Small Grants – Final Completion and Impact Report

Instructions: CEPF requires that each grantee report on project results and impacts at the end of their grant. To monitor CEPF's global indicators, CEPF will aggregate the data that you submit with data from other grantees, to determine the overall impact of CEPF investment. The aggregated results of all grantees will be reported on in our annual impact report and other communications materials. Your Final Completion and Impact Report will be posted on the CEPF website.

Ensure that the information provided pertains to the entire project, from start date to project end date.

Please complete all fields and respond to all questions listed below.

Organization Legal Name: *GTI – Geotehnicki Inzenering DOO Skopje*

Project Title: *Assessment of an Endemic Freshwater Snail in Dojran Lake – Population Status, Threats and Conservation Measures, North Macedonia*

Grant Number: CEPF-110711

Date of Completion of this Report: 30 June 2022

CEPF Hotspot: Mediterranean Basin Biodiversity Hotspot

Strategic Direction: 2 Support the sustainable management of water catchments through integrated approaches for the conservation of threatened freshwater biodiversity

Grant Amount: USD 32,330.76

Project Dates: 01 June 2020 to 31 May 2022

PART I: Overview

1. **Implementation Partners for this Project *(list each partner and explain how they were involved in the project)***

There were no project partners involved in this Project implementation. However, there were various Stakeholders engaged in the project with their particular roles within, as listed below:

- *Ministry of Environment and Physical Planning of RN Macedonia, having the role of an overall monitoring Agency with: general support to the Project, issuing of various field working permits, active participation on the Workshops (1-st and 2-nd), dissemination of the Projects outcomes among all of the interested parties (other State Agencies, Municipality of Dojran, Experts, NGO's and others);*
  - *Municipality of Dojran (including the Mayor himself) by giving a contribution of Project basics and acceptance of Project outcomes as important findings to be considered as assets towards future Municipal development plans;*
  - *Faculty of Natural Sciences and Mathematics,Institute of Biology "Ss.Cyril and Methodius" University of Skopje RN Macedonia, being the Project's scientific fundament by giving to GTI its Experts, expertize and laboratory capacities, to achieve Project goals, including students-volonteers participatinf on the 3-rd Workshop in Dojran;*
  - *NGO Milieukontact Macedonia with simultaneously having a Large CEPF Grant Project at the same KBA Lake Dojran, and sharing their bigger experience with the (almost) same targets and Stakeholders, as ours;*
  - *NGO "ED-Planetum"- Strumica with its Representative Mr.R.Gushev, as a fruitful participant to the 1-st Workshop;*
  - *MES, a CEPF Grantee, with their participation on the 1-st Workshop, comaping their work with the Project,*
  - *High School "Bogdanci" from Bogdanci (15 km from Dojran), with their magnificent biology Prof. M.Lisichkova, together with 4 of her best students, by giving their very active in-live presence on the 3-rd Workshop and Training Session in Dojran;*
  - *Elementary School "Kocho Racin" Skopje, lead by the biology Lecturer Prof. I.Shoreva (a GTI Team Member, as well) and her students, with their on-line active participation on the 3-rd Workshop in Dojran;*
  - *Local Individuals with their generous contributions during the Field Campaigns organizational issues and active interest for the Projects goals and outcomes*

2. Summarize the overall results of your project

The conducted activities within the project "Assessment of an Endemic Freshwater Snail in Dojran Lake – Population Status, Threats and Conservation Measures, North Macedonia (CEPF 110711)" were focused on the mollusks of conservational importance that inhabit Dojran Lake. Together with the Balkan endemic freshwater snail *Graecoanatolica macedonica*, the distribution, population status and threats were also detected for few other species such as: *Pseudobithynia ambrakis*, *Anodonta cygnea* and *Dreissena presbaensis*. Not less important was the identification of main threats through investigation of the status of eutrophication of Dojran Lake itself, including the inflowing artificial channel and their impact over the status of the above-mentioned mollusks. Within the planned activities, preparation of Outlines for designing of the Species conservation action plan focused on the endemic *Graecoanatolica macedonica*, as well as the other project target species.

The in-live Workshop No.3, including University, High-School and Primary School professors and students from Skopje and Bogdanci (Dojran Lake vicinity) was well realized. This resulted in gaining provisional public knowledge and awareness on increased knowledge of young people on freshwater biodiversity of KBA Dorjan Lake, especially Dojran Lake endangered species and threats towards, having the eutrophication as the heaviest one.

Once the GTI Project Team throughout the initial activities of this Project, recognized an opportunity to change the status category of the targeted Project species Gr*aecoanatolica macedonica*, it simply led to the next crucial Project activity as: submitting a Proposal for reassessment of the status category of *G. macedonica* in front of the IUCN Red List Authorities. Luckily, the GTI Project Team is comprised of scholars of the highest rank and acknowledgements in the Country and much broader, so one Scientific Paper outcome, based on researches carried out in this Project frameworks, and this may be acknowledged, titled as:

- ***Towards solving the mystery of the long lost endemite Graecoanatolica macedonica, Radoman & Stankovic, 1979 in Dojran Lake (approved for the journal Ecologica Montenegrina; currently under proof reading)***

All of the Project developments/followings and results were duly publicly posted and permanently upgraded on the following Facebook page:

https://www.facebook.com/Assessment-of-an-Endemic-Freshwater-Snail-in-Dojran-Lake-new-hope-110813777419162/?ref=page_internal

3. Briefly describe actual progress towards each planned long-term and short-term impact (as stated in the approved proposal)
List each long-term impact from your proposal

a. Planned Long-term Impacts - 3+ years (as stated in the approved proposal)

Impact Description	**Impact Summary**
Increase the knowledge on the distribution, population status, threats and implement the conservation measures for the protection of the endemic *Graecoanatolica macedonica* in Lake Dojran	- The GTI Project Team conducted 6 field campaigns where some fresh looking shells have been found at the lake. - Thorough lab investigations over collected samples were made, leading towards production of few Project Documents. - Lake's eutrophication, found as the major treat to *G. macedonica* and the other Project species, was particularly elaborated through samplings and lab analysis of both the Lake itself and the inflowing artificial channel. - Results were duly publicly presented (Workshops, Facebook page, Environmental web sites). - Such Project findings lead towards inevitable requirement of communication with IUCN Authorities and EU SC to officially start an Initiative to submit *Proposal for reassessment of the status category of G. macedonica in IUCN Red List*.

