The Rufford Small Grants Foundation

Final Report

Congratulations on the completion of your project that was supported by The Rufford Small Grants Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Alejandro Travaini
Project title	Patagonian wild foxes warning against poisoning by Conditioned Taste Aversion
RSG reference	30.06.09
Reporting period	Complete period, January 2010 – June 2011
Amount of grant	£5620
Your email address	atravaini@gmail.com
Date of this report	July 11, 2011

1. Please indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
To test if levamisole hydrochloride (the aversive) added to pet food baits induced learned aversion to untreated baits in two Patagonian free-living foxes			X	This aversive performed well in generating learned conditioned taste aversion. We were able to test this in only one of the two fox species, the Grey fox (*Pseudalopex griseus*). This was because the second species, the Culpeo fox (*Pseudalopex culpaeus*) was in an extremely low abundance in our original study area and also in a second protected area from our region, Southern Patagonia. Visitation rates to our experimental units by the Culpeo fox were too low to perform any analyses. Of course, we have no argument against considering similar potential results for the second fox when properly experimenting on it.
To test if levamisole hydrochloride masked within an ion interchange resin (Amberlite IRP-64) induced learned aversion to pet food baits in two fox species		X		We chemically obtained a resinate with the masked aversive (no smell or taste available to the target foxes). Nevertheless the amount of resinate needed to generate illness and learned aversion in foxes was too high in relation to the rest of the bait (pet food), so we had an unexpected palatability problem. This was because foxes can identify treated baits because of the high proportion of resinate in the mix, besides it has no smell or taste. An additional problem, partially obscuring our results, was the caching behaviour (take and hide the bait without consumption) observed in wild foxes.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).

Our former surprise was on the high expectation we had on the selected procedure to mask the smell and taste of the aversive. The use of the ionic interchange resin as a masking matrix don't performed well with our 30 g baits, because of the resinate proportion needed to generate temporary discomfort in foxes. We need to include as much as 40 % of resinate (resin plus aversive levamisole) in bait composition to attain recommended doses for our foxes. That makes a bait of different palatability, which partially obscured our results. After the field trials we actively searched technical assistance to prepare a different compound to mask the aversive. This was the microencapsulation of the levamisole. This is a chemical technique used by the pharmacological

industry and it was frequently recommended in the literature as a potential procedure to generate aversion. Nevertheless, to our knowledge there was no published experimental field with any wild species. Microencapsulation is a very specific technique. In our case, we need that the aversive (levamisole) reaches the stomach of the target species and dilute as fast as possible, to generate temporary illness shortly after consumption of the treated food and generate a negative association to it. Microcapsules should not dilute within the treated food. We obtained some help from a pharmacological laboratory from Buenos Aires. At this point thy have obtained levamisole granules (small particles) and now they are working on the specific coating for the previously mentioned performance.

Foxes used to cache (stole and hide) an important proportion of treated baits. We noticed that during our field work, by not registering the expected reduction in treated bait (those containing the aversive) consumption, assuming that the aversive was 100 % efficient in producing stomach illness in foxes. During field work we observed foxes performing this behaviour. The consequences of this were a reduction in statistical significance. The alternative to avoid the effect on bait palatability would have been to prepare a 120 g bait (our original bait was of 30g), which would had other unwanted consequences, formerly that would be too big to certainly assume that foxes would eat completely during field trials, and would not be accepted later for extended use in protecting foxes against illegal poisoning, the former application of our project. A potential way to consider this caching behaviour in future experiments is the use of small radio transmitters inside treated baits, and search for them after the fox takes it from the station. The former limitation of an experiment like this is its cost. I think that at least one fourth of bait stations should have a bait with radio transmitter, that is about 25-30 radio transmitters.

The Monte Leon National Park has an important guanaco (*Lama guanicoe*) population, the former native prey of cougars (*Puma concolor*). This could be the reason for the high cougar density inside the area and consequently a very low abundance of the Culpeo fox, the biggest of the two fox species, but an extremely high abundance of he smallest Grey fox (mesopredator release theory). We confirm this by receiving too many cougar visits to our bait stations, but too few from Culpeos. This was completely unexpected for us. To overcome this we evaluated Culpeo abundance at the Monumento Natural Bosques Petrificados, another national park about 500 km away from Monte León. During April 2010 we activated 18 bait station lines for three consecutive nights. We repeated this during September 2010. On both occasions Culpeo visits were too low as to run the aversion protocol with them.

Many of all these shortcomings are now being considered for research with captive foxes at zoos. Unfortunately, the most important zoos from Argentina have other fox species in captivity, but we will try to perform captive trials anyway.

