Report of the

FAO/TCF WORKSHOP ON ADVANCED METHODS FOR THE ANALYSIS OF HARD STRUCTURES OF FISH TO ASSESS FISH MIGRATION AND FEEDING BEHAVIOUR IN VIEW OF IMPROVED MANAGEMENT

Emt Camp, Bulgan Province, Mongolia, 7–13 October 2015
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PREPARATION OF THIS DOCUMENT

The first draft of the report of the FAO/TCF workshop on “Advanced methods for the analysis of hard structures of fish to assess fish migration and feeding behaviour in view of improved management”, held in the Emt Camp, Bulgan Province, Mongolia, in October 2015, was prepared by Mr Eldev-Ochir Erdenebat, consultant of the Taimen Conservation Fund (TCF) of Mongolia, together with Mr Andreas Zítek and Mr Thomas Prohaska (both from BOKU Vienna, Austria), resource persons of the workshop. It formed the basis for the present version jointly finalized by the afore-mentioned contributors and Mr Gerd Marmulla, Fishery Resources Officer of the FAO Fisheries and Aquaculture Department in Rome. The opening remarks included in the appendixes are reproduced as submitted.


ABSTRACT

The workshop “Advanced methods for the analysis of hard structures of fish to assess fish migration and feeding behaviour in view of improved management” was organized by the Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Taimen Conservation Fund (TCF) of Mongolia, and held in the Emt camp, Bulgan Province, Mongolia, from 7 to 13 October 2015. Workshop participants included representatives of the Ministry of Nature, Green Development and Tourism of Mongolia, the Environment Protection Department of Khovsgol Province, the Watershed Management Authority of the Delgermurun river, the Watershed Management Authority of Khovsgol Lake and Eg River, the Institute of Chemistry and Chemical Technology of the Mongolian Academy of Sciences (MAS), the Institute of Biology of MAS, the Mongolian National University, the Agricultural University of Mongolia, the Mongolian Educational University, the Egiin Gol Hydro Power Plant Project Unit (EGHPPPU), the private sector and the Taimen Conservation Fund (TCF). The workshop consisted of two parts, i.e. (i) classroom sessions to provide all participants with a broad overview of the background and theoretical aspects of the isotope analysis and (ii) some practical work in the field pertaining to identifying and characterizing relevant habitat structures as sampling sites, sampling of river fish and extracting, preserving and stocking of the hard structures under field conditions as well as water sampling and preservation and stocking of samples. The resource persons presented state-of-the-art knowledge on important aspects of the isotope analysis in fish hard parts with the aim to raise awareness of the power and the benefits of this advanced technique for the characterization of fish behaviour, including migration and feeding behaviour, and to stimulate informed discussions about fish migrations, fish protection, aquatic habitat and living aquatic resources management, and related issues in view of an improved integrated basin management. The ecological objectives underlying the use of migratory information are the protection and restoration of fish populations and, if required in this respect, the request for protection or restoration of the aquatic habitat or access to it, e.g. through the construction of fish passage facilities. The basics of fish ecology, the fish hard parts composition and ecogeochemistry were presented followed by a general introduction to the isotope and elemental analysis of hard structures of fish. The workshop then deepened into the principles and the methodology of analytical techniques for isotope and multi-element measurements related to applications in ecology with a specific focus on aquatic ecosystems and particular emphasis on the Eg/Selenge catchment in Mongolia. Examples of successful application of the isotope analysis in fish ecology in Europe, and implications of the resulting findings for management, were presented and discussed and the usefulness of, and the need for, integrated aquatic ecosystem research in the context of basin management in Mongolia examined. Especially during discussions in the last phase the workshop scrutinised local aquatic environmental issues, potential human-induced threats to the aquatic environment, and especially actual and potential impacts on fish and fisheries in the Eg/Selenge catchment, as well as potential assistance of science in supporting requests for change in environmental management and in designing improved solutions. There was agreement that further aquatic environmental studies would be beneficial to better understand river-related processes and potential implications of planned measures in the Eg/Selenge basin.
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EXECUTIVE SUMMARY

