

Final Evaluation Report

Your Details	
Full Name	Onur Doğan
Project Title	Assessing DNA-based biomonitoring applications in the Golden Horn (Istanbul, the Marmara Sea)
Application ID	28898-1
Grant Amount	£6,000
Email Address	onur.dogan@ogr.iu.edu.tr
Date of this Report	16.04.2021

1. 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
<p>Provide representations and fill in the gaps of the two most reliable reference databases (the BOLD and NCBI Gene Bank) of the known species in the Water Framework Directive (WFD) and the Marine Strategy Framework Directives (MSFD),</p>				<p>Tissue samples were obtained from various gelatinous species and PCR products corresponding to DNA barcoding workflow was achieved. PCR products were then sequenced, and the relevant gene regions (ITS1, partial small subunit ribosomal RNA and 5.8S ribosomal RNA genes – contain rRNA-ITS regions and COI) particular to these different species, were subsequently determined. A large number of samples were processed and labelled despite the lockdown procedures and regulations that have limited our ability (physically and mentally) to work regularly on those cases and achieve our aim. We have submitted our data into the NCBI repository (submission reference number SUB9469454 and SUB9475262). However, since this data will be included in our yet to be published article, submissions won't be available online until publication is out.</p>
<p>Testing the applicability of DNA-based biological monitoring methods within the Marine Strategy Framework Directives (MFSD) and prioritize the relevant gelatinous species for monitoring in future collaborative programs,</p>				<p>A variety of methods were tested at the beginning for both the "sampling" and the "PCR" parts. Plankton groups were detected, recorded and marked using a light microscope as well as DNA-based methods. Anything from common to rare species, as well as invasive species were detected.</p>
<p>Focusing on the crucial groups of zooplankton (like gelatinous jellyfish) which have adverse effects on the</p>				<p>Tissue samples were collected from a variety of jellyfish species, including <i>Aurelia aurita</i>, <i>Rhizostoma pulmo</i>, and <i>Mnemiopsis leidyi</i>. The latter has been a concern for fisheries in recent</p>

<p>economic process in water bodies, and aiming to reveal the biological inventory.</p>			<p>years. Many species including pollution indicator (e.g. <i>Pleopis polyphemoides</i>), <u>red-tide effect</u> (e.g. <i>Noctiluca scintillans</i>), invasive and non-local species (e.g. <i>Oithona davisae</i>) were recorded and photographed. Data on their abundance and variety were archived.</p>
<p>To contact the schools around the Golden Horn to involve children from these schools in the sampling and laboratory stages of our project so that they can try to understand the scientific process.</p>			<p>Although we communicated with schools and instructors, we were not able to achieve this step due to Covid-19 restrictions.</p>
<p>To raise local awareness by translating project outputs and the scientific process in many languages. In this context, digital or printed booklets and online website will be prepared.</p>			<p>A booklet written in several languages was published and distributed to the surrounding community. Also, the website (<u>hanar</u>) has now been launched. Moreover, our project has been published in the <u>EkoEvo Society's magazine</u>, and our grant was announced on <u>the official website</u> of Istanbul University's Institute of Graduate Studies in Science.</p>
<p>To communicate with the surrounding schools and cultural centres in order to share our work with the community. Moreover, we intended to organize an expedition in the Golden Horn area and share our experimental work with the public by simulating the sampling step.</p>			<p>We have been contacted with teachers and cultural centres, for which we have sent our project summaries. However, due to Covid19 restrictions, schools were closed and all kinds of organisations were cancelled. Furthermore, we could not carry out the activities we previously planned to do with the children (such as invite them to the lab were they can see and enjoy and discover the experimental part of our project). Nevertheless, children of the neighborhood showed interest and joined us spontaneously in the jellyfish sampling studies we conducted in and around the Golden Horn.</p>

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

Restrictions imposed by Covid-19 have limited and at some point forced us to stop the research as well as the social awareness activities. Companies selling laboratory and sampling equipments, and service providers such as sequencing companies have been closed for a certain period of time; subsequently, the whole process was delayed.

We overcame many problems by constantly communicating and interacting with our partners (i.e. multiple providers, research centres, and universities). During this time, working with open and straightforward colleagues kept us hopeful and the process ongoing. Considering Covid-19 and the subsequent safety restrictions, we, as a team, were mostly active online, focusing on raising social awareness, by publishing our recorded work videos.

Our activities were organised and published on our YouTube channel, Twitter accounts, and our website. Till now we were able to create an illustrative website, designed our own logo, and published our paper booklet. To accomplish our long-term goals, such as publishing children's books, board games, card games, and promotional videos, we approached a number of illustrators and obtained pricing offers for each product.