3. Briefly describe the three most important outcomes of your project.

Our experimental design, probably a bit complicated, performed well and allowed us to statistically confirm the generation of true learned aversion with pure levamisole and identify the palatability problem with the masking resin. We are still interested in microencapsulation because of the potential use of aversion in the conservation of endangered species, like ground nesting species, where absolute aversive masking is welcome.

We truly generated aversion to wild Grey foxes in natural conditions. This encouraged personnel from the protected are for our former but other potential uses. As an example, they were worried

because gull predation on a highly vulnerable bird species nests, and aversion could be an interesting option to reduce that.

We discarded the potential use of the resin as a masking substrate and started inquiring on an alternative procedure, the levamisole microencapsulation. Microencapsulation is an expensive procedure that should be developed for each particular substance and the chemical environment where it will be used. We contacted a pharmacological laboratory in Buenos Aires and they are experimenting with our substance. We need the levamisole to be used in many types of baits and that the micro capsules are diluted in the target species stomach, not before (in the bait matrix or in the fox mouth, for instance). Because of illegal poisoning use to be done in hen eggs by ranchers, and the already mentioned alternative to protect egg predation by gulls , they are working in a microencapsulation that don't dilute inside an egg, so it could be experimentally used following a similar procedures as our original design. At this moment they have prepared a granulated aversive, the next phase includes the preparation of a chemical coating to those granules so they don't dilute inside the eggs but dilute in the target species stomach.

4. Briefly describe the involvement of local communities and how they have benefitted from the project (if relevant).

Our results have two main recipients, the responsible authorities from protected areas and the sheep ranchers. During the field work at Monte León National Park we offered a brief talk to the guards and other people they invited, and we participated on a radio programme at the nearest town. People from the protected area were interested and participated during the whole experiment with us; they have considered other potential applications of aversion as a conservation tool (for instances, cougar predation is a new but important problem for the penguin colony inside the protected area). Ranchers, following your requirement to be completely honest, are always reluctant of any non lethal method because they hate foxes, irrespective of having real predation problems over their herds. Nevertheless some of them offered their properties for field evaluations of our methodology as a mean to selectively control the target species but also as a mean to generate some aversion against sheep predation. Some interesting work was done with coyotes in California. For future experiments like these, we should use pure levamisole, and test the microencapsulation option. From our results we should discard the use of the resin. I want to highlight that the poor performance of the resin was a very bad surprise for us. It was the most expensive component of all our work and it does not performed as expected based on the literature. Nevertheless, to our knowledge, this was the first time it was used in field and wild free-living carnivores.

5. Are there any plans to continue this work?

Yes there are. We incorporated a doctoral biologist, with a CONICET fellowship. She participated in the aversion field experiment at Monte León and is preparing some test with captive foxes. We planned to have some results by this time but unexpected problems with captive foxes at the zoo have delayed those results. We plan to perform at least one trial inside a sheep ranch, to generate aversion on Culpeo foxes as a mean to reduce lamb predation. For that we will use the pure levamisole but we would also like to test the microencapsulated substance. Finally, we have one additional interest, to test the microencapsulated levamisole to protect the Red cauquén (*Chloephaga rubidiceps*) from fox predation at their breeding areas. This is now an endangered species because of human persecution. This last interest involves some trials with captive foxes, to evaluate the potential of generating aversion to treated eggs, and then some field trials involving

artificial nests to reduce predation during the breeding season. All these generated after the Rufford support.

6. How do you plan to share the results of your work with others?

Our results have many application possibilities, mostly on species conservation and also on selective predator control. The alternatives mentioned above, evaluating the protection of some endangered species nests, are good applications of our preliminary results.

We prepared a brief report for the National Park authorities after completing the field experiment at Monte León National Park.

The results of the field experiment at Monte Leon National Park were presented at the SAREM (Sociedad Argentina para el Estudio de los Mamíferos) congress during November 2010. A copy of the poster is included with this final report.

Based on the previous report I submitted you, we are now preparing a manuscript for the publication of the field trial results.

7. Timescale: Over what period was the RSG used? How does this compare to the anticipated or actual length of the project?

The experimental field work was performed as originally proposed. It was completed during March 2010. Before that we made all preparative's, including the laboratory preparation of the resin (at a chemical laboratory from the Universidad de Mar del Plata), the elaboration of about 2500 baits and the preliminary survey of the study area, including the sampling unit selection for all three experimental treatments and the control.