The FAO/TCF workshop, held in Mongolia from 7 to 13 October 2015, was attended by government officials and representatives of the Mongolian Academy of Sciences, universities, the Eg River Hydropower Project Unit, the private sector and the Taimen Conservation Fund. Classroom sessions provided a broad overview of the background and theoretical aspects of the isotope analysis. Practical field work pertained to identifying and characterizing relevant sampling sites, sampling of fish and extracting and preserving of hard structures. Also water samples were taken. State-of-the-art knowledge concerning the isotope analysis in fish hard parts was presented to raise awareness of the benefits of this technique for the characterization of fish migration and feeding behaviour, and to stimulate informed discussions about fish migrations, fish protection, aquatic habitat and living aquatic resources management, and related issues in view of an improved integrated basin management. Local aquatic environmental issues, potential human-induced threats to the aquatic environment, and especially actual and potential impacts on fish and fisheries in the Eg/Selenge catchment, were scrutinised. Potential approaches based on science were discussed. There was agreement that further aquatic environmental studies would be beneficial to better understand river-related processes and potential implications of planned measures in the Eg/Selenge basin.
1. INTRODUCTION

The workshop on “Advanced methods for the analysis of hard structures of fish to assess fish migration and feeding behaviour in view of improved management” was organized by the Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Taimen Conservation Fund (TCF) of Mongolia, and held in the Emt camp, Bulgan Province, Mongolia, from 7 to 13 October 2015. It was organized for Mongolian scientists, including fish/fisheries biologists, river ecologists, geo-ecologists and chemists, as well as for administrators responsible for fisheries or watershed planning and management to familiarize with the advanced methodology and techniques of isotope analysis with the aim to stimulate informed discussions about fish migrations, fish protection, aquatic habitat and living aquatic resources management, and related issues. The workshop consisted of two parts, i.e. (i) classroom sessions to provide all participants with a broad overview of the background and theoretical aspects of the isotope analysis and (ii) some practical work in the field pertaining to identifying and characterizing relevant habitat structures as sampling sites, sampling of river fish and extracting, preserving and stocking of the hard structures under field conditions as well as water sampling and preservation and stocking of samples. The agenda of the workshop appears as Appendix 1 to this report.

2. OFFICIAL OPENING OF THE WORKSHOP

Owing to the fact that the practical field work in relation to habitat recognition and sampling of water and fish was not equally relevant to all participants and for practical reasons, i.e. logistically it would have been very difficult to accommodate all 22 workshop participants on the boats during the field work exercise on the rivers, as well as because Mr Marmulla could only arrive in the Emt camp on 10 October due to other commitments, it had been agreed during the planning phase of the workshop that only twelve scientists participate in the field work and that the official opening of the workshop was then done on day 5, i.e. after the field work part. The workshop was thus formally opened on Sunday 11 October following the gathering of all 22 participants and all resource persons in the Emt camp on Saturday 10 October.

Mr Eldev-Ochir Erdenebat, TCF consultant, who then very ably moderated or co-moderated the workshop sessions called the audience to order for the opening ceremony. In doing so, he made reference to the successfully completed first part of the workshop, i.e. the field work exercise, and expressed his thanks to FAO for allowing this workshop to happen and to Messrs Prohaska and Zitek for having accepted to be resource persons. He then gave the floor to Mr Shirendev Purevdorj, Executive Director of the Taimen Conservation Fund, who opened the workshop formally by welcoming the participants and resource persons to the workshop and by thanking the participants for accepting the invitation to attend this workshop despite their busy work schedules. On behalf of the TCF, he expressed sincere thanks to Mr Gerd Marmulla, Fishery Resources Officer, Marine and Inland Fisheries Branch, FAO Fisheries and Aquaculture Department, for his efforts, assistance and guidance in the preparations of this workshop, and to Mr Andreas Zitek and Mr Thomas Prohaska, both
University of Natural Resources and Life Sciences (BOKU\(^1\)), Vienna, for their availability to share knowledge through lectures and practical advice.

5. In his speech, Mr Purevdorj noted that FAO and TCF had successfully conducted the workshop “Fish passage design at cross-river obstacles – experiences from different countries, with potential relevance to Mongolia” at the Selenge Resort, Mongolia, in April 2014. The important results of this workshop included the recommendation that in case of building the Egiin Gol Hydro Power Plant a fish passage facility, which originally had not been included in the dam construction plan, must be created in order to protect the fish and preserve healthy fish populations. Mr Purevdorj expressed the hope that the current workshop would provide the participants with detailed knowledge concerning new methods for carrying out isotope analyses in water and in fish otoliths to better understand fish migrations and thus improve their knowledge on conducting comprehensive field research studies pertaining to fish ecology. His opening remarks appear in Appendix 3.