b. Planned Short-term Impacts - 1 to 3 years (as stated in the approved proposal)

Impact Description	Impact Summary
PROJECT BASIC PERIOD	
1. To determine the population status of the endemic *Graecoanatolica macedonica* (extinct/not extinct)	The analysis of the biological material collected from the all of the 6 field trips within the basic project period and the project extension, suggest that the species is not extinct. Although no live individuals were recorded, the fact that fresh empty shells are continuously being found is a strong indicator that this species still exists in Dojran Lake.
2.To determine the status of other important mollusks from Dojran Lake	Apart from collection of shells of *G.macedonica*, the other species targeted in this project were also recorded. Sampling for *Pseudobithynia ambrakis, Dreissena presbensis and Anodonta cygnea* was conducted on different sampling sites placed on different depths from muddy, sandy substrates and submersed vegetation. Live individuals of all three species were continuously being found in the lake, meaning that their populations in Dojran Lake can be considered stable. However, it should be noted that the swan mussel *Anodonta cygnea* was collected only from one sampling locality and despite the detailed search during the field trips, it didn't appear anywhere else. Although it is classified as Least Concern (LC) on the IUCN Red List of Threatened species its population in Dojran Lake may need more attention in the future.
3.To perform comprehensive examination of physic-chemical parameters in relation to eutrophication pressure, ecological status of the lake and bottom sediments, as well as the characteristics of the micro-habitats or refugia where mollusks have been encountered;	Performed analyses of Lake Dojran waters and sediments reveal intensive eutrophication processes which result in severe (most recent in July 2022) cyanobacterial blooms and toxin production. It is evident that this kind of pressure most probably influence all hydrobionts in the lake in an intensively negative manner. The bottom dwelling fauna in particular is forced to change the preferred habitats and move away from the most direct and critical impacts. Detected cyanotoxins pose serious problems to human, animal and environmental health.
4.To determine and confirm the impact of the artificial channel for water supply to the lake and its utilization	Being a source of additional water supply, the artificial channel constructed for the purpose of mitigating the adverse impact of water loss over the years, is now found to be a source for additional nutrient supply to the lake. At this point

	the channel significantly contributes to overall nutrient enrichment of the lake system.
5.To increase public awareness for the biodiversity importance and its protection	All the activities within this Project frames were conducted in order to increase the public awareness of the biodiversity and its protection. This task was successfully presented and performed in all the available media, either created or following the performance of the project *in-situ*. Many conversations, explanations and documentations were presented to local public and specifically to University and local high school students, including Elementary school pupils, attending the 3-rd Workshop.
6.To develop outlines for preparation of the Species conservation action plan focused on the endemic *Graecoanatolica macedonica*, but including *Pseudobithynia ambrakis*, *Dreissena presbensis* and *Anodonta cygnea*, as well	All the presented outlines completed within the framework of this project present the first step towards the finalization of a complete species conservation action plan for *Graecoanatolica macedonica*, *Pseudobithynia ambrakis*, *Dreissena presbensis* and *Anodonta cygnea*.
PROJECT EXTENSION PERIOD	
7.Current status of *G.macedonica* reassessed and confirmed	Biological samples from Lake bottom were taken during the 6-th field sampling conducted in May 2022. The sampling included few localities, the ones that during the previous 5 field trips in 2020 and 2021 were recognized as localities with the highest density of shells of *G. macedonica*. The analyses of the additional biological samples collected in the project extension period again showed presence of empty shells of the target species. Since fresh dead shells of *G. macedonica* are continuously being found, it is clear that the species cannot be extinct. It is possible that this species populations withdraw-escaped to some new localities in the lake or its surrounding waters and the species should be considered Critically endangered (Possibly extinct). Additionally in this research the Greek side of Dojran Lake as well as Axios River between Polikastro and Vafichori wasn't taken into consideration. Bearing in mind that *G. macedonica* was recorded in this part of Greece (Reischütz, 1988; Radea et al., 2013), detailed investigations are mandatory.
8.Increased knowledge of young people on freshwater biodiversity of KBA Dorjan Lake	At the final sampling campaign (May 2022), the specific workshop for university and high-school students was organized at Dojran Lake shore zone

	facilities. Many results of the project were presented and explained in order to underline the importance of biodiversity and its protection. Many answers were also given to all posted questions, and the overall impression is that the participating students have increased significantly their comprehension and knowledge on the subject.

4. Were there any unexpected impacts (positive or negative)?

During the complete Project period, no particular unexpected impacts were detected; especially not negative ones were a case.

PART II: Project Products/Deliverables

5. List each product/deliverable as stated in your approved proposal and describe the results for each of them:

#	Deliverable Description	Deliverable Update
PROJECT BASIC PERIOD		
1.1	1. Distribution, population status, threats and localities important for conservation for the endemic species *Graecoanatolica macedonica* in Dojran Lake fully investigated and corroborated;	The freshwater aquatic snail *Graecoanatolica macedonica* presents an endemic species to Lake Dojran. It was described from shells taken from the sampling site Kaldrma (type locality), on the Macedonian side of Dojran Lake. It was noted on stony and sandy substrates in the littoral zone of Lake Dojran (Kazan, Star Dojran, Nov Dojran), usually at depths of 0 to approximately 50 cm. The species population was noted to have suffered declines in the 1970s as the lakes water levels lowered from maximum depth of 10.4 m in the 1930s to 5.6 m in 1995. In 1987, densities were estimated at 2,500 individuals per m^2, however, in 1988 the lake's water level decreased by a further 60 cm as a result of a single outflow event. The species was not recorded in the area between 1992 and 2001. The species was thought to be extinct as a result of habitat loss through water abstraction. Water abstraction is still ongoing in the area as the lake's feeder streams and underlying aquifer continue to be used for agricultural purposes.

