

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. 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. 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	Elizabeth Grace Tunka Eronat
Project title	Can opportunistic sampling provide information for conservation of sharks and rays? Chondrichthyans Population genetics and breeding ecology in Turkish seas
RSG reference	17514-1
Reporting period	Final report
Amount of grant	£4950
Your email address	tunkaeronat@hotmail.com
Date of this report	28 th April 2016

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 obtain as much species and individuals as possible by opportunistic sampling methods		x		Due to having long coastline in Turkey and limiting time, we could not reach the all part of the Turkish Coasts, especially eastern part of the Black Sea. But we achieved to get samples from Black Sea to represent the structure in common species
To establish a network with fisherman and fisherwomen		x		Due to having long coastline in Turkey and limiting time, we could not reach the numbers of fisherman/woman for effective network. But, we achieved to get successful network for main geographical marine regions in Turkey.
To produce information and data on genetic diversity, feeding habits and reproduction biology on chondrichthyans of Turkey		x		Due to small sampling and some samples send frozen information on the species reproduction biology was limited. Also there were some problems during DNA sequencing which have delayed us and made the final report finalisation finish later than expected.
To produce information on reproductive biology of chondrichthyans of Turkey		x		Due to long distance, most of the samples were sent to us frozen. Frozen tissues don't give effective results for histological examinations. Even though to this problem, other features of reproduction biology were recorded and morphometric measurements and genetic analysis were fully achieved

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

Weather conditions limited us to travel as far as plan but this problem was solved by widening the network and finding more fisherman-fisherwomen from un-reached parts. Also because of the harsh weather conditions fishing activities were limited for many fisherman-fisherwomen. On the other hand even when they fished the bycatch chondrichthyans were not as much as they reported to be which a good thing for endangered species is. Since most of the samples were either send, preserved or brought frozen to us histological methodologies were not used as efficient as expected. But even though histology was not used as much as possible information of the species reproduction biology was gathered such as length at maturity, reproductive organs weight and where possible pup amount, weight and length of the pups. Also the DNA sequencing took more time than expected even though we have send our DNA sample long before unfortunately there were unexpected

delays.

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

- 15 species were obtained throughout the project. Critically Endangered *Squatina oculata*, *Isurus oxyrinchus*, *Gymnura altavela*; Endangered *Carcharhinus plumbeus*, *Rhinobatos cemiculus*, *Rhinobatos rhinobatos*; Vulnerable *Mustelus mustelus* and *Mustelus asterias*; Near Threatened *Raja clavata*, *Dasyatis pastinaca*, and *Myliobatis aquila*; and a Data Deficient species *Raja radula*. Obtaining these some rare and threaten species we were able to shed some light at least to these species genetic diversity and some aspects of their biology.

When the stomach contents were identified and analysed it was observed that shark species mainly consume bony fish but some species preferences were either towards crustaceans (*Mustelus mustelus*) or cephalopods (*Scyliorhinus canicula*). In batonoid or ray species the main preference was observed to be crustacean species and some molluscs as their body type also suggest but we have also identified that some species primary prey item is bony fishes (*Raja radula*, *Torpedo marmorata*).

The knowledge on the reproduction biology of the species showed that species *Mustelus asterias* give birth between February and March, and the species length at mature is 97.3 cm in Mediterranean coasts of Turkey (Figure 3). In *Isurus oxyrinchus* case we observed that the female specimen we obtain was in its second stage of in mature; developing according to ICES (2012) report. There were follicles in different stages of development, the histological sections can be seen in Figure 1a. In *Scyliorhinus canicula* it was observed that the species mating behaviour happens during spring-late spring but according to literature this species have an active reproduction system year long though we only were able to observe in spring-late spring. This could be the result of obtaining large or mature specimens during this period and obtaining small in mature, maturing individuals in other times and the photos of histological section of a maturing gonad can be seen in Figure 1b. We also observed that *Raja radula* also spawns between late spring-late summers. We were able to identify this since we only observed egg capsules almost mature and females who have already spawned (which were also identified according to ICES (2012) report). In species *Torpedo marmorata* it was observed that the maturation starts or length at maturity was observed to be at ~ 34 cm. In species *Rhinobatos cemiculus* it was observed that Gediz Delta was a spawning and nursery area for this species and the spawning occurs in late summer-early autumn. After the spawning the species neonates stay and grow steadily.

