Deadly deficiency at the heart of an environmental mystery

Researchers are puzzling over a widespread vitamin B shortage that appears to be killing wildlife.

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During spring and summer, busy colonies of a duck called the common eider (*Somateria mollissima*) and other wild birds are usually seen breeding on the rocky coasts around the Baltic Sea. Thousands of eager new parents vie for the best spots to build nests and catch food for their demanding young broods.

But Lennart Balk, an environmental biochemist at Stockholm University, witnessed a dramatically different scene when he visited Swedish coastal colonies during a 5-year period starting in 2004. Many birds couldn't fly. Others were completely paralyzed. Birds also weren't eating and had difficulty breathing. Thousands of birds were suffering and dying from this paralytic disease, says Balk. "We went into the bird colonies, and we were shocked. You could see something was really wrong. It was a scary situation for this time of year," he says.

Based on his past work documenting a similar crisis in several Baltic Sea fish species, Balk suspected that the birds' disease was caused by a thiamine (vitamin B1) deficiency. Thiamine is required for critical metabolic processes, such as energy production and proper functioning of the nervous system. This essential micronutrient is produced mainly by plants, including phytoplankton,

Researchers observed herring gulls, such as this one, that couldn't stand because of paralyzed legs. This bird and others also had squinting eyes, discolored pupils, and wings that didn't fold back close to the body. All are symptoms of thiamine deficiency. Image courtesy of Lennart Balk and Per-Åke Hägerroth (Stockholm University, Stockholm).

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bacteria, and fungi; people and animals must acquire it through their food.

Scenes such as the one in Sweden, seen again and again in recent years in a variety of species in Europe and North America, have Balk and other researchers worried that something in the environment is causing widespread thiamine shortages, which could explain these specific episodes—as well as possibly larger-scale wildlife population collapses. "This could be a very serious source of mortality," says Stephen Riley, a fish ecologist at the United States Geological Survey (USGS) Great Lakes Science Center in Ann Arbor, MI.

Researchers generally agree that the crises in seabirds, fish, and other marine species have thiamine deficiencies in common. But much remains unknown. Is a thiamine shortage the root cause of the problem in every case? What might be driving such a widespread environmental vitamin deficiency? As instances of sick and dying wildlife continue to arise, though, a sense of urgency is building among researchers trying to figure out what's going on. "I don't think we agree in our assessment of it other than that it is a real issue," says Clifford Kraft, a freshwater ecologist at Cornell University in Ithaca, NY.

Recurring Patterns

As a young researcher in the late 1980s, Balk started out examining the impacts of industrial pollution and endocrine disruptors on fish health. But he was so taken aback upon seeing the effects of thiamine deficiency on fish, he abandoned his earlier work to focus on this problem instead. In the late 1990s, Balk saw that several fish species in the Baltic Sea, including Atlantic salmon (*Salmo salar*), had trouble reproducing. Many of the larvae couldn't swim straight and were lethargic before dying. When he supplemented the larvae with thiamine, almost all survived. In contrast, almost all of the larvae not treated with thiamine supplements died. Balk concluded that the fish were suffering from a thiamine deficiency (1).

Just a few years earlier, researchers had begun seeing similar devastation among fish in the Laurentian Great Lakes. Since 1995, researchers including John Fitzsimons, then a fish biologist for the Canadian government and now retired, had documented a thiamine deficiency that devastated populations of lake trout (*Salvelinus namaycush*) and several species of salmon in the Great Lakes (2). The fish were not reproducing and had difficulty maintaining their balance while swimming. "It's like the fish were drunk. They lost their balance and would fall on their side," says Fitzsimons. At first, researchers thought pollutants were to blame, but the suspicion was not borne out in tests. In a moment of inspiration, Fitzsimons realized the fish must be lacking a nutrient.

