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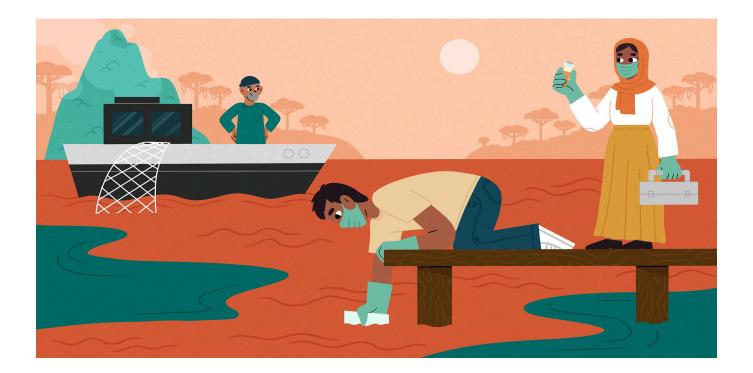
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HARMFUL ALGAE IN INDONESIA: SMALL IN SIZE, BIG IN EFFECT

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Sometimes the waters of seas, rivers, or lakes turn an odd color, becoming brown, red, or green. These colors are actually those of tiny algae that are invisible to the naked eye—but those algae are reproducing so fast that they stain the water. These events are called algal blooms, and they can be harmful because they produce toxins that kill aquatic animals and can cause poisoning in humans. Our group investigated the harmful algal blooms that have been occurring regularly in Lampung Bay, Indonesia, since 2012. We tracked down the algal species responsible for these blooms by analyzing seawater and the seabed sediments of the bay. We identified one species of algae, a dinoflagellate called *Margalefidinium polykrikoides*, as the culprit. This species can take on

multiple shapes, which makes its identification particularly difficult. Improving our knowledge about this algal species will eventually help us understand what triggers harmful algal blooms and how to prevent them.

WHAT GIVES THE SEA ITS COLOR?

While walking along the waterfront, you have probably observed that the waters of lakes, rivers, and seas are rarely turquoise or transparent. Water color depends on the colors of the tiny **algae** that live in it. These are not the large algae often seen washed up at the seaside—they are small algae, invisible to the naked eye, called **phytoplankton**. Like plants, phytoplankton use nutrients, and sunlight to get their energy. There are thousands of different species of phytoplankton in both marine and fresh bodies of water. Phytoplankton is essential for the aquatic animals that feed on it—it is the basis of the food chain in aquatic ecosystems. When there is enough light and nutrients are abundant, phytoplankton will reproduce a lot. Sometimes they become so numerous that the water takes on their color. Have you ever seen green, brown, or even red seawater? If so, you have witnessed an **algal bloom**.

Algal blooms are natural events that happen when waters are rich in nutrients. Blooms are occurring more frequently because of human activities. In fact, the nutrients in the fertilizers used in farming very often end up in rivers, lakes, and the sea. This is why algal blooms often occur in places that humans have polluted.

HARMFUL ALGAL BLOOMS

Some harmful algal blooms have disastrous consequences for aquatic animals and even humans [1]. There are several reasons why we call these blooms "harmful." First, during the bloom, there is less oxygen in the water because when algae die they are decomposed by bacteria that use oxygen. Aquatic animals need oxygen to survive, so they often suffer and die during harmful algal blooms (Figure 1A). Second, some phytoplankton species are toxic. Just as there are toxic plant species on land, there are also toxic species in the sea. Toxic phytoplankton release substances that are deadly to many fish, crustaceans, and shellfish. Third, some harmful algae can make a jelly-like mucus that sticks to fish gills, preventing the fish from breathing (Figure 1B). Lastly, fish and shellfish that survive can accumulate toxic substances from the bloom. Humans who eat these organisms will get sick, too (Figure 1C). These dangerous seafood products cannot be sold, so people who make a living from fish and shellfish farming suffer a great loss of money during harmful algal blooms (Figure 1D).