6. On behalf of FAO, Mr Marmulla welcomed the workshop participants and conveyed the greetings and best wishes for successful deliberations of Mr Kevin Gallagher, Deputy FAO Representative in Mongolia and Head of the FAO Office in Ulaanbaatar, who unfortunately was unable to attend the workshop due to other urgent commitments. Mr Marmulla expressed satisfaction that another important fish workshop could be held in Mongolia and voiced appreciation to the TCF for the preparatory work, the local logistics and the hosting arrangements, including simultaneous interpretation, made for the workshop. Thereby, he especially valued the personal commitment of Mr Purevdorj and Mr. Erdenebat. Mr Marmulla also noted with pleasure that the workshop participants came from a wide variety of governmental, non-governmental and educational institutions.

7. Mr Marmulla then made reference to the fact that Mongolia, especially in the upstream reaches of the watersheds of its northern and western parts, still had rivers that are essentially in such ecological conditions that can be defined as “pristine” or “close to pristine” or at least as “natural”. Many of these rivers had so far been largely spared from anthropogenic influences such as industrial or agricultural development, water development projects or urban discharges that cause water pollution. However, these intact aquatic environments are under increasing threat from human activities including hydropower development. In this context, Mr Marmulla made reference to the government’s plan to construct a large hydropower dam on the lower Eg River – one of the World’s cleanest rivers – which dewater into the Selenge River, a tributary to Lake Baikal which is the oldest freshwater lake in the world. He stressed that the Selenge River, which is formed by the Ider and the Delgermurun Rivers, and the Eg River, which flows out of Khovsgol Lake, were of great importance for sensitive fish species such as the taimen (*Hucho taimen*) and lenok (*Brachymystax lenok*) – species of high value, e.g. in the sport fishery but also for biodiversity, which are very vulnerable to human-induced habitat modifications.

8. As regards taimen, the largest salmonid in the world, Mr Marmulla pointed out that this fish is dependent on specific habitats for reproduction, feeding and larval growth which can still be found in the upstream reaches of a few rivers in Mongolia and that adult taimen undertake upstream migrations to reach the appropriate spawning habitat while young taimen and spent adult taimen then migrate back downstream. He recalled that, indeed, longitudinal and lateral fish passage in the form of movements or migrations was very important for many – and not only for the long-distance migratory – species to satisfy their physiological needs and successfully complete their lifecycles and to maintain genetic diversity. Especially where under threat, it was now very important that informed management measures were implemented to protect those species. Thereby, comprehensive knowledge of their behaviour, including the migration behaviour, was a basic requirement. However, on a global scale the knowledge of the migration behaviour of many species was scarce and this was particularly true for Mongolia where only recently – namely with the increase of human-induced environmental risks – the importance of the protection of the aquatic habitat and the living aquatic

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\(^1\) BOKU: abbreviation for “Universität für Bodenkultur“.
resources, including the fishery resources, was acknowledged and has led inter alia to the initiation of the studies and knowledge exchange mentioned by Mr Purevdorj.

9. In order to better characterize fish movements for improved management of fish populations, the analysis of isotopes (i.e. atoms of different weight of the same chemical element) in hard structures of fish – a new sophisticated technique that provides complementary evidence of the migration behaviour and that is usable in combination with other methods, e.g. radiotelemetry – has been developed and applied by renowned scientists in many parts of the world. As the ratio of these isotopes can vary in the water of different regions as a result of the different composition of the rocks and their weathering over hundred thousands of years, each river section can have a different pattern of isotopes, and this is then reflected in the isotope pattern of the hard structures of fish, e.g. in the ear stones (otoliths), fin rays or scales, where the isotopes accumulate. In analysing the isotope characteristics of hard structures, the geographical sequence of sojourns of a fish, that are interconnected by the upstream and/or downstream migrations in the river system, can be proven if the stay in different regions was sufficiently long. The isotope analysis also supports the assessment of seasonal habitat uses of different age classes of a sub-population, and can hereby provide essential information for an improved management of fishery resources.

10. Mr Marmulla pointed out that this workshop was organized with the aim to provide an opportunity for a broad spectrum of Mongolian scientists to familiarize with the advanced methodology and technique of the isotope analysis in fish to stimulate discussions about fish migrations, protection of fish as well as aquatic habitat and living aquatic resources management and other related issues. This workshop, which intended to provide the participants with a broad overview of the theoretical and practical aspects of the isotope analysis, was thought to be especially important for Mongolia as various human activities, including intensified industrialization, intensified agriculture, and increased hydropower development, known to have negative impacts on fish and fisheries now also developed very fast in Mongolia. Mr Marmulla then expressed the hope that this workshop would ultimately contribute to an improved and more sustainable management of fish and fish stocks. He ended by thanking Mr Prohaska and Mr Zitek for their availability to serve as resource persons and by wishing the workshop best success in its deliberations. His opening remarks are reproduced in Appendix 3.