		The freshwater snail *Graecoanatolica macedonica* was registered on six sampling sites located on different depths starting from 2 to 3.5 meters. The detailed laboratory analyses showed that this species presents part of the malacofauna of T26 Star Dojran – Izvrsen, T31 Star Dojran – Renesans, T36 Star Dojran - Beach Tomce-Sofka, T37 Star Dojran – Beach Fuk-Tak, T38 Crn potok, T39 Camp MVR and T42 Mrdaja. Unfortunately, the fifth field trip didn't reveal any new localities for the species' presence, nor showed any specimen's movement in the undertaken experiment. All collected specimens were also counted in order to provide information for further analyses concerning the population density of all target species. Regarding the number of detected specimens from each target species, our analyses have shown that the biggest number (63) of empty fresh shells from *G. macedonica* were noted on T42 Mrdaja (3.5 meters). Although the conducted field trips showed presence of only empty fresh shells of this species, information about their number presents a solid base for the future investigation's focus. It is possible that this species populations has withdrawn and survived on some new localities in the lake or its surrounding waters and its status should be determined as Critically endangered (Possibly extinct). Towards this statement is the fact that this research didn't take into consideration the springs around the Macedonian side of Dojran Lake, nor the Greek side of the lake and its surrounding waters as well (e.g. Axios River between Polikastro and Vafichori). Bearing in mind that *G. macedonica* was recorded in this part of Greece (Reischütz, 1988; Radea et al., 2013), detailed investigations are obligatory. All these results were adapted as deliverables in a form of a report that has been already sent.
2.1	The population status of other important mollusks in Dojran Lake (like *Pseudobithynia ambrakis, Dreissena presbensis* and *Anodonta cygnea*) determined	The obtained results have shown that despite providing suitable habitats for *G. macedonica*, the sampling localities T26 and T37 present preferable parts of the lake for other aquatic mollusks such as *Pseudobithynia ambrakis*. It seems that the sampling locality T26 really meets the ecological preferences of this species as it was found on

		depth of 3 and 4.5 meters. This species has also been noted on the sampling locality Toplec on depth of 0.5 and 1.5 – 2 meters, as well as on the locality Kaldrma (1.5 and 2.5 meters). The presence of the swan mussel *Anodonta cygnea* was detected only on three localities in Dojran Lake. Juvenile specimen was found in 0.5 meters depth on the sampling locality Toplec. Little deeper, on 1.5 meters depth this species was collected in adult stage. However, taking in consideration that *A. cygnea* is known to inhabit deeper part of the lakes, its presence was also confirmed in the part between Kaldrma and Nov Dojran on 7 meters depth. Three adult species were collected with dredger method, with help provided by the local community. The analysis of the bivalves in Dojran Lake showed that *Dreissena presbaensis* is the most distributed species among all four taxa targeted in this research. Out of all visited sampling sites, its presence was noted on 41 of them. Its population inhabit the lake starting from 0.5 meters to 7.5 meters depth, which makes this species dispersed throughout all the investigated area. Juvenile specimens were found on few localities such as T4 Nikolic (1-meter depth), T15 Star Dojran Renesans (0.5 meters depth) and T36 Star Dojran - Beach Tomce-Sofka (2 meters depth). Comprehensive analyses of the adult specimen's presence in the studied area are determined whether all collected specimens are viable. It turned out that the collected material contained viable specimens as well as empty shells of this species. It is evident that *Dreissena presbaensis* is the most widely distributed species in the lake. Three localities, T9 Toplec (0.5 meters), T26 Star Dojran – Izvrsen (3 meters) and T37 Star Dojran - beach Fuk-Tak (3 meters) were shown to be the only localities that contain specific microhabitats for even three target species. According to our analyses, *G. macedonica, P. ambrakis* and *D. presbaensis* live in sympatry at T26 Star Dojran – Izvrsen (3 meters) and T37 Star Dojran - beach Fuk-Tak (3 meters) as all three species were collected from these two localities. On the other hand, T9 Toplec (0.5 meters) locality present

		suitable area for *A. cygnea, P. ambrakis* and *D. presbaensis*. Although the populations of the swan mussel *A. cygnea* on the IUCN Red List of Threatened Species are estimated as Least Concern (LC) on a global level, the fact that this species was detected on only one locality in Dojran Lake is a little bit concerning. However, based on our comprehensive investigations it is evident that this species still inhabits the lake. The Balkan endemic species *P. ambrakis* requires special attention as it is listed as Vulnerable (VU) on the IUCN Red List of Threatened species. However, viable populations of the species were detected in Dojran Lake suggesting that this species has well adapted to the environmental conditions in the lake. Out of all target species, *D. presbensis* was found to be widespread all over Dojran Lake as its viable populations were detected on many localities. All these results were adapted as deliverables in a form of a report that has been already sent.
3.1	Eutrophication status of Dojran Lake determined	Status determined; Lake Dojran is found to be under severe pressure from excess quantities of nutrients, especially nitrogen and phosphorous compounds during the investigated period. This pressure evidently increases over the summer months clearly reflecting the influence of significant number of tourists whose waste material is directly discharged into the lake without any treatment. Prolonged period of negative influences is also recorded in the sediment analyses; mud samples being much more accumulative for different nutrient and pollution elements than sandy and/or calcareous sediments. Consequently, the lake's muddy sediments represent additional source of nutrient, heavy metal and toxin pollution. Being a source for additional water supply, the artificial channel constructed for the purpose of mitigating the adverse impact of water loss over the years, is now found to be a source for