Another interesting observation was that there were either predation or parasite damage on the fins and sexual organs (on males) of *Rhinobatos cemiculus* youngs. The damage made it difficult to determine the sex of the individuals sometimes. The photos of this damage can be seen in Figure

- This type of damage was observed on the individuals from October till the end of the projects. Tissue samples were stored at -20°C until DNA extraction. DNA isolation was performed with Tissue and Cell DNA Purification kit (GeneMark, Taiwan) and DNA quality and quantity were measured with NanoDrop spectrophotometer (Nanodrop, Thermo Fisher Scientific, USA). All the DNA was suitable for PCR (Polymerase Chain Reaction) analysis. Four different primer sets were used to amplify 3 different regions that commonly used for phylogenetic analysis (Table 1). Generally CytbL14841-CytbH15149 and COI_{fish}ff2d-COI_{fish}fr1d primers

pairs gave applicable bands for sequence analysis (Figure 3 and 4). PCR products purified and send for sequence analysis. The analysis have taken longer than we expect due to some technical problems. After getting the sequences of the selected gene regions Neighbour Joining (NJ) and Maximum Likelihood (ML) phylogenetic analysis will be performed and the results will be shared via scientific article and presentation in conferences. Updated information will going to be shared in the webpage of the project on the website of The Rufford Foundation.