3. ISOTOPE ANALYSIS OF HARD STRUCTURES OF FISH

3.1 Fish ecology and ecogeochemistry – practical field exercises

11. The official opening of the workshop was preceded by the practical field exercises in the Er and Selenge Rivers pertaining to identifying and characterizing relevant habitat structures as sampling sites, sampling of river fish and extracting, preserving and stocking of the fish hard structures under field conditions as well as water sampling and preservation and stocking of the water samples. As the practical field work was not equally relevant to all participants and for the practical reasons mentioned above, it had been agreed during the planning phase of the workshop that only twelve scientists participate in the field work exercise (cf. para. 3).

12. Workshop Day 1 started with a welcome to the participants and the resource persons, Mr Andreas Zitek and Mr Thomas Prohaska, by Messrs Erdenebat and Purevdorj. Mr Zitek and Mr Prohaska presented themselves, providing comprehensive information about their educational background, their affiliation (i.e. University of Natural Resources and Life Sciences (BOKU), Vienna), their professional achievements and the current projects they are involved in. They both expressed deep appreciation and satisfaction of being selected to serve as resource persons for this important workshop that can be seen as a milestone in aquatic ecology characterization and assessment training in Mongolia. Also the participants presented themselves and provided relevant personal information.
13. Mr Zitek and Mr Prohaska then gave a brief introduction with regard to the basic ideas that led to the organization of this workshop, making due reference to the importance of the knowledge of behaviour, including migration behaviour, of fish (and in particular taimen) for an improved, integrated and more sustainable management of river basins, water resources and fish (in terms of both species and populations) in Mongolia from a fish ecological point of view, but with broader societal benefits. They explained in detail the scope of the workshop, i.e. to enable workshop participants to familiarize with the advanced methodology and technique of isotope analysis in fish and to gain sufficient knowledge to understand the background and potential of isotope analysis in fishery management with the aim to stimulate further discussions about fish migrations, protection of fish, aquatic habitat and living aquatic resources management, and related issues in Mongolia and to develop and support the application of the isotope method in other river systems of Mongolia independently. They encouraged participants to ask questions whenever clarifications or further explanations were needed and reminded the participants that this workshop should be a truly participatory event, hence depended much on the participants’ contributions.

14. Mr Zitek and Mr Prohaska introduced the workshop agenda and time schedule, with particular emphasis on the course and timing of the delivery of the field training during the first days of the workshop. They also made due reference to practical aspects for the field work exercise and discussed those practical aspects with Messrs Erdenebat and Purevdorj and the workshop participants. They used a step-by-step approach in order to systematically address all important issues (e.g. detailed explanation of the equipment used to collect basic chemical and physical information (pH and conductivity); sampling instructions on how to achieve contamination-free sampling; scientific material and equipment to be packed, loaded and safely stored; the repartition of the participants on the boats; safety instructions) to be considered before embarking on the practical exercise, i.e. the exploration of the Eg and Selenge Rivers by boat for the characterization and selection of the sampling sites and training for sampling. All preparations were carried out in close cooperation between the resource persons and the workshop participants to make it as participatory as possible.

15. In deviation from the original plan to start the river descent on the first day of the workshop, it was agreed to spend the first day in the Emt camp to take advantage of the classroom and the safely anchored boats to give a short introduction to important issues, including safety instructions, to ease the understanding and handling of the practical work. The topics which were addressed included inter alia a very brief introduction to the methodology (isotope mass spectrometry and related techniques); the definition of “isotope” and an “isotopic pattern”; the definition of “elemental and isotopic fingerprinting”; the use and usefulness of the fingerprinting in fish-ecological studies; and the “conservation” of habitat information in fish hard structures and the use of this information in answering questions concerning fish migration, fish stock management and pollution monitoring. Examples from the Danube, where the methodology has been successfully applied, were provided and discussed. One of the examples offered information about fish migration in the Danube and showed how this information was gained by analysing fish otoliths that had “bunkered” the habitat information. The example was also used to introduce the concept of aquatic isoscapes and showed the variations of the strontium (Sr) distribution, i.e. the $^{87}$Sr/$^{86}$Sr pattern, in the river. It thus helped to elucidate the usefulness of this knowledge transfer to Mongolia in view of carrying out – based on this concept – a similar analysis in the Eg-Selenge river system and the use of the potential results in future fisheries management.