		additional nutrient supply to the lake. At this point the channel significantly contributes to overall nutrient enrichment of the lake system. Nutrient content in the lake was found to be continuously far beyond the Vth category of the water quality. This overall pressure has been detrimental in forcing the microflora changes in the lake plankton, favoring the massive dominance of cyanobacteria (more than 96%). Increased occurrence of the 'water blooms' consisted of numerous cyanobacterial taxa, mostly dominated by Microcystis spp., has resulted in frequent development of heavy floating growth on the surface of the water. Moreover, the bottom of the lake is covered by intensive growth of macrophytes in combination with the filamentous green alga *Cladophora glomerata* which is covering the stones and periphyton. Consequently, the lake is reflecting the intensive eutrophication pattern of an ecosystem under heavy anthropogenic influence, on the turning point towards swamp. Cyanobacterial toxins have been detected in the lake in the past. During this study their presence is firmly documented both in algal samples as well as in sediment where their concentration is quite high. Thus the lake has passed the threshold of a safe environment in regard to human and animal health safety.
4.1	The impact of the artificial channel for water supply to the lake and its utilization determined	Impact of the artificial channel determined; Concerning the calculations for Total P, Total N, dissolved P and condensed P, the input of water from the artificial channel severely contributes to the additional eutrophication process of Lake Dojran, thus leading to more accelerated algae growth. This statement can be confirmed by the ecological state of the area of Toplec where the water from the channel enters (investigated site T9 – Toplec, near the bridge). This area was massively dominated with gigantic, macroscopic colonies of *Microcystis ichthyoblabe*, with very high abundance, which were floating on the

		neuston and were maximally concentrated between the massive growth of macrophytes (*Potamogeton* sp., *Elodea* sp., and *Myriophyllum* sp. in large abundance) as metaphyton. The chemical analyses also confirmed that this investigating area (T9) had the worst conditions, in terms of Total N (1200 μg/L) and Total P (80 μg/L). In conclusion, the observed algal communities, the higher levels of Total P and Total N in the area of Toplec, near the bridge (T9, where the water from the channel enters), clearly depict the effects of the water from the artificial channel and its role as additional "booster" of the eutrophication. This can be confirmed by the chemical analyses and the calculations of Total N and total P input in Dojran Lake every second, from the water of the artificial channel.
5.1	Public awareness for the importance of mollusks, and overall biodiversity, for Dojran Lake increased	2 on-line workshops successfully performed; I-st Project Workshop (out of two-planned), was held on 15 September 2020. The workshop was organized by the GTI Team, hosted by our Main Researcher Prof.Dr. V.S. Stamenkovic. It was performed by the on-line based Zoom-platform, attended by the whole GTI Team and Stakeholders' Representatives from: MoEPP (Mr. Aleksandar Nastov), Milieukontakt (Ms. Petra Pop Arsova), CEPF grantee working on Conservation of National Endemic Plants in Macedonia (implemented by Macedonian Ecological Society – MES and represented by Prof. Silvana Manasievska) and NGO Strumica (Mr.R.Gushev). After a proper Presentation on the Project targets and further developments, prepared and performed by the Host, Stakeholders discussions were mainly oriented to some clarifications on the project species, their status and expected results from field trips and analyses. A significant support to the Project was given by all of the attendants. The Presentation is

		given as in Audio/Video records , available on demand; Having the previous experience with the 1-st Workshop with not so satisfactory attendance by the original stakeholders, the 2-nd Project Workshop/Results presentation was carefully planned and jointly organized with Milieukontakt-Macedonia (Mss Petra Pop Ristova and Maja Markovska). Their richer experience led towards joining all-kind of stakeholders and much higher extent of attendance. It was successfully conducted on 21 June 2021 by the on-line ZOOM platform. Milieukontakt-Macedonia took the role of Host, and our Project results and outcomes, together with theirs, were well presented. Having not enough time for long discussions afterwards, the attendants were kindly asked to send their additional questions/requests-for-clarifications by e-mail and to be replied at the same way. The Presentations were duly recorded as in Audio/Video records, available on demand.
6.1	Outlines for preparation of the Species conservation action plan for the endemic *Graecoanatolica macedonica* prepared	The outlines for preparation of the Species conservation action plan for the endemic *Graecoanatolica macedonica* included gathering of all gained knowledge on this species distribution, ecological preferences, potential threats and mitigation measures. The outlines present a pattern that should be followed during the preparation of the Species conservation action plan.
PROJECT EXTENSION PERIOD		
1.1	Database developed on the species distribution and ecology of G.macedonica	The analyses of the additional biological samples collected in the project extension period again showed presence of empty shells of the target species. Since only fresh dead shells of *G. macedonica* are continuously being found, it is clear that the species cannot be extinct.

		The results gathered were summarized and kept, presenting valuable data base that can be used in future investigations. The database is sent along with this FCIR.
2.1	Proposal for reassessment of the status category of G. macedonica prepared and elaborated	Within the project extension period the aquatic invertebrates team attended the Online Red List training by IUCN: (https://www.iucnredlist.org/resources/online). They became familiar with the IUCN Red List categories and criteria, assessment procedures as well as mapping standards, gained essential knowledge on the IUCN Procedures and received certificated at the end. The online course served as an addition to the knowledge already absolved at the IUCN Red List training by Prof. Dr. Valentina Slavevska Stamenković, few years ago. All this experience and new knowledge led to arranging the gathered data in a form ready to be delivered to the IUCN SSC Mollusk Specialist Group. We realized communication with the IUCN SSC mollusk specialist group and gotten response from the proposal submission; - Proposal submitted to IUCN; - Until completing of this FCIR, no response has been received from IUCN SSC; - Grantee undertakes a responsibility to inform CEPF on any later responses, whenever issued.
3.1	Young people are trained in sampling of molesculs in Dorjan lake	Public event/workshop Performed in Dojran; - Volunteering University (FNS) and local high-school students (one Professor and 3 students from local high school in live, including 2 on-line elementary school pupils and their Professor, were present); - Certificates of accomplishment were delivered;

		- Poster was produced and disseminated to stakeholders to be exposed on publicly visible places. Instead of a classic pop-quiz indicated as a "Mean of Verification" to the short-term impact "Increased knowledge of young people on freshwater biodiversity of KBA Dorjan Lake", an interactive discussion as quick question-answer drill was conducted. The GTI Scientific Team and monitored by the local Biology Professor (Ms. M. Lisichkova) performed a brief conformation of knowledge of the young students, gained to the whole-day workshop, comprised of field sampling (boat), site sample preparation, microscope analysis and the final video-beam in-live presentation. This became a basis of validation to (above mentioned) Certificates of Completion, issued to the attendants.