ALGAE

Microscopic organisms living in seas and fresh waters. They use light as a source of energy, just like plants. They are part of the phytoplankton.

PHYTOPLANKTON

Phytoplankton are microscopic algae and cyanobacteria. It is the base of the aquatic food chain in oceanic and freshwater ecosystems.

ALGAL BLOOM

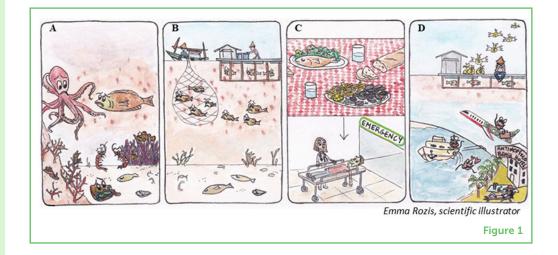
Rapid growth of phytoplankton due to excessive nutrients in the water. A bloom can be visible to the naked eye when it turns the water a red, brown, or green.

Figure 1

Harmful algal blooms have several negative consequences: (A) Aquatic animals can suffer and die from lack of oxygen, reducing an area's biodiversity. (B) Mucus produced by phytoplankton can stick to fish gills and prevent fish from breathing, decreasing the number of fish in the area. (C) Health problems occur in humans who eat fish and shellfish contaminated with phytoplankton toxins. (D) Fishers and fish farmers lose a lot of money and blooms are increasing as toxic phytoplankton are transported across the world on ships (Image credits: Emma Rozis).

INVASIVE SPECIES

Species that colonizes an environment far away from their natural habitats. These species can cause harm to ecosystems and threaten the species that live in the new habitats they colonize.



Harmful algal blooms have been occurring around the world in recent decades [2]. In fact, some toxic phytoplankton end up on huge ships that travel from continent to continent. In this way, they gradually invade the coasts of many countries, multiplying when they find large quantities of nutrients that suit them. For this reason, many toxic phytoplankton are considered to be **invasive species**.

Scientists around the world are working to better understand algal blooms and reduce their impacts. Now we will go back in time to 2012, when our investigation into Sumatra's first huge harmful algal bloom began.

THE FIRST HUGE HARMFUL ALGAL BLOOM IN SUMATRA, INDONESIA

The harmful algal bloom that we studied took place in Lampung Bay, located on the southeast coast of the island of Sumatra, Indonesia (Figures 2A,B). Fish farming, pearl farming, and fishing are very important in this region. In November 2012, Mrs. Muawanah, one of our scientists, discovered a huge bloom of a brownish phytoplankton near a fish farm (Figure 2C). This bloom caused the death of many fish. The fishers of the region lost a lot of money and consequently had difficulties feeding their families (Figure 2D).

Since 2012, harmful algal blooms have become increasingly frequent in this region, causing significant economic losses for fishers and fish farmers every year. Unlike the blooms occurring in other places, the phytoplankton responsible for these blooms had not yet been identified in 2012. So, our research team carried out an investigation [3].

Figure 2

(A) Location of the study site. Lampung Bay in Sumatra, Indonesia, is indicated by a red star. (B) Harmful algal bloom forming dark brown patches off Bandar Lampung. (C) Mrs. Muawanah, the research scientist, collecting brownish water samples. (D) Death of many fish on a fish farm in Lampung Bay, November 2012 (Image credits: Mrs Muawanah).

DINOFLAGELLATE

It is a specific group of brown algae.

REACTIVE OXYGEN SPECIES (ROS)

Reactive oxygen species (ROS) are highly reactive chemical compounds capable of damaging and destroying the cells of a living plant or animal.



TRACKING DOWN THE LAMPUNG BAY SUSPECT

Our team of scientists identified a suspect: *Margalefidinium polykrikoides*, which we will call *Mp*. This microscopic phytoplankton belongs to a group called **dinoflagellates**. It is about the thickness of a hair and has two tails, called flagella, that allow it to move in water (Figure 3). The cells of this microalgae divide and stick together to form short chains of 2, 4, or 8 cells.