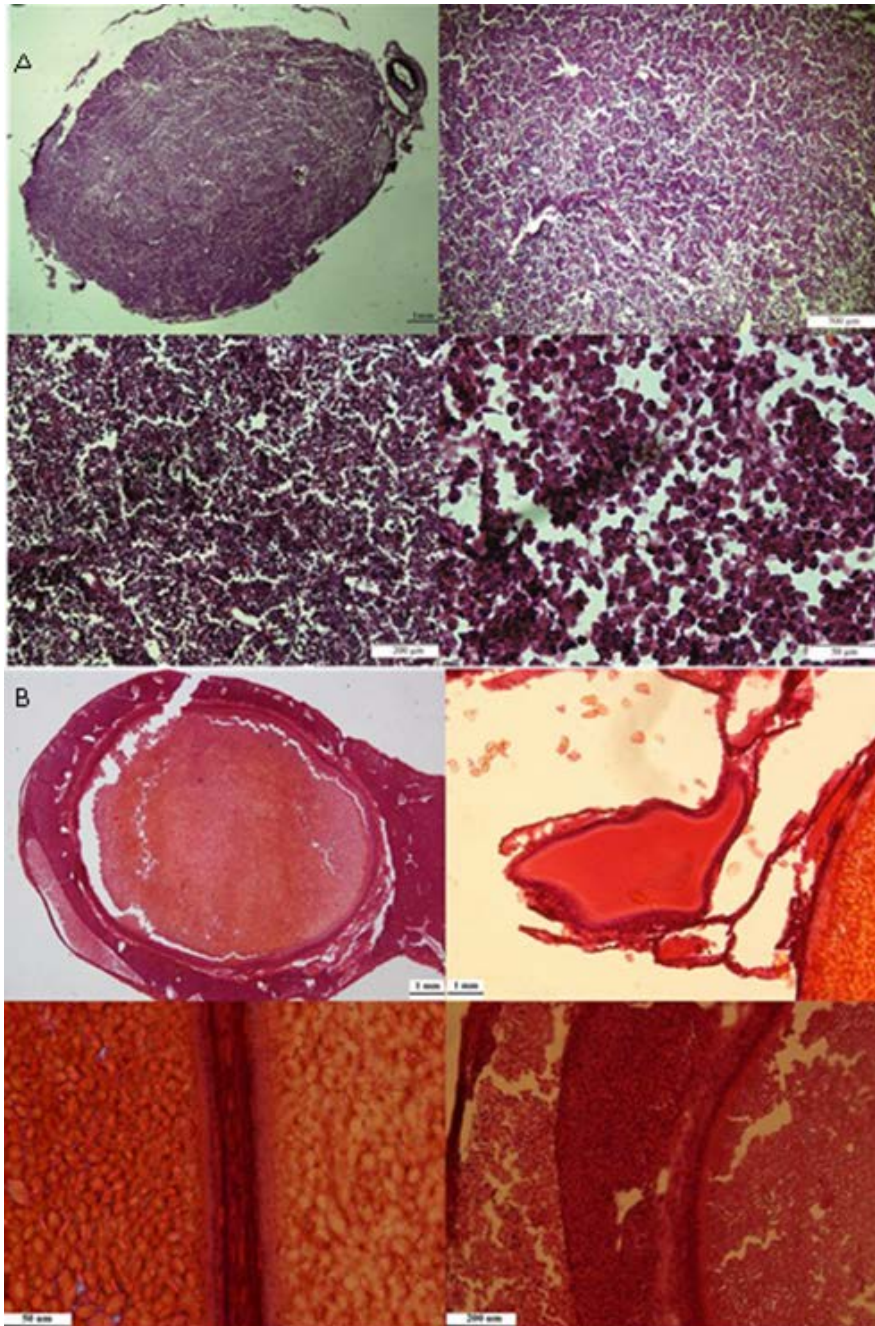


Figure 1. A: Histological section of a *Isurus oxyrinchus* gonad. From top to bottom and left right they are all showing that the gonad belongs to a second stage immature, B: Histological section of *Scyliorhinus canicula* egg and gonad. From top to bottom and left right an egg surrounded by connective tissue, an undeveloped egg next to a developing egg, two developing eggs with connective tissue in between, an egg with different type of connective tissue and cells surrounding it.

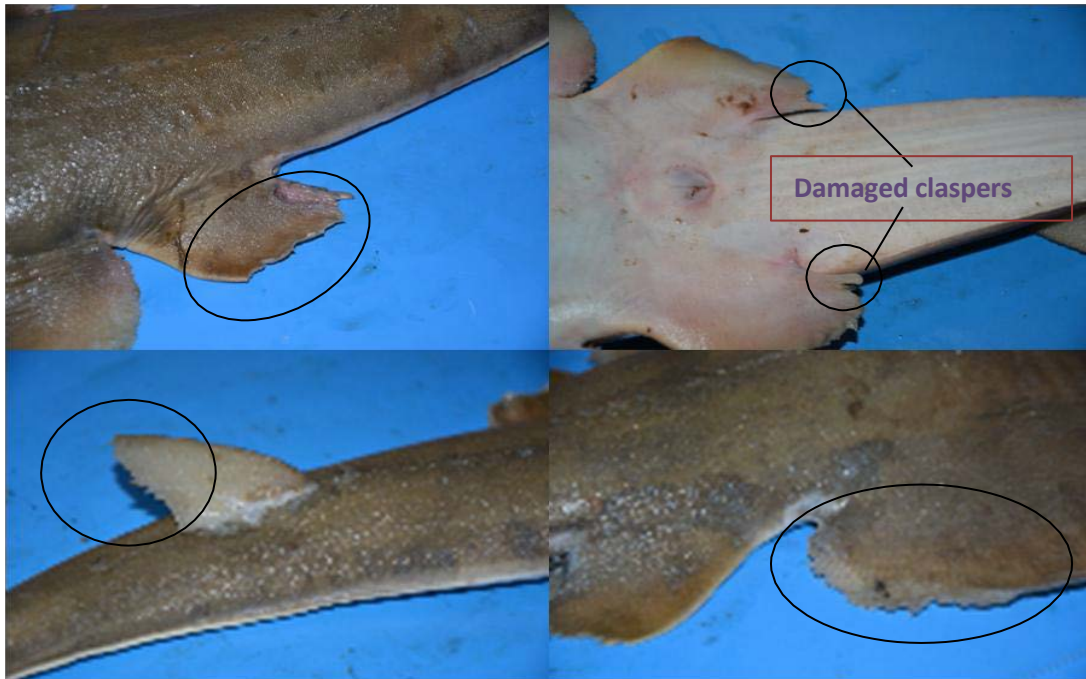


Figure 2. Evidence of the damage on the fins and clasper of *Rhinobatos cemiculus*.

