Illegal catch. Fisheries inspectors such as these can't nail all violators, but genetic tests may prove another deterrent.

To Fight Illegal Fishing, Forensic DNA Gets Local

A new generation of genetic tests could give authorities a much better idea of exactly where fish have been caught

IN 2003, GENETICIST EINAR NIELSEN OF the Technical University of Denmark got an unexpected phone call from Danish fisheries inspectors. They suspected that a fishing vessel had violated its quota by catching too many cod from the North Sea. But the captain claimed that he had caught the fish legally in the Baltic Sea. The difficulty for the authorities was that the fish from both places were the same species, the Atlantic cod (*Gadus morhua*), and they look alike. Nielsen had been studying the genetics of the Atlantic cod, however, and he used DNA markers called microsatellites to show that the fish in question were very likely from the North Sea. A judge agreed, fining the captain $8800 and confiscating his $44,000 catch.

It was a rare victory against the massive problem of illegal fishing. For technical reasons, however, microsatellite tests for identifying the local origins of caught fish haven't been widely adopted. A €3.9 million European research project, called FishPopTrace, aims to now lay the groundwork for a different kind of test that could be broadly useful not only for enforcing fisheries regulations but also for catching fraudulent labeling of fish in supermarkets.

The consortium, which began in 2008, is exploring a range of possible techniques, such as protein patterns in fish tissue and the composition and shape of ear bones called otoliths. But the group is betting heavily on genetic variants called single-nucleotide polymorphisms (SNPs). That's because SNPs should lead to tests that are faster, cheaper, and easier to scale up than those based on microsatellites. The ultimate payoff is the advent of reliable and widely used tests to determine not just which species of fish has been caught but which particular local population it came from. "Being able to take [enforcement] to the population level is a big step forward," says Michael Hirshfield, chief scientist of the advocacy group Oceana, based in Washington, D.C., who is not a member of the consortium.

Online
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Podcast interview with author Erik Stokstad.

At a meeting last month in Madeira, Portugal, the FishPopTrace consortium discussed its results, and although most of its work is unpublished, other fisheries experts there say the use of SNPs is promising. "This is going to explode," predicts Kevin Glover of the Institute of Marine Research in Bergen, Norway. "They've shown it's feasible." In February, the consortium will present final results to policymakers, environmental groups, and others at a meeting in Brussels.

Fishing fraud

Although it's difficult to get reliable information on the extent of illegal fishing, researchers estimate that this shadowy business is worth as much as $23 billion worldwide each year. Sometimes the violations consist of boats catching more fish than allowed. More egregious is the taking of fish from areas that have been closed to recover from overfishing. Another problem is consumer fraud: the intentional mislabeling of fish as a more valuable species or as coming from a more desirable location.

Government agencies fight illegal fishing using a variety of tools. Some place observers on larger vessels to keep an eye on what's hauled in, checking that only fish of the right size are caught, for example. In the European Union, larger boats must have GPS units to make sure they're not fishing in off-limits areas. But observers can't follow all fishing vessels, and monitoring systems can be bypassed. So when it comes to catching scofflaws, forensic genetic approaches that distinguish between species have been critical. For more than 20 years, DNA tests have been used to identify species that have been illegally caught or mislabeled.

It is much harder to track a fish to its native population. DNA tests look for unique markers, and there are fewer genetic differences between populations of the same species than between species. This is particularly true in the oceans, where populations tend to be large and overlap, which can wash out genetic differences between locales. The microsatellite approach, used by Nielson for cod, can find markers unique to populations, but it isn't particularly efficient or always feasible. And the technique hasn't been scaled up as an enforcement tool, in part because of calibration issues between laboratories.

In December 2006, the European Commission put out a call for proposals to develop tools to better characterize populations of marine fish and improve the traceability of fish products. FishPopTrace, coordinated by Gary Carvalho of Bangor University in the United Kingdom, won the competition.

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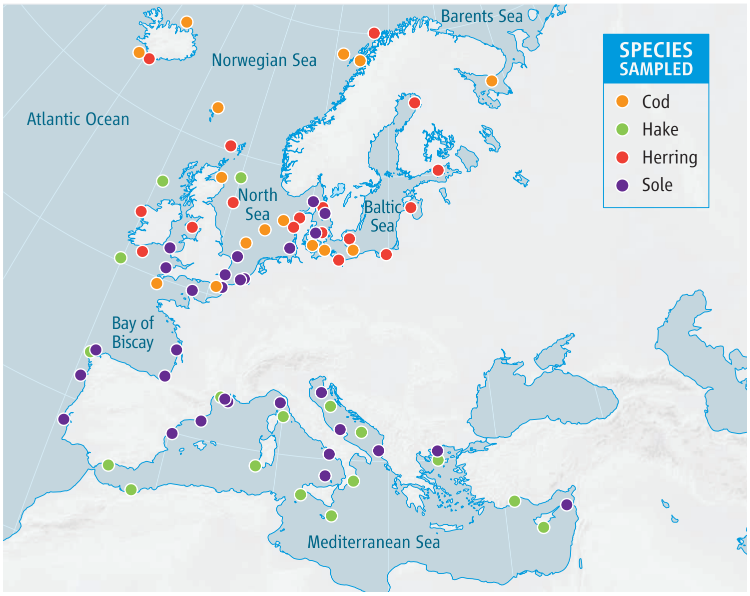
FishPopTrace is focusing mainly on SNPs, specific nucleotides in a DNA sequence that can vary and thus can help tell apart individuals or species. SNPs are much more common in the genome than are microsatellites, which improves the odds of finding patterns specific to different populations. This approach has been successfully used by managers to distinguish among Pacific salmon, whose populations spawn in particular streams and rivers and tend to be more genetically distinct than fish that spend their whole lives in the ocean.