By approaching recently graduated, freelance illustrators as well as artists who have been specialised in corporate work, we were able to negotiate the price for the products destined/designed to have an impact on children, families, and the society as a whole, either on the short and long term (ranging from basic drawings to children's books, including box games, card games, and animated film).

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

- (i) We introduced testable, environmentally friendly, and simple-to-implement methods for people to practice them in the Golden Horn area, in order for them to contribute to the biodiversity prediction by taking samples. We recorded, tracked and DNA-barcoded the collected samples. We, as well, enhanced the applied protocol for DNA-based tools from marine samples.



Photos taken by Esin Yüksel Durmaz, Ezgi Türkeri and Onur Doğan.

Despite the limitations, ongoing scientific projects, like ours, contributed to building a bridge between science and citizens or what we like to refer to as "seatizen (de Vargas, Colombar, et al., 2020)".

- (ii) We broadcasted live interviews related to jellyfish blooms to mainstream media (such as CNN Türk and TRT1 TV channels) during the lockdown period. We have also published statements in many newspapers.



The photos are taken from [CNN Türk](#) broadcast stream.

(iii) We have distributed free booklets to the surrounding communities. The booklet helps the readers better understanding the concepts of ecology and conservation biology. Booklets were written in a way that makes children and families interested in interacting and altogether exploring nature's wonders. A close attention was taken into account when translating the booklets into various languages as we kept in mind Istanbul's population structure, which has been influenced repeatedly by migrations.



A representation of the cover page of the booklet ([Arabic](#), [English](#), [French](#), [Turkish](#)).

Currently, there are versions of the booklet in seven languages and this number is increasing with the participation of volunteer and/or professional translators.

In addition, we have launched a website, based on the jellyfish theme, aimed at bridging the gap between people from all backgrounds and scientists. The website

is simple and easily participatable. Moreover, we have started a [YouTube channel](#) that includes videos of our field work and DNA studies.



In the same context, we have designed several jellyfish logos to be used as our signature in the present and future work (presented in the figure above).

Last but not least, we are planning to submit our work in peer review journals, for that matter we are currently working on finalising our manuscript detailing our research study.

4. Briefly describe the involvement of local communities and how they have benefited from the project.

We collaborated with local fishers, we rented their boats when working in the Golden Horn and the surrounding areas, and in return, they welcomed us as part of the team. We exchanged ideas about the effects of jellyfish blooms on fisheries. Particularly that jellyfish (Stabili, Loredana, et al., 2020) are considered as early warning "indicators" of harmful impacts, potentially affecting structure of trophic webs (Marine Strategy Framework Directive 2006/56).

Fishers hosted us as researchers in their local fishing cooperatives before and after our sampling activities.

On the other hand, we have been in contact with local visual artists to figure out how to present our work decently and in a way that is understandable by non scientists and the community as a whole. For that matter, we have consulted them on a regular basis about our project's goals and objectives.

5. Are there any plans to continue this work?

This project showed the importance of our workflow. We have been working on four stations within the Golden Horn region. In order to better understand the connectivity of the Marmara Sea and the Golden Horn, we will expand our sampling area to include the extensions of the Golden Horn.

By expanding our work to the Marmara shores, we will be able to observe events such as mucilage and red-tide in addition to jellyfish blooms. The latter was recorded by NASA in this image entitled "[Blooms in the Sea of Marmara](#)" in the summer of

2015. In our current study, we have also observed the *Noctiluca scintillans* that caused the red-tide phenomenon (check the [image](#)) at the Golden Horn and a junction station between Marmara sea and the Golden Horn sites.

This project is a great opportunity for us as it allowed us to meet with people from different backgrounds as well as many experienced conservation genetic researchers. I believe that, in addition to our good background in biomonitoring research, this work will continue to improve our abilities to establish a reliable and consistent sustained programme that can be used in routine check-up for changes in the dynamics of zooplankton communities.

In the next step, we intend to continue what we have started throughout this project. We are planning to enlarge our sampling to include all the nearby areas of the Golden Horn. For that matter, we intend to hire new teammates. Moreover, we are planning to illustrate our results, making them more understandable to everyone.

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

Our work has been shared through many means. As mentioned before, our project has been published via newspapers and TV channels. We are now seen in official announcements, magazines, and on cooperative, channels as well as within research and educational institutions (i.e. faculties and research centres).