6. Please describe and submit any tools, products, or methodologies that resulted from this project or contributed to the results.

- Production of facilitating materials for workshop(s), as leaflets, presentations, and other such as:
  - Leaflet for Workshop 1: Introduction of the Project;
  - Final Report on the Results (Mollusks), PPR, June 2021;
  - Final Report on Dojran Lake Eutrophication Status; PPR, June 2021;
  - Outlines for Species conservation action plan; PPR, June 2021;
  - Scientific paper: Towards solving the mystery of the long lost endemite *Graecoanatolica macedonica*, Radoman & Stankovic, 1979 in Dojran Lake (Taking into consideration that the presented results in the scientific paper should be accompanied by proper literature review and discussion, its preparation takes time, several proof readings and review from the journal. When ready, the scientific paper is planned to be published in the journal *Ecologica Montenegrina*.)
  - Poster (as a Project product), and
  - Certificates for the attendees and stakeholders

MATERIAL and METHODS

Field measurements included *in situ* determination of the basic physicochemical parameters (temperature, pH, dissolved oxygen, oxygen saturation, conductivity, total dissolved solids) by means of portable equipment Senso Direct 150 Multimeter. The Secchi depth was

estimated by using a field Secchi disk, while the maximal depth was measured with weighted marked line.

For the measurements of basic nutrients and chlorophyll *a*, an integrated water sample was collected by using Ruttner water sampler. For chlorophyll *a* analyses and cell-bound toxin analyses, 250 mL of the integrated sample was filtered through Glass fiber filters GF/C (47 mm) *in situ*, using vacuum filtration device; the filtrate was aliquoted for determination of dissolved toxins and dissolved nutrients (dissolved nitrates, nitrites, orthophosphates). Aliquots of the unfiltered integrated sample was also taken for determination of total nitrogen, total phosphorus and total toxins. All samples (glass filters for cell-bound toxins, glass filters for Chl *a*, filtrate for dissolved nutrients and toxins, unfiltered samples for total toxins, total N and total P) were transported on cold and kept frozen at -20°C till the day of the analysis.

The plankton material was collected by means of a plankton net (pore size 10 μm) by slowly dragging using a motor boat, or by means of vertical column mixing. Benthic mud was collected by using Ekmann grab. One part of the collected material was immediately preserved in 4% formaldehyde, while the other was transported to laboratory on cold for inoculation into Z media for cyanobacterial *in vitro* growth in culture.

In laboratory, the total N, total P, nitrate, nitrite and orthophosphate levels were determined by using spectrophotometer Lovibond Tintometer®, with the appropriate kits (total sets) from Lovibond. Chlorophyll *a* analyses will be performed according to laboratory protocol standard methods (APHA, 1998).

The untreated collected material was transported in the room for *in vitro* culture and isolates from different cyanobacteria will be aseptically inoculated on plates with ½ Z media solidified with 0.8% agarose. By subsequent inoculation, different strains of cyanobacteria were isolated, kept in culture and analyzed for toxin content individually.

All plankton and mud material was analyzed by standard light microscopy. Microphotographs were taken both from native materials, culture isolated strains and fixed samples with Nikon eclipse E800M microscope coupled with Nikon Coolpix 4500 camera. The taxonomic identification of the species were performed by using standard literature.

Lastly, the quantitative detection of cyanobacterial toxins in the lake samples, as well as in the individual isolated cyanobacterial strains were performed by using suitable ELISA kits from Abraxis. More precisely, the Microcystins-ADDA ELISA kit (Product No. 520011) was used for determination of microcystins, the Anatoxin-a (VFDF) ELISA kit (Product No. 520060) for determination of anatoxins, BMAA ELISA kit (Product No. 520040) for determination of BMAA, Saxitoxins (PSP) (EC 2002/225 Compliant) ELISA kit (Product No. 52255B) for determination of saxitoxins and Anabaenopeptins ELISA kit (Product No. 520070) for determination of anabaenopeptins.

During the last fieldtrip (September, 2020), surface samples from the Dojran Lake bed substrate (mud, sediments) were collected from 8 sampling sites. At the Faculty of Natural Sciences and Mathematics - Skopje, the samples were analyzed for their total Nitrogen and total Phosphorus content; moreover, the mud composition of macronutrients, microelements and heavy metals was assessed by using Atomic Absorption Spectrometry (AAS).

In order to achieve all postulated objectives of this project, in the period from 01 June 2020 to 31 May 2022, a comprehensive investigation of mollusks' populations in Dojran Lake was performed. The goal was to determine the status of the possibly extinct snail *Graecoanatolica macedonica*, but also to establish the population status of threatened mussels *Pseudobithynia ambrakis*, *Dreissena presbensis* and *Anodonta cygnea*. Total length of the Macedonian side of Lake Dojran is divided on 48 sampling sites that represent the littoral habitat which is considered as the prime

environment for most of the stated mollusks. Biological samples for collection of the target species were performed by Ekman grab collection and Kick-net sampling. All sampled materials went through precise sediment separation. The present mollusk specimens in each sample were separated, checked under stereomicroscope and marked as alive or dead, depending on whether the whole specimen is present in the sample, or there are only empty shells detected. All specimens were properly labeled and stored for further examination and taxonomic identification. Special emphasis was dedicated to the collected *Graecoanatolica macedonica* specimens. Namely, after separating them into Petri dishes, they were detailly examined, under stereomicroscope. The analyses included shell breaking and checking for soft tissue inside. The analyses continued with making microscope slides out of the mollusk remains and taking microscope pictures with a microscope digital camera.

PART III: Lessons, Sustainability, Safeguards and Financing

Lessons Learned

7. Describe any lessons learned during the design and implementation of the project, as well as any related to organizational development and capacity building.

"Lessons learned" are experiences you have gained that you think would be valuable successes worth replicating or practices that you would do differently if you had the chance. Consider lessons that would inform project design and implementation, and any other lessons relevant to the conservation community. CEPF Lessons Learned Guidelines are available here: https://www.cepf.net/sites/default/files/cepf-lessons-learned-guidelines-english.pdf.