16. Sufficient time was also devoted to describe and explain the sampling strategies and to explain and demonstrate the sampling itself. Issues such as how to recognize and select the proper sampling sites in a river (or river system) and how to collect meaningful samples, e.g. of water, were discussed. As regards the selection of the sampling sites, due reference was made to the importance of the local geography and geology, and most importantly the change in geology, and in particular to the location of tributaries as those might bring about a change in water chemistry depending on the geology in their catchments. Moreover, sites needed to be selected according to their importance in fish migration (e.g. spawning habitats). Based on the available map of the river network, the workshop participants, under the guidance of Mr Zitek, then tried to define and preselect sites that
might qualify for sampling and that would have to be further scrutinized when passing by boat on day 2 and day 3. This was followed by hands-on training in the Eg River pertaining to the measurement of water conductivity, temperature and the pH value, the correct sampling of water and the preservation, marking and correct storage of the samples.

17. In addition, two individuals of grayling *Thymallus arcticus* and two individuals of lenok were caught by angling near the Emt camp, and the extraction of otoliths, scales and fin rays was demonstrated to the participants. All samples were cleaned, preserved and stored in an appropriate manner. The otolith extraction procedure, the otolith structure and growth as well as the “preservation” of the chemical information in the otoliths were explained and illustrated. It was also explained how to determine the sex of the fish and how to analyse the gut content. Questions raised by the participants were answered.

18. On day 2, the participants and resource persons descended the Eg River by boat from the Emt camp to the TCF research camp. The preselected sampling sites were visited and thoroughly assessed for appropriateness under the aspects discussed on day 1. Thereby special emphasis was given to tributaries which (i) have water of a different chemical fingerprint as they come from a different geological area and (ii) might be potential spawning habitats and therefore could be of utmost importance for some of the fish species and the preservation of the local biodiversity. On sites retained as sampling points in the Eg River and in some tributaries, the participants then carried out conductivity and pH measurements and took water samples. Also, fish samples were taken by fishing with rods on selected sites. Upon arrival in the TCF research camp, where accommodation for the overnight stay was available, further handling of the samples was done, e.g. subsamples were filled into extra vials to conduct oxygen isotope ratio measurements and then all samples were carefully stored. In addition, the collected data (GPS and physicochemical parameter of the waters of the sampling sites) were transcribed into adequate tables.

19. Day 3 started with a visit to a freshwater spring feeding a tributary of the Eg River approximately 5 km upstream of the TCF research camp. Conductivity and pH measurements were carried out and water samples were taken in the tributary. Then the confluence of Eg and Selenge Rivers was visited, assessed and sampled. As by that time the participants had gained sufficient knowledge and practice with sampling, two groups were formed to assess and sample the Selenge River downstream and upstream of the Eg River confluence independently and simultaneously under the guidance of one resource person. All samples were taken to the TCF research camp for further handling. Upon arrival in the TCF research samples were stored and data transcript was accomplished accordingly.

20. On day 4, the participants and resource persons returned to the Emt camp by boat. There, the water samples were filtered and transferred into fresh vials by the workshop participants under the guidance of the resource persons. An exact numbering of the samples was done and data listed in a table. Then, all data, including earlier assessed data, were transcribed into a computer-based table. The additionally sampled fish (two European perch *Perca fluviatilis* and one lenok from the Selenge River approximately 12 km upstream of the confluence with the Eg River, and one grayling *Thymallus arcticus* from the Selenge approximately 15 km downstream of the confluence with the Eg), were dissected and otoliths extracted; fin ray and scales samples were taken. As already described above, all samples were cleaned, preserved and stored in an appropriate manner.