Since 2013, we've been participating in seasonal expeditions on a regular basis, and we keep in contact with fishers, fishing cooperatives, sailors ("planktonauts"), students, and locals who were involved in the process. Furthermore, research assistants and scientific consultants who are on board currently teach undergraduate students' courses at Faculty of Aquatic Sciences at Istanbul University. Thus, they are able to share our current work with students.

As PhD candidates, we are able to officially share our work through the communication channels of the scientific communities that we are currently a member of. For instance, our work was also published at the official magazine of the Ecology and Evolutionary Biology Society of Turkey.

We are trying to improve our team by sharing our project scope with the researchers in the laboratories we work in and collaborate with.

We are trying to discuss our work with people from different disciplines in order to create communication networks with them (e.g., from art and social sciences).

We use traditional media, such as newspapers and television stations, as well as live sharing from our social media accounts, to disseminate our work.

We have been in contact with freelance providers/service suppliers as well as with corporate companies while carrying out the project, this has contributed to the natural spread of our project among community.

In addition to all these, we established a website and coordinated our actions with the social media accounts we frequently use.

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

The Rufford Small Grant was used throughout the entire project timing, which is from February 2020 in April 2021. However our work flow was disrupted due to Covid-19.

There are three main phases in our scientific workflow: **(i)** marine sampling, **(ii)** wet-lab, and **(iii)** dry-lab. The money has been disbursed on these steps as planned. In addition, we have created an illustrative booklet to raise local awareness about ecological and conservation genetics. We've made it available in a many languages, both online and in print.

Marine sampling the budget destined for this part helped pay for the following: transportation, food and beverages, water-resistant clothing for researchers (for tough weather conditions), supply and transportation of sampling equipment (fuel consumption), requirements for different filtering methodologies, various preservatives to be used for advanced physical (formaldehyde) and molecular analysis (96 EtOH and RNAlater) of seawater samples. Additionally, the money was used to buy various sampling equipments (from Sterivex filters to WP-2 plankton net), boat rental, waterproof camera for recording fieldwork, as well as to pay the researchers for their work on sampling days.

Wet-lab: Wet-lab is about storing the collected samples under suitable conditions for further physical and molecular studies.

While working on this part, and due to covid crisis, suppliers or companies providing materials required for physical and molecular analysis were closed. To prevent delays, we got in touch with next-door laboratories from which we had to borrow the needed materials.

Once the lockdown was over, we have returned the borrowed materials.

The budget destined for this part was spent, in part, on materials/equipments and, the rest was paid to researchers undertaking this workload.

Dry lab: It includes the acquisition of physical parameters and DNA data, the processing of the data, and the fees paid to researchers involved in the relevant steps

To summarise, in order to raise awareness in conservation genetics, ecology and related themes, we have worked in coordination with many stakeholders, from journalists to reporters, freelance translators to professional illustrators. Therefore, scientists and everyone else who was involved in the project have received their wages based on their tasks.

8. Budget: 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. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Sampling	£950	£950		In order to perform expeditions, we rented boats. The amount of money was spent on the transportation of researchers and equipment that have been purchased and used in the sampling area.
Sampling equipment	£950	£1097	+141	This section includes the amount spent on filters, various filtration systems, solutions used to preserve samples, and the design of plankton nets.
Salaries	£650	£980	+330	We decided to increase the wages to be paid to the members of project in order for them to be motivated and therefore accomplish the tasks in more efficient way.
Publishing	£750	£750		We worked with a freelance illustrator who designed the booklet and a freelance journalist who helped making our work more understandable. This section also includes the fees paid to professional and freelance translators working on various foreign languages as well as the amounts to be paid to platforms for licensed illustrations purchased when booklet designed.
Videography service	£500	£473	-27	Due to the Covid-19 restrictions, we haven't been able to negotiate with videography service supplier. We had to find an alternative way to get a camera in order to record our

				actions. So, we bought a camera with waterproof features.
Daily wages	£200	£600	+400	Due to the Covid-19 restrictions, we had to go sampling many more times than expected. Same thing was encountered when performing laboratory essays and experiments. An increase in our team's expenses was eventually caused by the situation of additional health and safety measurements.
Mapping services	£500		-500	We used the open source <u>R studio</u> for this work package. We didn't need any additional service for mapping. We decided to use the money destined to be spent on this part for increasing the daily wages and salaries of our project stakeholders.
Workshop	£550	£375	-175	Due to Covid-19 disruption, we haven't been able to organize a workshop where we could present our work to the surrounding community. For the moment, this workshop is still on hold until further notice. We decided to transfer some money in this part for increasing the wages of our project stakeholders. (Pending)
Sequencing	£950	£775	-175	The part related to sequencing did not proceed smoothly: Due to the pandemic, the sequencing services over the country (and I assume over the world as well) has been prioritized for Covid-19 related research studies and/or tests. In addition to this, the companies providing this service have suspended their work, for a certain period of time during the lockdown period. During this difficult time, we stayed in contact with some

				laboratories that were able to help us finish our sequencing and helped us finalize this step. After lockdown restrictions were limited and things got back to normal, we paid the labs we worked their services from the budget already set for that matter.
Total	£6000	£5625	-375	This amount of money will be used to organizing workshop in future time.

