here and down to the 6th image, by Steve Haddock, with green spots, on the left edge of this web page) would glow green on each *Aequorea* medusa in a real picture of the luminescence or fluorescence of this species, and the rest of each animal would be invisible to you in the dark.

A false-colorized version of one of my photos of *Aequorea victoria* appeared on the August 1995 cover of TRENDS IN GENETICS. This photo was submitted to the journal by Dr. Douglas Prasher, without my permission or knowledge, as his own. About ten years earlier I had given a print of that *Aequorea* photo to Dr. Prasher, who apparently used it so often that he forgot that he had never taken a good photo of *Aequorea victoria*. My original black-and-white image had metamorphosed into a misleading and fakey green-colored medusa that ended up as a cover photo. I should have been thrilled to have a cover photo - I was not. The myth of all-over jellyfish bioluminescence got a big boost in the serious science literature with that double dose of scientific dishonesty.

I have been told that what is probably the same photograph of *Aequorea* has moved around the world in scientific talks, being used by people who I have never met or known about. There is little doubt that whatever is said about the photograph in such contexts is not likely to be completely accurate or true.

In another article about jellyfish GFP, SCIENCE NEWS (July 26, 1997), on bad advice from an unknown scientist, published a reflected-light, blue-tinted image of the medusa *Aurelia*, captioned as the "The Pacific Northwest jellyfish, *Aequorea victoria*, glows bright with GFP, an intriguing fluorescent protein". In the opening words of the article, the author further used the phrase "glowing with a cool inner light" to describe luminescent jellyfish. In fact, they do not glow. Not only was this reflected-light photograph not an image of either fluorescence or luminescence, but it was a photograph of *Aurelia*, a species that is **not** bioluminescent.

I am increasingly concerned that people studying green fluorescent protein recognize neither the animal from which it derives nor natural fluorescence when they see it. The "cool inner light" (phrase used in opening text of the SCIENCE NEWS article, vol. 152, p. 55) of the above-mentioned photo was actually reflected light from the photographer's flash, not fluorescence. This article has inadvertently highlighted the growing gap between the reductionist and the naturalist in science. I would think that even the most reductionist scientist should be expected to avoid this sort of misinformation and should also be expected to be able to recognize his own subject material as a living animal.

In December 1999 another *Aequorea* bioluminescence/fluorescence blooper came to my attention. In the December 1999 print version of BioProbes 32, a subcatalogue produced by Molecular Probes, p. 10 features a most-bizarre "enhanced" photograph of *Aequorea* (fortunately not one of my shots), with brilliant and shamelessly lime-green tinted radial canals, whose caption reads "The luminescent jellyfish *Aequorea victoria*. Image from GFP in Motion, CD-ROM supplement to Trends in Cell Biology, B. Ludin and A. Matos, Eds.,

Elsevier Science, 1999. Used with Permission." Used with permission, perhaps, but probably without the slightest idea of the deception and inaccuracy depicted. This blooper is all the more surprising since Elsevier's Trends in Genetics had already done about the same thing in 1995 with one of my photos and had apologized in print for it (see above). Apparently the lime-green *Aequorea* was also on the COVER of the Trends in Cell Biology CD-ROM.

Still in October 2008, Bio-Rad Laboratories is (probably unknowingly) using a similar shamelessy green-tinted *Aequorea* image in the description of their Life Science Education Biotechnology Explorer kits. Bio-Rad has also for years circulated a lovely poster advertising such kits, with a similar false-colored green *Aequorea* on it.

Another confusing use of an *Aequorea* photograph in the scientific literature is the December 1999 cover of Trends in Biotechnology. Although the caption never actually says that the photograph by J. Blinks used on the cover is a bioluminescent image, it is somewhat implied by the large word *Bioluminescence* splashed across the twice-iterated image. Trends in Biotechnology might be given some credit for not altering the photo with false-colors, but with this cover presentation their editors seem to subscribe to the myth of all-over bioluminescence of the "cool-white" variety. This cover does not show *Aequorea* luminescnce, which is green, and only occurs around the bell margin. *The highly cited "Trends ..." journal series now rates #1 for repeated misuse and misinformation about Aequorea bioluminescence in the scientific literature.*

Another *Aequorea* image blooper in the scientific literature is the photo labelled *Aequorea aequorea* at the top of page 292 in *Nature*, volume 405, 18 May 2000, which is in fact *Mitrocoma* (was *Halistaura*) *cellularia*. This image (which makes no claims whether or not it shows luminescence - it does not - and is probably interpreted different ways by different readers) accompanies a News and Views commentary about the newly-imaged tertiary structure of the aequorin photoprotein described elsewhere in the same issue of *Nature*. (*Mitrocoma cellularia* makes a related photoprotein, which has also been characterized and

named *halistaurin*, after the old genus name.)

In the popular press, the Seattle TIMES newspaper used one of my *Aequorea victoria* photographs to illustrate a 14 July 1998 newspaper story about Dr. John Blinks' long-time study of the luminescent aequorin molecule. My Kodachrome slide was digitized by a local business, who apparently "enhanced" the bluish color of the bell in my photograph, although I hadn't realized this at the time. A very lovely, but inaccurately-turquoise image of *Aequorea* was printed at nearly half-page size in the newspaper. Aware of the potential abuse of any *Aequorea* image, I had given the SeattleTIMES explicit one-time-only, not-on-the-Internet, rights to that photograph. Not happy with the misleading turquoise color in a story about jellyfish bioluminescence, I nevertheless assumed that it was a mistake not to be repeated. Well, images have a way of staying in files, and thus history repeats itself, as I soon learned.

The following year Pacific Northwest Magazine, a supplement to the Sunday Seattle TIMES, used that same enormous *Aequorea victoria* image for their June 27, 1999 cover photo in a story about jellyfish. I provided the Pacific Northwest Magazine with several other jellyfish photos for use in this article - the image of *Aequorea* was intentionally not included in that set. I was told that the editors were using an image "found in their files" for their cover. I was never told that it was my photograph of *Aequorea*, which should not have still been in their files, but just to be safe, I sent a message to the photo editor stating that *Aequorea* was a colorless animal and in the event that they were using it, they needed to take the color out of that digitized image. I never received an answer, so assumed that my note was irrelevant - some other people take nice photos of jellyfish, too. Well, I have again become part of the jellyfish bioluminescence misinformation campaign as my (now slightly-less) turquoise-tinted photograph was printed a second time, with the following inaccurate caption which I never saw or approved of: "Bioluminescence shows off the delicate-looking *Aequorea* jellyfish ..." The cover looked great (as did the smaller come-on photograph on the bottom left of the Sunday front page), but this interpretation of the photograph was dead-wrong.

If you have asked to use one of my photos of *Aequorea* and I have not responded postively, maybe the above narrative will help you understand why. Every time one of my *Aequorea* photos is used in someone else's story, something bad and wrong happens.

For more about the facts and myths of bioluminescence, see The Bioluminescence Web Page.

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