### 3.2 Fish ecology and ecogeochemistry – classroom sessions

#### 3.2.1 Getting started

21. On day 5, for the first time all participants jointly attended scheduled activities. After the official workshop opening ceremony (cf paragraphs 4 through 10), all participants and resource persons presented themselves in a round-table session moderated by Mr Erdenebat. Both Mr Zitek and Mr Prohaska welcomed now all participants to the classroom sessions and expressed again their...
satisfaction of being resource persons for this workshop. Mr Erdenbat then also presented the workshop agenda which was unanimously adopted without changes. Subsequently, Mr Purevdorj presented relevant information concerning TCF and described in detail the goals, structure and achievements of TCF as well as the future plans and research activities for the protection and preservation of fish species, especially the endangered taimen, in the Selenge River basin. Particular emphasis thereby lies on the stretch from Huvsugol Lake down to the confluence of the Eg with the Selenge River. TCF’s objectives thereby extended from the general research on river fish and taimen protection and rehabilitation to promotion of the development of eco-friendly tourism, with eco-camps, especially in the context of recreational fisheries on taimen. In this respect, he underlined that TCP explicitly promoted the more sustainable taimen catch-and-release fishing. Mr Purevdorj specifically stressed TCF’s cooperation with local, national and international partners from the educational system (e.g. University of Mongolia and universities of the United States of America), the administration and the private sector (e.g. Mongolian tour operators) as well as with TV channels and FAO (e.g. on fish passage issues).

22. Mr Purevdorj presented examples of successful TCF studies and activities that were completed or ongoing. In particular, he mentioned a study on food items ingestion by large fish and fish tagging and telemetry studies in the Eg-Uur-system. He emphasized that TCF activities not only helped protecting the fish but also had a social component, i.e. it benefited families that live along the Eg River as TCF paid salaries to families that engaged in fish protection work. Furthermore, TCF supported families by buying them motorcycles in order for them to better carry out the protection work. Mr Purevdorj then proudly mentioned that now already 21 fish protectors worked in different regions emphasizing, however, that this was not enough but a promising interim result. He furthermore briefly touched upon TCF’s hatchery operations at the Ider and Delgermurun confluence for the production of salmonid fingerlings for stocking rivers. He also made reference to reports, e.g. on monitoring of the fisheries, illegal fishing and research work, that TCF submitted to research partners and ministries. Last but not least, Mr Purevdorj made reference to an agreement between the EGHPPPU and TCF pertaining to fish research in the Eg River in preparation of the construction of the Eg River hydrodam.

23. Asked how serious a problem was IUU fishing on taimen, Mr Purevdorj replied that fortunately IUU on taimen was not a significant problem in the Eg River region due to the intensified patrolling activities in which the protector families, financially supported by TCF, played a key role. Also, the relatively severe monetary fines when getting caught were believed to constitute deterrence. He mentioned that most IUU concerned other species than taimen and that in 2014 only one incidence related to taimen had been reported. Making again reference to the importance of surveillance, Mr Purevdorj said that the taimen population was now relatively stable in this region due to the successful efforts to combat IUU.

24. Before delving into the technical issues of this part of the workshop, both Mr Zitek and Mr Prohaska provided more detailed information on their educational background and their professional work and achievements in river or fish ecology and analytical ecogeochemistry, respectively, as well as on the general focus and work of the University of Natural Resources and Life Sciences (BOKU) in Vienna where they do research work and teaching. Mr Zitek made particular reference to his work on *Hucho hucho*, the Danube salmon, in Austria and promised to elucidate in the course of the workshop how this research also could be benefitting work to better understand issues in the Eg-Selenge river system. Mr Prohaska pointed out that the English name of BOKU made direct reference to “nature” and that the university’s activities focussed on nature and natural processes in the fields of agriculture, forestry, fisheries, bio-technology and environment, with a particular emphasis on protecting nature. Thereby, one priority area was to develop methodologies to better study the environment, with a particular focus on aquatic habitats. He then made brief reference to the Research Group on Analytical Ecogeochemistry (VIRIS)² of the BOKU Department of Chemistry which does work on the development and application of analytical methods to investigate

dynamic processes in biological and ecological systems. Mr Prohaska concluded by stressing that this workshop was designed to provide guidance and assistance to Mongolia in the important attempt to develop strategies for future aquatic habitat research.

25. Mr Zitek briefly recapitulated the scope of the workshop, i.e. to share knowledge about methodologies, methods and techniques, e.g. mass spectrometry, in fish ecology research with the ultimate objective to allow informed decisions to be taken in river basin management. Whether fish would benefit or be negatively impacted by management measures will depend on the decisions taken by the managers, but at least pros and cons of any decision would be transparent and could be discussed in the course of decision-making. He highlighted the importance of the collaboration in the field of the conservation of fish species, especially when endangered, and the application of fingerprint methodology in aquatic ecosystems as a lot of research, on which to build on, had already been conducted in other countries. With particular reference to research carried out in the Danube catchment, he stressed that the principle of interaction between water and rocks was the same everywhere in the world, i.e. the geology, through weathering of the rocks, influences the water characteristics which are then to a certain extent recorded in the fish body. This can be a helpful feature in fish ecology research for fish and fisheries management as was to be shown in the course of this workshop.

