SERENDIPITY

WHY IS THE FISH BLUE?

NAVODITA JAIN & SWAGATHA GHOSH

The accidental discovery of the novel property of an enigmatic blue protein answered a question that had nagged a fish biologist for two decades. This is the story of Wayne Schaefer and his pursuit of an observation – a blue fish in a lake famous for its yellow ones.

The large number of lakes in North-eastern America and Canada (the Great Lakes region) may explain the huge popularity of fishing as a recreational activity in these regions (refer **Fig. 1**). And, sometimes, when a person with a keen sense of observation goes fishing, she (or he) might just come back with a scientific breakthrough. This is the story of one such observant angler – Wayne Schaefer.

Fig. 1. The Great Lake region of North America and Canada.

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Fig. 2. The yellow walleye

(a) The Fish.

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(b) The Mascot (Wally the Walleye).

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Schaefer, now a Professor Emeritus at the University of Wisconsin, United States, is a fish biologist with a special interest in one species – *Sander vitreus* or the yellow walleye (refer **Fig. 2a**). Every year, for the last 25 years, Schaefer has been heading down to a cabin he owns in Ontario, Canada, to study these fish in their natural habitat.

What is the walleye?

This fish is believed to get its unusual name from the fact that its eyes are opaque and glassy. Native to Canada (and North America), the walleye is a local delicacy in the region. With a length of about 76 cm and a weight of about 9 kg, this fish is a golden catch indeed! Interestingly, an ice hockey team – the Toledo Walleyes of Ohio, United States, has Wally the Walleye as its mascot (refer **Fig. 2b**)!

"Hey! I caught a blue walleye!"

In the summer of 1992, something unusual happened – Wayne caught what seemed like a new species of fish with bluish scales (refer **Fig. 3a**). On handling the fish, however, he found that the blue colour came from a mucus-like substance that rubbed-off on the palms of his hands (refer **Fig. 3b**), leaving behind a steel-grey fish with an uncanny resemblance to the yellow walleye. This story may have ended here, but for Wayne's acute sense of enquiry.

With his experience in fishing in those waters, Wayne identified the catch to be a walleye. Back in his lab, he used DNA studies to confirm this conclusion. But, what was the blue-coloured substance? Why was it there? Was this a one-time occurrence? To the scientist in Wayne, this anomalous observation was an irresistible challenge.

As other reports of blue walleye sightings started pouring in from both recreational and professional anglers fishing in the same region, Wayne discovered that this was no rare occurrence. He himself continued to catch more of these fish on subsequent fishing expeditions. In 2006, Wayne decided to compile his data on the walleye into an online database. Then, he invited other people to add to this database with details of their own sightings, and requested that mucus samples of each specimen be shipped to him. Looking through these records, Wayne and his group discovered two interesting patterns – most sightings of the blue walleye were in the summer (June to August), and none of them were from lakes in Minnesota or Wisconsin, United States. These patterns were supported by the field observations of Wayne and his research group over a period of 16 years. Based on these parallel sources of data, Wayne went on to conclude that blue walleyes were found at latitudes north of 45° and, therefore, only in lakes on the Canadian side.

Fig. 3. The blue walleye.

(a) As compared to the yellow walleye.

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(b) With mucus that rubs off while handling.

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Wayne's group also continued to analyze different samples of the blue mucus that rubbed off from the fish's surface, arriving at many different hypotheses to explain it. One of these attributed the blue colour to the acidity of the water in this region; another speculated that the mucus may be the result of algae or bacteria growing on the skin of the fish. Finally, through collaborations with expert chemists, Wayne managed to isolate the specific substance that gave the mucus its blue hue. This substance turned out to be proteinaceous in nature, ruling out the possibility of it being a bacteria or algae surviving on the skin of the fish.

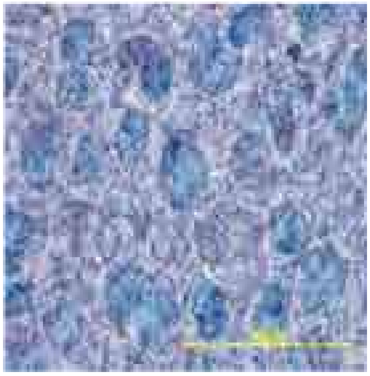
The fishy blue protein

The blue protein, much like the fish, has an interesting story of its own. Through extensive chemical analyses, Wayne and his collaborators discovered that its blue colour came from a protein-chromophore complex. The protein turned out to be unique to the fish, and was named Sandercyanin after it. The chromophore turned out to be Biliverdin — a chemical that is highly conserved across species. Biliverdin is produced by the breakdown of heme (during the recycling of hemoglobin in red blood cells). Given the patterns of occurrence of the blue fish, this gave rise to the question — why was this protein-chromophore complex produced only during summers, and only in walleyes found in Canadian lakes?