"One day the fish would be fine, and the next they would be lying on their side. It got me wondering if they had some sort of deficiency," he recalls. Fitzsimons supplemented eggs from sick fish with thiamine and other B vitamins. He found that just less than 10% of fish eggs injected with thiamine died. The other B vitamins had no therapeutic effect. In contrast, he saw greater than 75% mortality in the control specimens that received no added vitamins. Fitzsimons and his colleagues concluded a thiamine scarcity was behind the paralysis and population declines of fish in the Great Lakes (2, 3).

Researchers witnessed eider ducklings, apparently weakened because of thiamine deficiency, being preyed upon by herring gulls. Reprinted with permission from ref. 14.

After witnessing similar symptoms resurface in wild birds in 2004, Balk concentrated on exploring how far the disease reached. In 2009, his team documented symptoms in European herring gulls (*Larus argentatus*) and the common starling (*Sturnus vulgaris*) in addition to *S. mollissima* from sites across northern Europe. The researchers saw that the thiamine concentration in *L. argentatus* egg yolk was as much as 41% lower in birds from the Baltic Sea area compared with birds taken from around Iceland. The researchers also saw a drop in several biomarkers of thiamine activity, including thiamine-dependent enzymes, indicating that the vitamin was scarce in some birds around the Baltic Sea.

Balk's team treated sick birds with thiamine injections and found that all but 1 of 10 paralyzed adult *L. argentatus* recovered over a 2-week period. None of the untreated birds showed signs of improvement. In addition, 10 young birds fed a thiamine solution on the first and second days after hatching were vigorous and active, whereas 10 young birds fed a saline solution were lethargic and apathetic and began to die approximately 4 days after hatching. Similar tests on *S. vulgaris* and *S. mollissima* also suggested that individuals were suffering as a result of thiamine deficiency. Balk reported that the deficiency was hampering the birds from breeding, which could be contributing to declines in local seabird populations (4).

Telltale Signs

It was certainly not the first time such symptoms had been seen in birds. In the 1880s, Christiaan Eijkman, a doctor working in the Dutch East Indies, observed paralysis in chickens, noting that they were experiencing weakness in their legs reminiscent of a disease

known as beriberi, which was afflicting people across Asia at the time.

Eijkman linked the paralysis to the chickens' diet of predominately cooked white rice, which he believed must be toxic; but his colleague, Gerrit Grijns, later came to suspect the rice had been stripped of some vital protective substance by modern milling processes. In some parts of Asia where people's diets also relied largely on white rice, beriberi affected nearly 30% of the population. Eijkman shared a Nobel Prize in 1929 for his observations that aided the discovery of thiamine—the first vitamin to be identified (5). Today, instances of thiamine deficiency among people are rare because the vitamin is added to a wide range of processed foods, such as breakfast cereal. But deficiency does still occur among refugees with poor diets and among those who have alcoholism because their bodies have difficulty absorbing the vitamin.

Still another group at risk are people who eat a lot of fermented fish, notes Riley, because the fish are rich in the enzyme thiaminase, which breaks down thiamine. In fact, a diet of thiaminase-rich fish, it turned out, was the culprit in the case of thiamine-deficient Great Lakes trout and salmon.

In 2005, a team including Don Tillitt, an environmental toxicologist at the USGS, reported that salmon and lake trout were eating mainly alewives (*Alosa pseudoharengus*), an invasive species of fish that is rich in thiaminase. Tillitt and his colleagues fed 17 female lake trout, from a hatchery in Michigan, a diet consisting of only alewives. The team found that the fish laid eggs with a total thiamine concentration of approximately 2.5 nmol/g. In contrast, 13 fish were fed only bloaters (*Coregonus hoyi*)—prey fish lacking thiaminase—laid eggs with approximately 12 nmol/g. The researchers saw that nearly 20% of the young fish died when their mothers were fed only alewives. But all the young survived when their mothers were fed bloaters (6, 7).

"Thiamine deficiency almost completely stopped all reproduction in some fish species in the Great Lakes, causing huge population declines," says Tillitt.

> "We found that thiamine deficiency is much more widespread and severe than previously thought."
> —Lennart Balk

Tip of the Iceberg?