On January 9, 2020, the money transferred by [the Rufford Foundation](#) has reached the [EkoEvo Society's](#) bank account. A portion of this money (~£30) was deducted by the bank as a fee service and another portion (~£86) was donated to [the EkoEvo Society](#). In January 2020, 1 euro was equal to ~0.85-pound sterling; and now (April 2021), 1 euro is equal to ~0.87-pound sterling. In January 2020, 1 euro was equal to ~6.74 Turkish lira and now (April 2021) 1 euro is equal to ~9.74 Turkish lira.

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

Many steps must be taken following the end of this project. below I list the most important ones:

- 1- Ensure the continuous monitoring of plankton in designated stations (whether the old or the new ones).
- 2- Use the project results in our doctoral dissertation and publish articles in scientific journals. We plan to present our project at the [2nd International Aquatic Biotechnology Symposium](#) soon.
- 3- Expand our communication channels.
- 4- Expand our stations to include many more within the Golden Horn and the Marmara Sea in order to understand population genetic structures and better establish genetic connectivity between populations.
- 5- Enrich our project by collecting new samples of gelatinous species, within the Golden Horn area.

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

We have used the Rufford Foundation logo. It can be seen on our website and on the cover of [the booklet](#) as well as in [the 3rd issue of EkoEvo e-bulletin](#).



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The
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Mart 2020

Sayı 3

Onur
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Rufford Müttevelli Heyeti tarafından yapılan değerlendirme ile 2020 Ocak ayı içerisinde desteklenmesi yönünde onay alan projemiz, ekolojik olarak anahtar rolere sahip olan çeşitli zooplankton gruplarının antropojenik faaliyet baskısı altındaki Haliç'te (İstanbul) DNA temelli biyo-izleme hizmetleriyle uygulanabilirliğinin bir sınanmasıdır. Su kaynaklarının yönetiminde, AB Su Çerçeve Direktifi gereğince üye ve aday ülkelerin, biyolojik kalite unsurlarının izlenmesi amacıyla çalışmalar yürütmesi sahip oldukları su kaynaklarının iyi ekolojik duruma getirme amacıyla yönetim planları hazırlaması büyük önem taşımaktadır. Bu bağlamda kısa DNA fragmentleri kullanılarak sucul türlerin referans kütüphanelerinin oluşturulması özellikle önemlidir. Geleneksel ekolojik yöntemlerle bilinen türlerin tanımlanması uzun yıllar birçok araştırmacı tarafından çalışılmıştır. Biz de daha önce TÜBİTAK 1002 projesi tarafından desteklenen çalışmamızla Haliç'teki çeşitli zooplankton gruplarını klasik yöntemler kullanarak test ettik ve bu çalışmaya dair makalemiz [1] çok yeni yayınlandı. DNA barkodlama yöntemleri ile bilinen türler için referans kütüphanelerin oluşturulma süreci ise son yıllarda gerek ulusal gerekse uluslararası fonlarla desteklenmekte ve kapsamlı databankaları (BOLD, Genbank, ABOL, FinBOL, GBOL, BFB, ibol, NorBOL, SwissBOL, LusoMarBol) ile biyoçeşitliliğin kaydı tutulmaktadır. Salt bilimsel çıktı edinimi gagesinin ötesinde beceri ve imkanlarımız dahilinde yerelin sosyokültürel dinamikleriyle de etkileşime girme çabasında ya da Jacques Monod'un deyimiyile bu iletişim ağı içerisindeki bileşenlerle birlikte çoğunlukla ...anlamaya çalışıyor... hâlinde bulunacağımız bu süreci uzun seneler sürdürülebilir kılmaya niyetindeyiz.

[1] Işıl M. Doğan O. Zooplankton Biodiversity in the Golden Horn Estuary after the Opening of the Water Channel from the Strait of Istanbul, Turkey, Turkish Journal of Fisheries and Aquatic Sciences, 2020 Vol 20 Num 2, Pages:147-158

https://www.rufford.org/projects/onur_dogan

EKOEVO BÜLTEN

04

The cover of our designed illustrative booklet (left) and our introduction in EkoEvo e-bulletin (right).