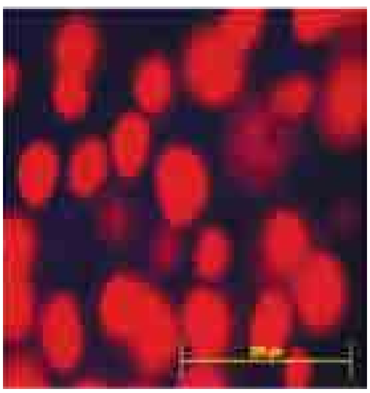
One piece to this puzzle came from a high school student who pointed out that regions above 45° latitude received unusually high amounts of ultraviolet (UV) radiation in summer. A study from southern Sweden had recorded a similar increase in UV radiation during summer in regions above 55° latitude. The authors of the study had attributed the increase to a seasonal depletion of ozone near the poles. The second piece came from studies on the synthesis of Biliverdin. While the degradation of heme is usually mediated by enzymes, it is also known to occur on exposure to UV radiation. With this key bit of information, Wayne and his colleagues seemed closer to forming a hypothesis — higher amounts of UV radiation in Canadian lakes could be triggering the increased breakdown of heme. This in turn could lead to the increased production of the Sandercyanin-Biliverdin complex or the blue mucus.

The final piece of the puzzle came purely by chance. A collaborator suggested that Wayne view the blue mucus under a bright-field microscope (white light). On trying this, Wayne was surprised to see many bright red spots in the sample. Hearing this, his collaborator wondered if Wayne was "sure that the incident light was set to white". This was when Wayne realized that he had been looking at the sample under UV (with a wavelength of about 375 nm) light in a fluorescence microscope. Excited, he illuminated the sample first with white light, and then with UV light. Every time he did this, he got the same result. The sample looked almost uniformly blue under white light (refer **Fig. 4a**) but appeared to have bright red spots under UV light (refer **Fig. 4b**). Some component of the mucus was capable of absorbing UV light (of a lower wavelength) and emitting red light (of a higher wavelength) — in other words, it could fluoresce. Wayne hypothesized that the fluorescing component was Sandercyanin. This was confirmed when he and a collaborator,

Fig. 4. The blue mucus under a fluorescence microscope.



(a) Observed under white light.



(b) Observed under UV light.

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S. Ramaswamy, managed to isolate the protein.

This very naturally led to the next question — was the mucus simply a by-product of a seasonal reaction, or did it provide some kind of survival advantage to the blue fish?

The serendipitous discovery that Sandercynanin could absorb UV light helped provide clues to this mystery. While Wayne could not find any genomic differences between the yellow and blue walleyes, he inferred that the blue mucus could be a seasonal 'adaptation'. By absorbing the high levels of UV radiation at the surface, the Sandercyanin-Biliverdin complex in the mucus could be protecting deeper tissues from its damaging effects. Ramaswamy suggests another possible advantage. Since water preferentially absorbs light of higher wavelength, only lower wavelengths of light (in the blue and UV spectrum) are able to penetrate deeper waters. Fish that inhabit deeper waters have adapted to this by developing the ability to 'see' objects by the UV light they reflect (called UV vision). Pikes, natural predators of walleyes in Canadian waters, tend to rely on their UV vision to hunt for prey. Thanks to their seasonal adaptation, blue walleyes absorb UV instead of reflecting it. Thus, the blue mucus acts as an invisibility cloak — making the fish invisible to their primary predators! But, walleyes also inhabit deeper waters and rely on UV vision. Does this mean that the blue walleyes become invisible not only to pikes but also to a potential mate? Ramaswamy hypothesizes that external fertilization in the walleye may rule out the necessity of 'being visible' to a potential mate.

It's not just the fish that find this protein useful. Swagatha, a researcher in Ramaswamy's laboratory, was part of the team that studied the fluorescent properties of Sandercynanin in detail. This team found that Sandercynanin is quite small in size — perhaps, the smallest red fluorescent protein known till date. However, because of its chemically unique nature, it can fluoresce for long periods of time. Keeping these two properties in mind, Swagatha plans to explore Sandercynanin's role as a fluorescent tag in research and clinical diagnosis. This will involve identification of the coding regions of the Sandercyanin gene, and engineering it into live cells and tissues of other organisms to track their activity in real-time.

To conclude

The spark of curiosity that led one scientist to question why some fish looked blue instead of yellow marked the beginning of a long process of scientific research. This process has involved scientists from many different disciplines and fishing enthusiasts from across the Great Lake region in Canada. It has also led to the 'accidental' discovery of a new fluorescent protein — one that is currently being studied for its potential in unlocking other mysteries of life through its applications in research and diagnostics. Serendipity, most often, strikes minds sharpened by hard work — offering an explanation and leading the way. Did you spot something today that seemed out of the usual? Did you follow it and discover pieces of a greater puzzle?

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Navodita Jain is a cell biologist by training with an interest in the latest in therapeutics and an inclination towards science writing. She is associated with IndiaBioscience as their Science Education Coordinator. Navodita can be contacted at navodita@indiabioscience.org or navod12@gmail.com.

Swagatha Ghosh is a Visting Researcher at the University of Gothenburg. She is interested in improving the quality of Indian school education and promoting higher education. Swagatha can be contacted at swagathag.gu@gmail.com.