So far, no such clear explanation has emerged for the other cases of thiamine-deficient wildlife that researchers have documented, even as the tally grows. In 2016, Balk showed that several other species across northern Europe, including blue mussels (*Mytilus* sp.) and eels (*Anguilla* sp.), were also suffering from the deficiency. He further analyzed correlations between deficiency-induced biochemical changes and long-term health effects, such as increased parasite infection and impaired growth (8).

"We found that thiamine deficiency is much more widespread and severe than previously thought," Balk says. Given its scope, he suggests that a pervasive thiamine deficiency could be at least partly responsible for global wildlife population declines. Over a 60-year period up to 2010, for example, worldwide seabird populations declined by approximately 70%, and globally, species are being lost 1,000 times faster than the natural rate of extinction (9, 10). "He has seen a thiamine deficiency in several differ phyla now," says Fitzsimons of Balk. "One wonders what is going on. It's a larger issue than we first suspected."

In this year's *Trends in Ecology & Evolution* "Horizon Scan of Emerging Issues for Global Conservation," a prominent group of conservation researchers also flagged increasing evidence of thiamine deficiencies in "a range of taxonomic groups" and in ocean waters as "a possible driver of wildlife declines" (11). "It seemed to us that this could be an important area that needs more research in the future," says William J. Sutherland at Cambridge University, one of the authors.

Critics, however, say more research is needed before declaring a global crisis or implicating a thiamine shortage. Contaminants such as lead and diseases such as botulism, caused by the bacterium *Clostridium botulinum*, can cause paralytic symptoms similar to those of thiamine deficiency. By blocking the production or release of acetylcholine, botulism causes a loss of muscle function and flaccid paralysis in the legs, wings, and neck. As a result, infected birds often drown. The animals typically ingest the botulism toxin directly or eat invertebrates, such as insects, that contain it. "The links between thiamine deficiency and impacts at the population level are not well established," writes wildlife ecotoxicologist Christian Sonne of Aarhus University in Denmark (12, 13). Sonne, who declined to comment for this article, has written multiple critiques of Balk's 2009 paper on wild birds.

Tonie Rocke, an epizootiologist at the USGS National Wildlife Health Centre in Madison, WI, notes that outbreaks of botulism are common in water birds, easily killing 100,000 individuals globally in a given year. Balk's study omitted tests needed to rule out other possible causes of death, Rocke says. "There was no postmortem examination of the birds. He just decided they died of thiamine deficiency." Rocke doesn't dispute Balk's evidence that the animals were suffering from low thiamine but does "take issue with the sweeping conclusion" that the deficiency was reducing population sizes, she says. Balk acknowledges that the birds, such as eider ducks, probably were suffering from other diseases and health effects—which he contends were most likely caused by the thiamine deficiency.

To bolster the argument that thiamine deficiency can be severe enough to wreak havoc on whole populations, Balk and a team led by Torsten Mörner, a wildlife pathologist at the National Veterinary Institute in Uppsala, Sweden, further examined eider duck reproduction. The team found that thiamine-deficient eider ducks on Vållholme in southern Sweden were

not laying as many eggs as expected: The average clutch size across 16 nests was 3.8 eggs per nest compared with a normal average of 4.6 eggs. Tests showed the eggs were deficient in thiamine as well.

In the field, Mörner also observed the unusual sight of hundreds of adult female birds alone on the water. Typically, females are surrounded by young birds, he says. On closer inspection, the team discovered the young birds survived only a few days after hatching. On average, the number of young birds was just 6% of the expected population size. The researchers monitored nests with cameras and saw that the eggs hatched normally and were not robbed by other birds. Instead, upon reaching the water, the weakened ducklings were preyed upon by herring gulls (14).