1. *Identification of sampling sites (spots)*: Within the PDP planning period, 48 sampling spots were identified as the most representing ones to the purpose, thorough review of all the previously Studies, Reports and related documents carried out, thus contributing of these sampling spots definition. But yet, it came out that investigations on the current Lake eutrophication status including the artificial channel role in, gave another picture of *G. macedonica* habitats spread. So, always take case of the both climate changes and pollution factors compared with those from previous studies/materials/investigations, when selecting sampling points;

2. Significant water fluctuations have led to drastic habitat loss for mollusks in the lake, thus being the main reason not to detect them in the traditional sampling spots, areas and habitats where they were thriving in the past. Additionally, the documented intensive eutrophication processes in the frame of this project (but also in the past) have also contributed to their slow re-colonization and/or habitat change in relation to more distant habitats from the lake's shore, or in the light of having only empty shells of *G. macedonica* at very limited sampling sites and its disappearance from the traditional ones. Identifying as a possibility to give this Project a bit more sense and contribute towards slightly more precise outcomes, the GTI Team conducted the 6-th field trip=sampling, gaining additional results and knowledge on *G. macedonica*. Final sampling and *in-situ* and lab examinations were done then. Additional research is needed to resolve this issue;
3. *Communication/Stakeholders' Participation:* Apart from a thorough and serious period with preparation activities (all of the Stakeholders were duly informed by e-mails and phone conversations, materials distributed on-hand by Currier), the 1-st Workshop was not

attended by all of the liked and expected visitors. This might had been interpreted through the following:

- Not enough available human resources at Institutions (Ministry, Municipality) to participate, in case of special occasions (Covid-19 pandemic);
- Lack of sense for the Project narrow and wider goals;
- Requirement of harder promo actions by the Team;

Due to 1-st Workshop lesson learned, and attempt to establish denser communication, in terms to more inform all of the Stakeholders of the Project favors, and as-much-as-possible involve them into the Project developments, the Team seriously considered this as a possible constraint and in organization of the 2-nd workshop, a coordination with Milieukontact- Macedonia took place, thus producing much better involvement and interest of joint stakeholders, having Milieukontakt's better experience and relations with. It produced a very well performed and successful joint 2-nd Workshop. Additionally, having the previous experience, the GTI Team performed seriously well-based and prepared 3-rd workshop, having University, local High-school and Elementary school students, attending. It resulted in inclusion of students in boat sampling procedures and dissemination of the Project outputs in later in-dry session.

4. *Interactivity of the Project Team with Local Scientific Institutions:* The initiative to focus on the Dojran Lake endemic freshwater snail opened many opportunities such as collaboration with the local high school in Bogdanci and the Museum of Natural History of the Republic of Macedonia in Skopje. Contacting the high school in Bogdanci introduced the local educational institutions to the efforts of conserving the unique Dojran Lake biodiversity and contributed in raising the awareness about the importance of the lake between the local populations. Concerning the collaboration with the Museum of Natural History of the Republic of Macedonia in Skopje, the Laboratory for Invertebrates at the Institute of Biology in Skopje have been working with the Museum Invertebrates department on many projects and manuscripts before. Thanks to the mutual enthusiasm, the expert team was able to check the museum collection of *G. macedonica* specimens collected in 1989 containing not only shells but soft tissues also.

Sustainability / Replication

8. Summarize the success or challenges in ensuring the project will be sustained or replicated, including any unplanned activities that are likely to result in increased sustainability or replicability.

Regarding the proposed goals of this project, such as: a) to determine the status of *G. macedoniaca* in Dojran Lake, b) to determine the status of other mollusks in the lake, c) to determine the intensity of eutrophication and its consequences on the lake's ecosystem, d) to examine the hitherto known facts of the habitat distribution, species composition and status of mollusks in the lake, we do consider all of our efforts successful.

Gained knowledge in the frames of this Project, offers a solid basis for future applications of similar activities that can be regarded as sustainable. But, having had a very little response from the local self-government or wider public, it sustainability remains in the realm of the future positive prospects regarding the protection of Dojran Lake.

On the other hand, more research is needed to resolve the problem this project has emphasized regarding the extinction of *G. macedonica*. Our results show quite positively that

this small endemic snail has succeeded to survive the unfavorable conditions in some other refugia in the lake's vicinity, mostly on the south side towards Mrdaja locality, where a constant flow of dead fresh specimens are delivered into the lake from. It is from these localities that future research should be based on in order the real source of living snails and their correct status to be determined, as well as a proposal for conservation of this (and many other) species to be proposed.

Safeguards

9. **If not listed as a separate Deliverable and described above, summarize the implementation of any required action related to social or environmental safeguards that your project may have triggered.**

   No recognizable Safeguards issues were raised and/or impacted by the Project during the Implementation period.

Additional Funding

No additional funding apart from CEPF originals/basics applied to this Project.

10. **Provide details of any additional funding that you have secured to support this project.**

    a. **Total additional funding (US$)**

    b. **Type of funding**

    Please provide a breakdown of additional funding (counterpart funding and in-kind) by source.

Donor	Type of Funding	Amount

Additional Comments/Recommendations

11. **Use this space to provide any further comments or recommendations in relation to your project or CEPF.**

    Being a scientific project, the methodology and applicability are easy to replicate taking into account that certain aspects are highly professional and can be only reproduced by skilled scientific workers.
    Relations and facilitation coming from the CEPF RIT is being on a very high level, appreciated very much by the Project team. It is to be noted that the Team would like to thank CEPF RIT for the support through the project implementation.

PART IV: Impact at Portfolio and Global Level

Contribution to Portfolio Indicators

12. In order to measure the results of CEPF investment strategy at the hotspot level, CEPF uses a set of Portfolio Indicators which are presented in the Ecosystem Profile of each hotspot. Please list these below and report on the project's contribution(s) to them.