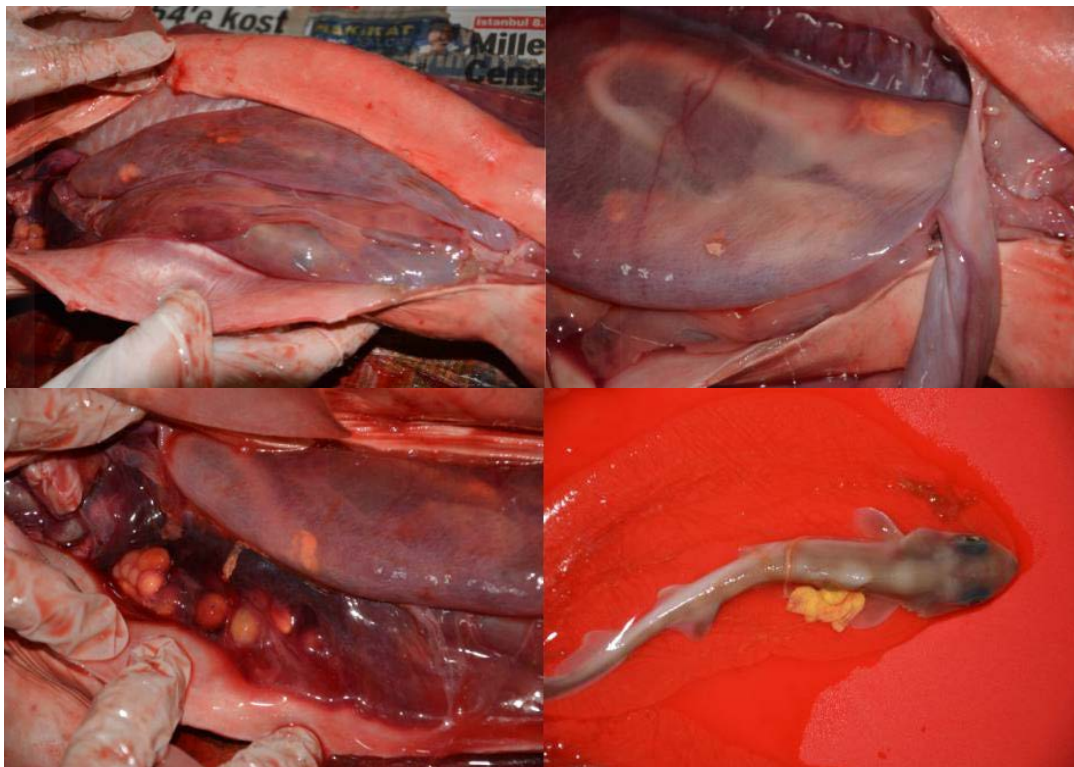


Figure 3. Photo of the pregnant *Mustelus asterias* which was bought from fisherman. Babies can be seen easily.