To see whether they could expand the technique to exclusively marine fish, Carvalho's team picked four economically valuable species as test cases: Atlantic cod, European hake, common sole, and Atlantic herring. These species represent different life habits and geographic ranges, and all suffer from illegal fishing. The researchers obtained samples from about 50 fish from each of 20 populations around European waters, then team members at Aarhus University in Denmark sequenced the samples and identified possible SNPs. By June 2009, they had created one "SNP chip" for each of three species: sole, hake, and herring. (Canadian researchers had already created a SNP chip for cod to assist in aquaculture research.) These DNA-covered microchiplike devices can test the identity of 1536 possible SNPs. For each population, they tallied up the frequencies of all the SNPs, creating what they hoped would be a diagnostic pattern.

The next step was to find out if the SNP chips could accurately and reliably distinguish among samples from different populations: a North Sea cod from a Baltic one, as Nielsen had done with microsatellites, for example. An important part of this process was figuring out how few SNPs were needed; minimizing the number will lead to cheaper, simpler tests.



Population control. Genetic analysis of fish populations from across European waters is revealing patterns that can be used to identify the source of fish.

SOURCE: FISHPOPTRACE

Fish and chips

The researchers investigated questions relevant to fisheries and to European consumers. A common concern for the latter is the source of Atlantic cod. Fish from the Baltic are worth less because they tend to have lower quality flesh and higher levels of contaminants. The cod team used its SNP chip to examine, without knowing the source, samples from both locations. By looking at 20 SNPs, the researchers correctly identified every sample's origin. With just 10 SNPs, 96% of the samples were still correctly identified. "The results look very promising," Nielsen says.

The SNP chip for sole (*Solea solea*) also performed well. This flatfish fetches the highest price of the four species and is severely overfished in Europe. A key question is whether sole from the North Sea can be distinguished from populations in the Mediterranean, which are considered to be higher quality. Just one SNP could reveal which sole was which with 96% accuracy. "Technically, it's a piece of cake," says Filip Volckaert of the Catholic University of Leuven in Belgium.

European hake (*Merluccius merluccius*) has been a contentious species because of differing regulations. Atlantic hake must be 27 centimeters long to be legally landed. But in the Mediterranean, vessels can catch hake that are only 20 cm. So fishing vessels in the Bay of Biscay sometimes catch smaller fish and then misreport their origin as the Mediterranean. The FishPopTrace team showed that just 10 SNPs could reveal the origin of hake with near-perfect accuracy.

The most demanding test case was herring. An abundant, migratory species with a wide distribution, European herring have long resisted attempts to tease apart genetically distinct populations. By looking at SNPs, however, it was possible to accurately distinguish herring, such as those in the northeast Atlantic and those in the North Sea—a goal important to a joint E.U.-Norwegian management plan. But more SNPs were required to identify fish from various sources around the United Kingdom, where there is substantial misreporting of catches. Further refinements could reduce the number of SNPs, says Sarah Helyar, a postdoctoral researcher at Bangor University.

FishPopTrace is also checking the rate at which local fish populations evolve, a determinant of how long their SNP tests may be useful. For each species, the teams are using computer models and looking at decades of tissue samples to see if specific fish populations will maintain the same patterns of SNP frequencies over time.

Rob Ogden of TRACE Wildlife Forensics Network, a nonprofit in Edinburgh, U.K., is validating the consortium's new genetic tests. This involves confirming that they work under a range of conditions and creating standard operating procedures for other labs to use. "It's absolutely essential if you want to move from an academic environment to testing," Ogden says. And there is growing interest among regulators; a major E.U. fishing law passed last year mentions genetic tests explicitly as possible enforcement tools. "I'm pretty convinced that they will be much more extensively used," says Jann Martinsohn, who studies marine policy at the European Commission's Joint Research Centre in Ispra, Italy.

How SNP-based tests are ultimately used—by port inspectors, for example, or commercial labs testing frozen fillets—will depend on their accuracy, cost, and ease of use. SNPs that end up on the witness stand may remain the exception, Ogden predicts. He says that just the act of random testing will encourage compliance, at a fraction of the cost of litigation.

That view matches Nielsen's courtroom experience, which was the first introduction of genetic testing of populations to the Danish fishing fleet. "The case did certainly have a deterrent effect," he says. "They know that we can test, so they are probably less likely to put themselves in situations where testing could reveal fraud." The task now is to create genetic tests that will broaden the scope of this deterrence.

–ERIK STOKSTAD