26. Mr Zitek than also gave a brief overview of the work and achievements during the field work part of the workshop for the benefit of those who did not participate in the practical field exercises. He mentioned in particular (i) the characterization and selection of sampling sites; (ii) the learning about the chemical composition of the river water and measurements of water characteristics; (iii) the sampling of water for further analysis in Austria; (iv) fish sampling for determination of the relationship between water chemistry and substances accumulated in the fish; and (v) the recording of data in a protocol.

3.2.2 Basic biological and ecological principles relevant in fish migrations

27. Mr Zitek started by explaining why migrations were important for fish and why it was important to understand the significance of fish migrations. Migrations are an adaptation of fish to the most dynamic three-dimensional environment, i.e. rivers, are the result of biological evolution and are important, or even critical, for the survival of the species. Basically, fish can be anadromous (i.e. they ascend rivers from the sea for breeding, e.g. salmon, sturgeon), catadromous (i.e. they migrate from freshwater down into the sea to spawn, e.g. eel) or potamodromous (i.e. they migrate within freshwater only). Migrating allows fish to move to habitats relevant for the completion of their lifecycle or actively avoid unfavourable or bad conditions in their immediate environment. The distance travelled can be anything from only a few kilometres to several hundred or thousand kilometres. Mr Zitek noted that, while the best known migrations were the long-distance migrations of emblematic species such as salmon, sturgeon and eel, also many freshwater fishes require open migration corridors in upstream and downstream directions, as well as in the lateral dimension, in order to complete their life cycle. Reasons for migration include _inter alia_ the change of habitat for spawning, feeding, wintering and shelter and concern adult fish (spawning/post-spawning), juveniles or even larvae (downstream). Most migrations are thus directly linked to the species’ survival.

28. Mr Zitek then cited D.S. Pavlov’s description of “migrations as migratory rings” as fish lifecycles correspond to “rings”. The migrations of different fish species in upstream and downstream direction can take place over different spatial and temporal scales, including long-distance migrations between marine and freshwater habitats. Different fish species thereby might exhibit very different migration patterns due to their different life histories. These different migration patterns evolutionarily developed as a central basis for successful reproduction and maintenance of sustainable populations.

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If these “rings” are broken, e.g. by loss of even only one habitat type or by loss of access to critical habitats due to artificial connectivity interruptions such as dams and weirs as is often the case in regulated rivers, the natural fish behaviour is negatively affected and fish populations might be severely impacted and species endangered or even threatened by extinction. In this respect, Mr Zitek mentioned the example of the effect of a dam on the fish biodiversity in the River Inn, Austria, where 27 out of 29 species had disappeared after dam construction, i.e. 29 species were recorded in 1920, but only 2 in 1983 as a result of dam construction. At different geographic scales, different species might be affected as is the case for the Danube where the long-distance migratory beluga (*Huso huso*, the European sturgeon) disappeared at catchment scale and where at sub-catchment scale for example trout is threatened by river fragmentation, especially the loss of connectivity between the main river and its smaller tributaries that served as important spawning places.

29. Mr Zitek stressed the importance of gaining knowledge of fish behaviour, including migration behaviour, and mentioned that otoliths were an excellent data storage with respect to information on migrations as they bunker chemical elements, e.g. strontium isotopes, and thus can tell much about migrations. He mentioned that he himself and Mr Prohaska were the first experts in Austria to work on otoliths of different fish species to reconstruct migration patterns to discern migration behaviour.

30. Mr Zitek furthermore gave an overview of the methods used to assess fish migrations and presented more results of his work. He pointed out that both capture-independent and capture-dependent methods existed. Capture-independent methods include (i) acoustic and (ii) video monitoring. They also can be used in combination with fish traps to extract fish for further examination. The camera can be installed above the water surface or under water at an obligatory passage that fish have to use, e.g. a weir or a monitoring channel. Counting techniques use infra-red (IR) sensors or hydro acoustics using sonar technology. In particular, Mr Zitek mentioned the Vaki counter and the Didson hydrosonar. Capture-dependent methods rely on fishing with active or passive gears and include (i) telemetry (namely both hydroacoustic telemetry and radiotelemetry), (ii) genetic analyses and (iii) extraction of trace elements and determination of element and/or isotopic patterns. Mr Zitek stressed that this workshop was addressing exclusively the trace-element or isotopic method which are used to discern information about migration stored in fish hard structures such as otoliths, fin rays, bones or backbones. It was discussed in detail that with fin ray clipping or use of scales also non-lethal methods were available to monitor the life record of a fish by the elemental and isotopic signatures stored. Knowledge-sharing concerning other methods would require separate workshops.

