What droppings can tell us

June 22 2015

Eurasian otters (Lutra lutra. L.) are only rarely seen in daylight. One reason why our knowledge about the threatened species is still very limited. Credit: André Künzelmann, UFZ

If you want to find out about the shy Eurasian otter, its droppings are a fascinating source of information. By isolating DNA from otter droppings – known as spraint – researchers can not only identify

individual animals but also estimate the size of the population. But it's important to know how to interpret the information correctly. A team led by Simone Lampa from the Helmholtz Centre for Environmental Research (UFZ) in Leipzig reports on the benefits and drawbacks of the method in science journal *PLoS ONE*.

Eurasian otters are elusive creatures. Only rarely do researchers manage to observe these shy animals directly in the wild. Trampled vegetation at the water's edge, slides into the water and accumulations of spraints are often the only clues to their presence. So figuring out how many otters there are in a given locality is very difficult. "But with threatened species, this is very important information," says Simone Lampa from the Department of Conservation Biology at UFZ. Only with reliable figures can we tell whether protective measures are having any impact or whether new problems are emerging.

In the case of the otter, all the signs seem to indicate a positive trend. In the 19th and 20th centuries, hunters massively reduced otter numbers and the species completely disappeared from many parts of central Europe. But since the 1990s, its distribution has gradually been increasing again. Most of the clues have been provided by analysing spraint. The animals leave their droppings in certain places in order to communicate with other otters. The standard method of otter research is therefore to look for these marks and conclude whether or not otters are present in the area.

However, this tells us nothing about the number of animals present, because several otters may be living in the same area and leaving droppings in the same places. What's more, otter droppings look very similar to those of the introduced North American mink, which can easily lead to confusion. So for a number of years biologists have been using genetic analysis, which allows them to identify each individual animal. Spraint contains intestinal cells from which the genetic material

DNA can be isolated. The DNA contains certain segments typical of otters, and the length of these so-called microsatellites varies from one individual to another.

These unique genetic 'fingerprints' are analysed by Simone Lampa and her colleagues in the biosphere reserve of Oberlausitzer Heide- und Teichlandschaft in eastern Saxony. "The otter never disappeared from this area," she explains. "And from here it's spreading westwards." This makes it even more interesting to find out more about the development of this population. The researchers at UFZ spent six years collecting and genetically analysing otter spraint. They discovered that around 20 otters are living in the investigated area of about 35 square kilometres. The number fluctuates from year to year depending on how many ponds are being farmed and therefore offering rich yields of fish.

To arrive at these figures, the researchers don't need to find spraints from each individual animal. Instead, on each collection trip they determine how many spraints come from known individuals and how many from unknown ones. Using this ratio, they can apply mathematical formulae to estimate the probable size of the population. This method, known as non-invasive genetic mark-recapture, has become increasingly popular in conservation research in recent years.

During their investigations, however, the UFZ team have discovered some problems with the technique. The method is based on the assumption that every otter exhibits the same marking behaviour. "As far as the amount of spraint is concerned, that is correct," says Simone Lampa. But there are differences in quality. Otters generally leave different types of spraints: as well as dry droppings and those covered with a slime layer, they also produce lumps of anal secretions, so-called anal jellies. These are often found on rocks and in other exposed places where they are easy to spot. Because it is also easier to isolate DNA from this material than from normal droppings, many studies prefer the

analysis of this type of sample.

But the Leipzig-based researchers have now discovered that male otters produce anal secretions more frequently than females. As a result, spraints from males are investigated more frequently than spraints from females, distorting the results. Consequently, several otter studies conclude that investigated populations have a surplus of males which probably does not exist in reality. "We shouldn't focus too much on anal secretions," says Simone Lampa. Other spraint samples are more time-consuming to collect and analyse, but they yield more representative results.

Another problem is that the analysis of the DNA sequences does not always produce 100% accurate results. Otter DNA passes through the extremely aggressive environment of the animal's digestive tract, and components may be easily misidentified. Just one mistake of this kind could result in a known individual being identified as an unknown one. This creates 'phantom' animals in the data which do not actually exist. Although Simone Lampa has analysed every sample between three and 26 times to minimise such errors, three or four 'virtual' otters remain. Computer models are available to eliminate errors of this type from the data. "It's essential to use these models in genetic mark-recapture studies," she says. Otherwise the size of the population will be overestimated, which in the case of threatened species like the Eurasian otter could be fatal.

The animals themselves don't make the researchers' job any easier, either. Although the analysed spraints are left where they are found, an otter seems to notice if someone has been tampering with them. Animals whose spraint has been analysed display particular eagerness to mark, adding another error to the statistics which needs to be eliminated. "But even when you take all of this into account, genetic mark-recapture studies are a very good method of finding out more about otter

populations," says Simone Lampa. For the elusive otters, it's getting harder to keep their secrets to themselves.

More information: "Non-Invasive Genetic Mark-Recapture as a Means to Study Population Sizes and Marking Behaviour of the Elusive Eurasian Otter (Lutra lutra)." *PLoS ONE* 10(5): e0125684. journals.plos.org/plosone/arti … journal.pone.0125684

Provided by Helmholtz Association of German Research Centres