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Esin Yüksel Durmaz – Researcher (PhD candidate at Istanbul University): She participated in fieldwork and laboratory studies. Esin, who has a bachelor degree in science and technology teaching, took part in determining the ways to be followed while explaining the project outputs to children.

Ezgi E. Türkeri – Researcher (PhD candidate at Istanbul University): She participated in fieldwork, made evaluations regarding species identification, determination, enumeration, abundance and diversity under microscope. She took part in organizing and conducting field studies.

Maha Salameh – Project Developer, scientific writing (PhD student at Bogazici University): Maha Salameh, who worked as a scientific writing process, also made valuable contributions in the development of project outputs.

Dr. Mohamed Camara Project Developer (Engineer): Dr Camara contributed to the development of our filtration systems in bringing our method to a more robust form.

Onur Dođan - Principal Investigator (PhD candidate at Istanbul University): I have organized the field studies using different scenarios based on the many hypotheses set. Throughout the project, I have been in contact with researchers from both our research unit and/or from other institutions. In addition, I have invited people from different disciplines to get involved as they may enrich our experience and be an added value to the project in general.

Şeyma Eren Msc. (Researcher): She participated in fieldwork and worked on the development of our methods.

Ufuk Gürbüzdal – An independent filmmaker and a Research Assistant at the Faculty of Communication at the Hasan Kalyoncu University. He designed the booklet of this project.

12. Any other comments?

As young researchers, we are grateful to be part of this interdisciplinary project. We would like to thank the Rufford Foundation for granting us the funds and helped us realize and run our own plankton monitoring study. This project as important as it is can be a steppingstone towards bigger and more specified future research studies related to the marine sciences.

Special thanks

We are grateful to my advisor, Prof. Dr. Melek Isinibilir Okyar (Istanbul University), for her valuable contribution, for sharing her wisdom, and for all the facilities she has provided throughout this project.

We are grateful to Prof. Dr. Raşit Bilgin (Bogazici University) for his scientific consultancy and cooperation. Throughout the process, Dr. Bilgin was tremendously helpful and always available when needed. His collaboration is highly appreciated especially when it comes to his guidance in solving both existent and anticipated problems.

We would like to thank the Ecology and Evolutionary Biology Society of Turkey association for motivating us to keep our scientific enthusiasm despite the circumstances.

We are grateful to the Republic of Turkey Ministry of Environment and Urbanisation for providing a field work permit and a favourable working environment for us as researchers.

They are presented in alphabetical order.

We would like to thank captains, Mr. Ahmet Yavuz (President of Eminönü Fishery Cooperative) and Mr. Mustafa Fıçıcı, for their valuable assistance during the sampling step, and for sharing their wisdom while being on board.

We are grateful to Dr. Bilgenur Baloglu (Lead bioinformatician), who shared her experience and knowledge with us in robusting our methods and work flow during the process.

We would like to thank freelance journalist Eylem Altınışık for her dedication and cooperation in simplifying our scientific terminologies, making them more understandable to readers from different backgrounds.

We would like to thank Dr. Ezgi Altınışık (Hacettepe University) for her scientific advice. She was an important source of motivation for us with her scientific enthusiasm and energy throughout the process.

We are grateful to Dr. Ezgi Özkurt (Earlham Institute) for being accessible at any time during the process and adding value to her knowledge in both solving instant problems and shaping our long-term work flow chart.

We are grateful to MSc. Halldis Ringvold for translating the booklet into Norwegian and for her valuable comments in further readings.

Many thanks to Msc. İnci Şıxaliyeva (PhD candidate at the Molecular Biology department at Istanbul University) for translation and redaction of the booklet.

We are grateful to Dr. Nadia G. El-Sarraf Nasr (Researcher & Instructor) for sharing her scientific advice and experiences in the scientific writing part.

We are grateful to Nurbahar Usta Baykal (Hacettepe University), a Rufford small grant executive, for sharing her experience with us.

We are grateful to Dr. Vasco Elbrecht (ETH Zurich), who did not hesitate to share own knowledge and experience with us in the development of the scenarios we followed regarding our lab techniques and methods for the fieldwork.

References

1. Stabili, Loredana, et al. "The Microbial Community Associated with Rhizostoma pulmo: Ecological Significance and Potential Consequences for Marine Organisms and Human Health." *Marine Drugs* 18.9 (2020): 437.
2. de Vargas, Colomban, et al. "Plankton Planet: 'seatizen' oceanography to assess open ocean life at the planetary scale." *bioRxiv* (2020).