Indicator	Actual Numeric Contribution	Actual Contribution Description
2.4 Number of Freshwater KBAs in priority CMZ with improved information on biodiversity, shared with stakeholders	1	Within KBA Lake Dojran, MKD04, together with the Balkan endemic freshwater snail *Graecoanatolica macedonica*, the distribution, population status and threats were also detected for few other species such as: *Pseudobithynia ambrakis*, *Anodonta cygnea* and *Dreissena presbaensis*, through this Project developments towards improving information on freshwater biodiversity;

Contribution to Global Indicators

Please report on all Global Indicators that pertain to your project.

13. Benefits to Individuals

13a. Number of men and women receiving structured training.
Report on the number of men and women that have benefited from structured training due to your project, such as financial management, beekeeping, horticulture, farming, biological surveys, or how to conduct a patrol.

# of men receiving structured training *	# of women receiving structured training *	Topic(s) of Training
	2	IUCN Red List Structural Training of Students – GTI Team Members; https://www.iucnredlist.org/resources/online

4	7	Public event/workshop-structural training session of University/High School/Elementary School, combined in-situ/on-line, performed in Dojran In the frame of the project, a scientific workshop has been organized on which students from the regional schools participated. The students have been introduced to working with collected field samples, microscopic observations, as well as to follow the full process of working with the collected material (detection of *G. macedonica* specimens, their transfer to specific lab dishes for evaluation of their viability, etc).
4	9	Total 13

**Please do not count the same person more than once. For example, if 5 men received structured training in beekeeping, and 3 of these also received structured training in project management, the total number of men who benefited from structured training should be 5.*

13b. Number of men and women receiving cash benefits.
Report on the number of men and women that had an increase in income or cash (monetary) benefits due to your project from activities such as tourism, handicraft production, increased farm output, increased fishery output, medicinal plant harvest, or payment for conducting patrols.

N/A

# of men receiving cash benefits*	# of women receiving cash benefits*	Description of Benefits

**Please do not count the same person more than once. For example, if 5 men received cash benefits due to tourism, and 3 of these also received cash benefits from increased income due to handicrafts, the total number of men who received cash benefits should be 5.*

14. Protected Areas
Number of hectares of protected areas created and/or expanded
Report on the number of hectares of protected areas that have been created or expanded as a result of your project. Protected areas may include private or community reserves, municipal or provincial parks, or other designations where biodiversity conservation is an official management goal.

No protected area(s) have been created and/or expanded. Data filled consider basics, presented in the GTI's (the Grantee) initial LoI, Part A.

Name of PA*	Country(s)	Original # of Hectares**	# of Hectares Newly Protected	Year of Legal Declaration/ Expansion	Longitude***	Latitude***
Dojran lake	*North Macedonia*	*2.696*	*N/A*	*N/A*	*41.2218° N*	*22.7510° E*

** If possible please provide a shape file of the protected area to CEPF.*
*** Enter the original total size, excluding the results of your project. If the protected area was not existing before your project, then enter zero.*
**** Indicate the latitude and longitude of the center of the site, to the extent possible, or send a map or shapefile to CEPF. Give geographic coordinates in decimal degrees; latitudes in the Southern Hemisphere and longitudes in the Western Hemisphere should be denoted with a minus sign (example: Latitude 38.123456 Longitude: -77.123456). To obtain the latitude and longitude of your protected area, use googlemap, right click on the center of your protected area, and select "What's here?", and copy the latitude and longitude appearing in the popup window.*

15. Key Biodiversity Area Management

Number of hectares of Key Biodiversity Areas (KBA) with improved management

Report on the number of hectares in KBAs with improved management, where tangible results have been achieved to support conservation, as a result of your project. Examples of improved management include, but are not restricted to: increased patrolling, reduced intensity of snaring, invasive species eradication, reduced incidence of fire, and introduction of sustainable agricultural/fisheries practices. Do not record the entire area covered by the project - only record the number of hectares that have improved management.

If you have recorded part or all of a KBA as newly protected for the indicator entitled "protected areas", and you have also improved its management, you should record the relevant number of hectares for both this indicator and the "protected areas" indicator.

Name of KBA	KBA Code from Ecosystem Profile	# of Hectares Improved *
Lake Dojran	MKD04	*No improvement*

** Do not count the same hectares more than once. For example, if 500 hectares were improved due to implementation of a fire management regime in the first year, and 200 of these same 500 hectares were improved due to invasive species removal in the second year, the total number of hectares with improved management would be 500.*

16. Production landscapes

Number of hectares of production landscape with strengthened management of biodiversity

Please report on the number of hectares of production landscapes with strengthened management of biodiversity, as a result of your project. A production landscape is defined as a landscape where commercial agriculture, forestry or natural product exploitation occurs.

- For an area to be considered as having "strengthened management of biodiversity," it can benefit from a wide range of interventions such as best practices and guidelines implemented, incentive schemes introduced, sites/products certified, and sustainable harvesting regulations introduced.
- Areas that are protected are not included under this indicator, because their hectares are counted elsewhere.
- A Production Landscape can include part or all of an unprotected KBA.

Name of Production Landscape*	# of Hectares with Strengthened Management**	Latitude***	Longitude***	Description of Intervention

** If the production landscape does not have a name, provide a brief descriptive name for the landscape.*

***Do not count the same hectares more than once. For example, if 500 hectares were strengthened due to certification in the first year, and 200 of these same 500 hectares were strengthened due to new harvesting regulations in the second year, the total number of hectares strengthened to date would be 500.*

**** Indicate the latitude and longitude of the center of the site, to the extent possible, or send a map or shapefile to CEPF. Give geographic coordinates in decimal degrees; latitudes in the Southern Hemisphere and longitudes in the Western Hemisphere should be denoted with a minus sign (example: Latitude 38.123456 Longitude: -77.123456). To obtain the latitude and longitude of your production landscape, use googlemap, right click on the center of your production landscape, and select "What's here?", and copy the latitude and longitude appearing in the popup window.*

17. Benefits to Communities

CEPF wants to record the non-cash benefits received by communities, which can differ to those received by individuals because the benefits are available to a group. CEPF also wants to record, to the extent possible, the number of people within each community who are benefiting. Please report on the characteristics of the communities, the type of benefits that have been received during the project, and the number of men/boys and women/girls from these communities that have benefited, as a result of your project. If exact numbers are not known, please provide an estimate.