Why did we suspect this phytoplankton? First, the brown color of the water indicated that a dinoflagellate was certainly responsible for the bloom. Second, the way the fish died led us to suspect that *Mp* was the culprit—this phytoplankton causes massive fish kills in other places in Asia. In fact, *Mp* releases a toxic substance called **reactive oxygen species (ROS)**. When phytoplankton cells penetrate fish gills, the toxic ROS released prevents the fish from breathing. Lastly, during blooms, we found a great quantity of *Mp* in a single drop of water. However, when blooms were not happening, *Mp* was nowhere to be found...

We wanted to verify *Mp* as the culprit, and we also wanted to be able to predict other harmful algal blooms. So, we analyzed samples of water and sediment from other locations in the Bay. Phytoplankton in water were collected with a plankton net. Sediment from the sea floor was collected with a tool called a grab. We looked for *Mp* in

Planktonic stage

in the water column

Figure 3

The life cycle of Margalefidinium polykrikoides in seawater and in seabed sediments. This algae can take many forms. In its planktonic stage, it can exist as (A) a single cell, (B) two-cell, (C) four-cell, or even eight-cell chains that swim freely in the water. These algae can release reactive oxygen species (ROS) that can harm fish and other animals. (D-F) In its benthic stage, in the sediment, Mp cysts of three separate shapes have been discovered in Lampung Bay (Image credit: Emma Rozis).

Image: specific displayed displayed

Flagella

these samples by examining drops of water and samples of sediment under a microscope. We also extracted DNA from phytoplankton cells in the water and sediment samples, to try to detect *Mp* through its genetic identity.

A DIFFICULT SUSPECT TO CATCH

This study was difficult for several reasons. Lampung Bay is very large, so many water and sediment samples had to be examined. Further, *Mp* is not always present in the water and it is not easy to detect or recognize, specifically because it changes its appearance during its life cycle [4]. It has two main stages in its life: a planktonic stage, in the water, and a benthic stage, in the seabed sediment (Figure 3). This is why we analyzed both water and sediment.

After a bloom, when nutrients in the water are scarce, *Mp* cells meet each other and reproduce. The new ones do not look like their parents—they look like tiny resistant balls, which are called **cysts**. This shape allows them to survive inside the seabed until the amount of nutrients in the water increases again.

But the situation is even more complicated because there are several forms of cysts—and not all of them have been described by scientists yet. We found three separate cyst forms in Lampung Bay sediment

CYST

A life form that some organisms adopt to survive during stressful times, like when there is no food or water. When conditions become favorable again, the cyst sprouts like a small seed. (Figure 3). This large reservoir of cysts hidden in the seabed is called a cyst bank. When the conditions are right again (enough nutrients and light), the cysts start to sprout and multiply. The cycle starts again, as a new bloom appears.

We found *Mp* cysts in many locations within Lampung Bay. So, *Mp* is not only present during harmful algal blooms, but also at other times of the year. This was the first time that *Mp* was found in such large quantities in Indonesia. There is a huge cyst bank in the sediment that can sprout when conditions become favorable!

We also found many cysts of other dinoflagellate species. To identify them, we need to learn more about the forms that those phytoplankton species can take. Scientists have a lot of work to do to identify all the cysts found in the Lampung Bay sediment.

AQUACULTURE It refers to all animal or

plant production activities in an aquatic environment. Aquaculture is practiced in rivers or in ponds, on the sea shore.

BALLAST WATERS

Ballast waters are used on board ships to stabilize them. They carry thousands of marine or aquatic microbes, plants, and animals, which are then transported around the world. New methods and technologies are being developed to better detect, identify, and track dinoflagellates, but it is impossible to control harmful algal blooms. Toxic algae often settle in places where ecosystems have been modified by humans. For example, in locations where **aquaculture** facilities/structures are present, if too many animals are crowded together, their excrement (poop) will enrich the environment with natural fertilizer that helps algae grow. Toxic algae can also settle in confined or semi-confined areas with stagnant water, such as in harbors or behind dykes, or in ports where ships empty their **ballast water** and the invasive species that water contains. Finally, when the seabed is damaged by removal of corals and seagrass, algae can proliferate.