Our original plans considered a similar proportion of both fox species in the study area, the absence of one of them forced us to consider another study area where to duplicate the experiment. The alternative area, the Monumento Natural Bosques Petrificados, about 500 km away from Monte León has a bit higher Culpeo presence, but not as much as to repeat the whole one month experiment. As an alternative we considered some captive trials inside zoos. Unfortunately, there are only one Culpeo at the Buenos Aires zoo, and no one at other ones. Anyway we are preparing some captive trials with another fox species, mostly aimed to experimentally evaluate the final size the bait should have to effectively mask the resin within its meat matrix, and if possible, the caching behaviour of foxes.

The other problem we had, that of the resin, is still in a resolution phase. We are working on the elaboration of the levamisole microcapsules. As I told you before, a pharmacological laboratory from Buenos Aires is helping us in this preparation. They reacted with enthusiasm when we told about the potential conservation application.
These were the main reasons because we don't consider as finished our experiment just after completing the field trial.

8. Budget: Please provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Edibles	2.750.00	1.650,51	1.099.49	We plan to use the difference for the payment of extra levamisole and resin, or the microencapsulation inputs
Bibliography	180.00	116.68	63.32	We bought some books and we hope we can buy some additional one during the doctoral development of the person I mentioned before
Publication costs	220.00	0.00	220.00	Our manuscript is still at an elaboration phase, this money will be used if the manuscript is accepted for publication in a journal with charges. Many related journals from USA (e.g. Journal of Wildlife Management) charge as much as 150 Us dollar each printed page.
Trips and maintenance	1.420.00	1.062.05	357.95	We are still using this surplus for the person who is preparing the captive trials at one zoo in Batán, province of Buenos Aires
Equipment	1.050.00	250.00	800.00	We received the video cameras from IdeaWild (USA), so we don't buy them. We bought a laboratory shaker, for the resinate preparation (unfortunately probably we will no use it much more). We didn't use the fish bait, as originally planed, so we didn't need the freezer.
Total	5.620.00	3.079.24	2.540.76	

We prepared an Excel spreadsheet with all expenditures. We used an exchange rate of 6,24 Argentine pesos for each Sterling Pound, taken from the internet at the beginning of the work. We plan, if possible, to continue using the rest of the money in the experimental preparation of the microencapsulation (at least its experimental preparation will be a result of this proposal) and the captivity trials aimed to supply the shortcomings produced during our field trial.

9. Looking ahead, what do you feel are the important next steps?

An important step is to complete and submit the manuscript with our field results.

The use of microencapsulation as a mean to deliver aversive to wildlife is widely encouraged in the scientific literature. Nevertheless there was no experimental use of it. The use of the resin was

tested in laboratory conditions, where there was no volume or weight limitation in the offered food. Our bait should be used in the field and its size is a limiting problem, it could not be too big because of many logistical constraints. We also planned to use our results on future field experiments, but using those substrates preferred by ranchers (eggs, pieces of meat, wildlife carcasses, etc.), the resin will not be a good option (the amount of resin needed to generate egg aversion to a big fox would make the egg unpalatable and easily identified as treated). That is, because it is needed a high volume of it to attain aversive doses for wild predators. The alternative of microencapsulation should be an important step in our research.

Finally, once fitted the logistical problems just described, we should perform at least two different kinds of field tests. One of them, to generate predator true aversion towards lambs and sheep, or the use of aversion to reduce non target species damages during predator control campaigns. And the second group aimed to protect endangered species from predation, like protecting red geese predation from Culpeo foxes during their nesting season, or penguins from cougar predation.

10. Did you use the RSGF logo in any materials produced in relation to this project? Did the RSGF receive any publicity during the course of your work?

We printed some stickers with the Rufford logo and putted them on all the materials and equipment bought with the Rufford money. We also printed the logo in the poster presented at the SAREM meeting last year.

11. Any other comments?

I would like to mention that my delay in sending this final report was because I wanted to include at least one captive trial and a more advanced status on microencapsulation. Unless they were not included in the original proposal, they were palliatives for the absence of results for one of the fox species and the low performance of the resin-levamisole compound. Both issues are being under development and will be informed when completed. Remaining funds are in use for these purposes. I would like to ask for a little some more time to spend all remaining money.

I really appreciate your initial paragraph encouraging us to also comment our negative experiences within the framework of the proposal. I will be glad to provide any additional material you request, as a detailed spreadsheet of our expenses and the original vouchers.

Finally, it is in our intention to submit a prosecution application to the Rufford Small Grants, for the development of both the captive but formerly the field trials outside protected areas just described in the present report.

I want to thanks the Rufford Small Grant support, it was an excellent starting point for this line of research.