Not only did thiamine deficiency cause the ducks to lay fewer eggs, Mörner says, more than 90% of the ducklings that did hatch were lost through herring gull predation. "They just lay there and looked at the gulls, and of course they were eaten." Mörner's results do advance the evidence of harm to populations, says Rocke. "It suggests a link to population declines," she notes, adding the study also effectively rules out botulism as the cause of paralysis in the ducks. "Botulism was very unlikely in this case."

A Sea of Possibilities

Even as researchers agree to disagree about some specific examples of wildlife in distress, Balk and others are investigating what might be the root cause of such a widespread environmental thiamine deficiency.

Balk fears that a single pervasive factor, such as an atmospheric pollutant, may be depleting the environment of thiamine at its sources, including phytoplankton and bacteria, affecting the entire food chain. To see how far the problem reaches, he is now looking at upstream terrestrial wildlife such as elk (*Alces alces*). Balk is also investigating whether any of several pollutants might interfere with the oxidation, hydrolysis, or synthesis of thiamine.

Tillitt, too, is casting a wider net, searching for thiamine deficiencies in water birds in the Great Lakes and moose in Minnesota. Although he is confident that alewives were the cause of fish declines in the lakes, he's not certain what might be driving cases of thiamine deficiency seen in species elsewhere. "If there is a chemical that somehow affects thiamine, that could be extremely dangerous," he says. "It is very important for us to understand more about it."

But researchers need not invoke a pollutant to explain thiamine deficits, says Sergio Sañudo-Wilhelmy, an environmental biogeochemist at the University of Southern California in Los Angeles. Imbalances in phytoplankton and bacteria, both of which are primary producers of thiamine and other B vitamins, could account for the problem (15).

Sañudo-Wilhelmy has measured very low levels of B vitamins, including thiamine, in coastal waters around California. Other researchers have estimated similar scarcities in some areas of the open ocean (16). Warming waters due to climate change could explain the seawater vitamin scarcity, he says. Warmer temperatures speed bacterial growth, making the microbes consume more B vitamins than they produce—gobbling up the vitamins before the phytoplankton can take their share.

Sañudo-Wilhelmy suggests that a slightly different imbalance could have caused thiamine deficiencies around the Baltic Sea, where nitrogen and phosphorous pollution likely play a role. Large blooms of cyanobacteria—toxic blue-green algae—are common in the Baltic Sea during the summer because of eutrophication. Work from researchers at Linnaeus University in Sweden found that zooplankton—tiny aquatic animals that feed on phytoplankton—were starved of thiamine during such blooms (17). As a result, the vitamin no longer gets passed up the food chain to small fish that feed on the zooplankton or to top predators that feed on the fish, says one of the study's authors, aquatic ecologist Samuel Hylander. Sañudo-Wilhelmy says that the growing number of toxic cyanobacteria blooms occurring around the world could cause similar thiamine deficiencies elsewhere, suggesting another potential route for the problem to become widespread.

But Sañudo-Wilhelmy says it's too early to say if the ocean vitamin shortage he's documented along the Pacific coast is happening elsewhere. Limited data exist on dissolved vitamin concentrations across different geographic areas and during different seasons so the researchers lack evidence of broad trends in environmental vitamin levels.

If various kinds of imbalances are involved in causing thiamine deficiencies in different settings, the thiamine mystery might be broken down into more manageable pieces. For example, the deficiency in fish of the Great Lakes was managed by adding thiamine to the water in fish hatcheries; then the artificially bred fish were released into the lakes to boost populations. Eventually, alewife numbers also started to decline, and natural trout and salmon reproduction in the lakes began to recover (18). And if cyanobacterial blooms are playing a part, efforts to curb agricultural pollution could help prevent deficiencies from spiraling up the food chain.

This much seems clear: A one-size-fits-all fix for the wider problem is unlikely. Thiamine supplements are not a realistic solution to ameliorate whole ecosystems, says Balk.

Whether thiamine alone is the culprit or not, Balk and others are intent on collecting more clues in hopes of solving a mystery with major implications for multiple species and ecosystems. "The most important thing to do," says Balk, "is to find the cause."

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