It is possible to do a better job of preventing harmful blooms and the animal deaths they cause. For example, regular monitoring of phytoplankton levels in the waters and sediments of Lampung Bay can help prevent the algae-related health problems seen in fish, shellfish, and humans. In summary, algal blooms have always existed but now the increasing appearance of toxic algal blooms is a catastrophe for wildlife and human health. Researchers have been warning for a long time that only decisions for the conservation and the respect of nature accompanied by drastic changes of human activities will be able to stop these phenomena.

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Africa Science Platforms) of JSPS (Japan). Figures 1 and 3: drawings were performed by Emma Rozis, scientific illustrator (http://illumer.fr). Figure 2: Mrs. Muawanah's photos taken in November 2012.

ORIGINAL SOURCE ARTICLE

Thoha, H., Muawanah, Bayu Intan, M. D., Rachman, A., Sianturi, O. R., Sidabutar, T., et al. 2019. Resting cyst distribution and molecular identification of the harmful dinoflagellate *Margalefidinium polykrikoides* (Gymnodiniales, Dinophyceae) in Lampung Bay, Sumatra, Indonesia. *Front. Microbiol.* 10:306. doi: 10.3389/fmicb.2019.00306

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YOUNG REVIEWERS

ISABEL, AGE: 12

Hello, I am Isabel. I am 12 years old and I really like writing stories. I also like reading and playing drums. I am really interested in diplomacy.

KINABALU INTERNATIONAL SCHOOL, AGES: 10-11

Hello world! We are science-passionate Kinabalu International School's students. We love to review scientists' work and help explain their hypotheses and research. Teamwork is very important to everyone's education. Therefore, we hope to inspire other children to love science as much as us by clarifying the information and making sure the language is understandable for others. We enjoy participating in environmentally friendly actions and we feel lucky to have the chance to work alongside experts. Get ready to be scienced because science rules!

MARGARIDA, AGE: 15

My name is Margarida, I am 15 years old and I like reading, climbing, and writing. I love science, especially astrophysics and I have absolutely no idea what I want to do when I grow up. I also really like biology.

AUTHORS

MUAWANAH

Mrs Muawanah is a engineer in aquatic ecology at the Main Center for Marine Aquaculture of Lampung, DGA, Lampung, Indonesia. She monitors harmful algal blooms and water quality in Lampung Bay. She trains people, including students and the owners of aquaculture facilities, to monitor water quality. She ensures that the waters of Lampung Bay are healthy for the fish and shrimp growing in aquaculture ponds, and safe from harmful algal blooms.

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Mariana D. Bayu Intan is a junior researcher at the Research Center for Oceanography, National Research and Innovation Agency (RCO-BRIN). She is currently doing her Ph.D. research at the University of Technology Sydney (UTS), which focuses on the ecology and dynamics of *Margalefidinium polykrikoides* (planktonic form and cysts) in Lampung Bay. She has always been interested in understanding the ecology and biogeography of harmful algal blooms and the ability of these species to adapt and initiate blooms in changing environments.

























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Elyse Boudin received a degree in ecology engineering from the University of Montpellier in 2019. Now, she is involved in several environmental and educational projects in the South of France. These projects combine research in marine and terrestrial ecology with the creation of environmental education tools.

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Arief Rachman is a marine plankton researcher at Plankton Laboratory, Research Center for Oceanography, National Research and Innovation Agency (RCO-BRIN). His research is mainly about the ecology and community dynamics of coastal and oceanic phytoplankton in tropical ecosystems. He also works on the emerging harmful algal bloom species in Indonesia, including species diversity, distribution, cyst bank deposits, and he applies artificial intelligence technology in his studies.

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