N/A

Please provide information for all communities that have benefited from project start to project completion.

Name of Community	Community Characteristics (mark with x)							Country of Community	Type of Benefit (mark with x)									# of Beneficiaries	
	Small landowners	Subsistence economy	Indigenous/ ethnic peoples	Pastoralists / nomadic peoples	Recent migrants	Urban communities	Other*		Increased access to clean water	Increased food security	Increased access to energy	Increased access to public services (e.g. health care, education)	Increased resilience to climate change	Improved land tenure	Improved recognition of traditional	Improved representation and decision-making in governance forums/structures	Improved access to ecosystem services	# of men and boys benefitting	# of women and girls benefitting

*If you marked "Other" to describe the community characteristic, please explain:

18. Policies, Laws and Regulations

Report on policies, laws and regulations with conservation provisions that have been enacted or amended, as a result of your project. “Policies” pertain to statements of intent formally adopted or pursued by a government, including at sectoral or sub-national level. “Laws and regulations” pertain to official rules or orders, prescribed by authority. Any law, regulation, decree or order is eligible to be included.

N/A

18a. Name, scope and topic of the policy, law or regulation that has been amended or enacted as a result of your project

No.		Scope (mark with x)			Topic(s) addressed (mark with x)															
	Name of Law, Policy or Regulation	Local	National	International	Agriculture	Climate	Ecosystem Management	Education	Energy	Fisheries	Forestry	Mining and Quarrying	Planning/Zoning	Pollution	Protected Areas	Species Protection	Tourism	Transportation	Wildlife Trade	Other*
1																				
2																				
…																				

*** If you selected “other”, please give a brief description of the main topics addressed by the policy, law or regulation.**

18b. For each law, policy or regulation listed above, please provide the requested information in accordance with its assigned number.

N/A

No.	Country(s)	Date enacted/ amended MM/DD/YYYY	Expected impact	Action that you performed to achieve this change
1				
2				
3				

19. Biodiversity-friendly Practices

Number of companies that adopt biodiversity-friendly practices

Please list any companies that have adopted biodiversity-friendly practices as a result of your project. While companies take various forms, for the purposes of CEPF, a company is defined as a for-profit business entity. A biodiversity-friendly practice is one that conserves or uses natural resources in a sustainable manner.

N/A

No.	Name of Company	Description of biodiversity-friendly practice adopted during the project	Country(s) where the practice has been adopted by the company
1			
2			
…			

20. Networks & Partnerships

Number of networks and/or partnerships created and/or strengthened

Report on any networks or partnerships between and among civil society groups and other sectors that you have created or strengthened as a result of your project. Networks/partnerships should have some lasting benefit beyond immediate project implementation. Informal networks/partnerships are acceptable. Examples of networks/partnerships include: an alliance of fisherfolk to promote sustainable fisheries practices, a network of environmental journalists, a partnership between one or more NGOs with one or more private sector partners to improve biodiversity management on private lands, or a working group focusing on reptile conservation.

Do not list the partnerships you formed with others to implement this project, unless these partnerships will continue after your project ends.

No.	Name of Network / Partnership	Year established	Did your project establish this Network/ Partnership? Y/N	Country(s) covered	Purpose
1	MILIEUKONTAKT Macedonia	2021	Y	RN Macedonia	Data/results/workshops sharing on both familiar and compatible CEPF projects in the Dojran Region. To cherish and develop partnership/cooperation through all the future joint and/or similar projects in the Region.

21. Sustainable Financing Mechanism

List any functioning sustainable financing mechanisms created or supported by your project. Sustainable financing mechanisms generate funding for the long-term (generally five or more years). These include, but are not limited to, conservation trust funds, debt-for-nature swaps, payment for ecosystem service (PES) schemes, and other revenue, fee or tax schemes that generate long-term funding for conservation. To be included, a mechanism must be delivering funds for conservation.

21a. Details about the mechanism

No.	Name of Financing Mechanism	Purpose of the Mechanism*	Date of Establishment**	Description***	Countries
1					
2					
3					

**Please provide a succinct description of the mission of the mechanism.*

***Please indicate when the sustainable financing mechanism was officially created. If you do not know the exact date, provide a best estimate.*

****Description, such as trust fund, endowment, PES scheme, incentive scheme, etc.*

21b. Performance of the mechanism

For each Financing Mechanism listed previously, please provide the requested information in accordance with its assigned number.

NO.	Project intervention (mark with x)			Has the mechanism disbursed funds to conservation projects?
	Created a mechanism	Supported an existing mechanism	Created and supported a new mechanism	
1				
2				
3				

22. Red List Species

If the project included direct conservation interventions that benefited globally threatened species (CR, EN, VU), as per the IUCN Red List, add the species below.

Examples of interventions include: preparation or implementation of a conservation action plan, captive breeding programs, species habitat protection, species monitoring, patrolling to halt wildlife trafficking, and removal of invasive species.

Genus	Species	Common Name (Eng)	Status (VU, EN, CR or Extinct in the Wild)	Intervention	Population Trend at Site (increasing, decreasing, stable or unknown)
Graecoanatolica	*macedonica*	/	Extinct	- Additional field sampling in order to gather more data	Unknown- Although populations with high number of fresh death shells of *G. macedonica* were constantly recorded, no live specimens of this species are registered. Therefore, the population trend of this species is still unknown.

Part V. Information Sharing and CEPF Policy

CEPF is committed to transparent operations and to helping civil society groups share experiences, lessons learned, and results. Final completion and impact reports are made available on our Web site, www.cepf.net, and publicized in our e-newsletter and other communications.

Provide the contact details of your organization (organization name and generic email address) so that interested parties can request further information about your project.

Organization Name: GTI – Geotehnicki Inzenering doo Skopje, RN Macedonia
Generic email address: gti@unet.com.mk