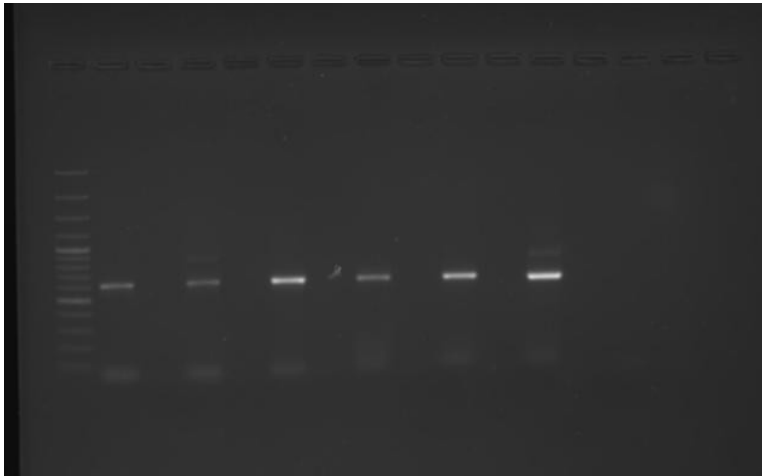


Figure 4: PCR results with the primers CytbL14841-CytbH15149

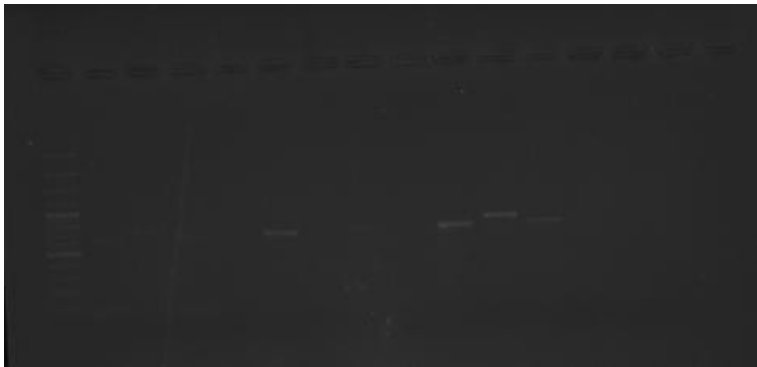


Figure 5: PCR results with the primers COI fishff2d-COI fishfr1d

Table 1: The amplified regions of elasmobranch species and their PCR results.

Primers	CytbL14841-CytbH15149	COI fishff2d-COI fishfr1d	L2510-H3080	LCO1490-HCO2198
Gene region	Cyt b	COI	16S	COI
1	+	+	+	-
2	+	-	-	-
3	+	-	-	-
4	+	+	-	-
5	+	+	-	-
6	+	+	+	-
7	+	+	-	-
8	+	+	+	-
9	+	+	-	-
10	+	+	-	-
11	+	-	-	-

12	+	-	-	-
13	+	-	+	-
14	+	+	-	-
15	+	+	-	-
16	+	+	-	-
17	+	+	-	-
18	+	+	-	-
19	-	-	-	-
20	+	-	+	-

- A wide network of fisherman-fisherwomen was established which worked successfully for most part of it. During these interactions the fisherman-fisherwomen were really interested on learning these species biology. They were amazed to hear that there were wide range of elasmobranch species in Turkey and learn that some of the species they caught which they tough were bony fish or in their terms "regular fish" were actually elasmobranch.
- Also by sharing the result with national and international symposiums people from all around the world were able to take a glimpse of the species inhabiting eastern Mediterranean and Turkish waters and some information on not just popular species but some other uncommonly known species biology and biodiversity. Another important outcome was that there will be at least 2 scientific articles published from the result we have obtain which will be another valuable resource on chondrichthyans biology especially for eastern Mediterranean. Where among these chondrichthyans are mostly Critically Endangered, Endangered or Vulnerable.

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

Even though the network was focused on fishermen and fisherwomen we got help from local people of the areas we visited such as market owners, local elders and even students were willing to help us with our sample collecting. They were also eager to hear and learn about the species in their region and some information on their biology. Especially, where we came across, the children of the people we met were really amazed to learn about sharks and rays and to be able to see and sometimes touch to an individual without fear. Also fisherwomen and fishermen were interested to read and listen about the species in their area and learn about species status and need for conservation.

5. Are there any plans to continue this work?

Yes there are plans to continue since a network has been already established and we are more experience we hope to focus on the areas which were not as satisfactory as others. Especially now we have a data set of biodiversity on the species we obtained and some information on their genetic diversity, feeding and reproduction biology it is important to have a continues flow of information feeding this data set for feature local small scale management or for large scale conservation measures.

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

We are planning to publish our results at least two articles in international journals. We are also going to upload genetic information of the species to GenBank. We have already presented two posters in one national symposium and one international symposium. One of the poster was on a neonate female *Isurus oxyrinchus* individual caught from Foca, central Aegean Sea, and the other poster was about the project which was presented in annual European Elasmobranch Association (EEA) symposium in Portugal (Figure 2). We are also planning to present the result as oral and poster presentations in 2016 EEA symposium. Also we plan to publish at least two articles especially one on *Isurus oxyrinchus* since it is a rare species for these coasts and there were two specimens obtained

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

The grant was used through out the length of the project. As proposed first we established the network of fisherwomen-fisherman. At the same time samples were collected by visits and some were sent by the fisherwomen-fisherman. During the samples dissection where possible tissue samples were preserved and gonad samples suitable for histological examinations were preserved. Histological examinations were conducted throughout the project. Tissue sample preparations started for DNA extraction in February and were sent for barcoding after all DNA samples were ready.

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
Histological work package	315	242	73	Histological equipment and substance costs were reduced since we did not do as much histological examination as we anticipated.
Dying chemicals	630	683	-53	Due to exchange rates some of the chemical prices increased.
Genetic study work package	2150	2305	-155	Due to technical problems during services we had to increase replications
PCR services	375	375	0	.
Travel and accommodation expenses	1200	1373	-173	We had to travel to some areas more frequently since the fisherman-fisherwomen were not able to either preserve the individuals or were not able to send us. Some of the accommodation expenses were higher than we expected due to seasonal price increase.
Shipment of samples	200	223	-23	

Presentation	80	80	0	
TOTAL	4950	5281	-331	

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

The project was the first systematic and wide range project on chondrichthyan species of Turkish seas. It was also the first genetic study for many chondrichthyan species especially for eastern Mediterranean. This showed us that studies on these threatened species should continue and a regular data flow should be integrated.

The sampling and recent catch records showed us that for *Isurus oxyrinchus* Turkish coasts are this species new breeding ground. Also from samplings for *Rhinobatos cemiculus* Gediz Delta is a breeding and nursery area for this species. Since we obtained regular by-catch individuals from fisherman in Gediz we have observed that there is an increase in the length of the species in the area and the species gave birth between summer-late summer. With these results Gediz Delta should be closely monitored since this species is only found in Gediz Delta, in central Aegean Sea and Iskenderun Bay, in southeast of Turkey.

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 have used the foundations logo in our posters which were presented in one national and one international symposium and fliers which were given to fishermen-fisherwomen. We are also going to use the logo and the name on Mediterranean Conservation Society Bulletin, as an article about the project and some of its results.



Figure 6. The poster presented in annual European Elasmobranch Association (EEA) symposium in Portugal

BU BALIKLARI NE KADAR TANIYOR SUNUZ?

Köpek balıkları ve vatozlar bilimsel adları ile "kıkırdaklı balıklar" yaygın olarak bildiğimiz ve tükettiğimiz diğer balık türlerinden çok farklı olarak kemik değil tamamen kıkırdaktan oluşan bir iskelet sistemine sahiptir. Ülkemiz denizlerinde dağılım gösteren kıkırdaklı balık türleri toplamda 66 tanedir. Bu türlerin Türkiye denizlerine göre dağılımı göz önüne alındığında; Karadeniz sularında 8, Marmara Denizi sularında 35, Ege Denizi sularında 58 ve Akdeniz sularında 60 tür yaşamaktadır. Yaşayan bu türlerin bir çoğunun nesli kirlilik, aşırı avcılık ve yaşam ortamlarının daralması gibi nedenlerden dolayı yok olma tehlikesi altındadır.



Ülkemizde kıkırdaklı balıklar genelde hedef dışı olarak adlandırılan amaç dışı av aracına takılmasıyla yakalanmaktadır. Hedef dışı yakalanmalarından dolayı av takımlarına ciddi zarar verebildikleri için pek sevilmeyenler fakat buldukları ortamların dengede kalabilmeleri ve verimliliği için çok önemli canlılardır. Bir anlamda bu canlıları bahçenizdeki sebzelerinizi yiyen farenden kurtulmak için aldığınız kedi gibi düşünebilirsiniz. Kedi fareleri kontrol altında tutarak çoğalmalarını engellerken sebzeleriniz de daha az zarar görecektir. Bu nedenle bundan sonra bir kıkırdaklı balık ile karşılaştığınızda lütfen öldürmeden denize geri bırakın.

Bu broşür, **The Rufford Foundation** tarafından desteklenen "Fisatçı Omurgasızların Yitirilmesi, Köpekbalıkları ve Vatozların Korunmasına Yönelik Birgi Açılışları mı? Türkiye Denizlerindeki Kıkırdaklı Balık Türlerinin Popülasyon Genetiği ve Strüktürel Ekolojileri" adlı proje kapsamında köpekbalıkları ve vatozlar hakkında genel bilgi verme amacıyla düzenlenmiştir.
http://www.rufford.org/projeksiyelizaketh_graca_turka_ecozel

Figure 7. The flier in A5 size which we have gave to fishermen-fisherwomen (in Turkish).