31. The ecological objectives underlying the use of migratory information are the protection and restoration of fish populations and, if required in this respect, the request for protection or restoration of the aquatic habitat or the access to it, e.g. by keeping rivers free of obstacles or through the construction of fish passage facilities. Not constructing cross-river obstacles is the best solution while passage facilities would help maintain sustainable fish populations upstream of a dam or a weir or facilitate recolonization of lost river stretches. Stable populations, implying the need for a minimal population size that must not fall below a certain critical limit, are essential to the survival of a species and hence crucial for both fishery and biodiversity whereby biodiversity is one element in sustainable fisheries. Based on the information gained through the analysis of fish behaviour, including migration behaviour, improved and more sustainable management measures can be taken through an informed decision-making process which ultimately benefits fisheries, livelihoods and food security. In this connection, Mr Zitek made reference to the EU-LIFE project “Living space of Danube salmon”. The study had shown that only about 10 percent of the original distribution area of *Hucho hucho* were left and that this fish was thus endangered in the Danube catchment. The study also showed that tributaries were utmost important for the survival of the Danube salmon as there is still spawning habitat available.

32. Mr Zitek continued by explaining that the study results gained in Austria were useful for Mongolia though the species composition in both countries was not the same. The key issue was that the objectives of the studies in Mongolia and in Austria were similar, i.e. protect and restore endangered species, and that the basic principles were the same. Therefore the concept of studies
carried out in Austria can be applied, with some adaptations, also in Mongolia. He then presented the overall fish species-richness map of Mongolia, with 76 native fish being reported in the river systems, pointing out that most of these species were found in the Selenge/Eg area. He also showed the map of threatened species of Mongolia, emphasizing that, unfortunately, most of those (and amongst them *Hucho taimen* and lenok) were likewise found there.

33. The presentation was followed by a discussion during which the participants asked questions. It was *inter alia* asked whether the size of fish matters as regards migrations. Mr Zitek explained that size did not matter and that there was no correlation between the fish size and the distance fish migrate – it was the fish’s strategy that matters. In several rivers, e.g. the Mekong and Danube, small or very small fish migrate far. But also the several-metre-long sturgeons migrated far in the past. As small fish often feed on small prey, e.g. plankton, these fish often chose shallow rivers with low water velocity where the appropriate prey size can be found and ingested. As the fish grow and need bigger prey, they move to habitats with other characteristics. In this connection, Mr Zitek stressed that stomach content analyses were an appropriate means to gain information about habitat use. There was also a question whether fish could be transferred from one watershed to another if the rivers drained to different seas. This is possible in principle but genetics will most likely be different and the transfer might lead to a new genetic mix, with the risk of losing autochthone genetic diversity. Therefore, such activities had to be carefully planned and executed. If there were a decision of transferring fish, this should preferably be based on a high number of parent fish to increase genetic diversity of the transferred fish. In such transfer, bacteria and diseases could be an issue but are usually less of a problem; the real issue is genetics, i.e. being adapted to the new conditions or not.

3.2.3 General introduction to the analysis of hard structures of fish to assess the fish migration and feeding behaviour in view of improved management

34. Mr Zitek started by recapitulating the significance of – the sometimes complex – migrations in fish ecology and briefly recalled the principles of “lifecycle and migration rings”. He then elaborated on the recording of chemical “signatures” in fish hard parts emphasizing that habitat shifts are recorded in fish hard structures, e.g. ear stones (otoliths), by using the chemical fingerprint. By analysing this chemical material, useful ecological information can be gained about the fish migrations, the habitat use, population structure, population size, origin of fish (wild fish or hatchery-raised) and even pollution monitoring but also information about the management of fish release. As regards the need for ascertaining the origin of fish, he gave an example of a practical application, i.e. addressing the problem of escaping fish (e.g. farmed salmon, with different genetics and hence genetic pollution of the wild salmon) and the necessity to identify the owner of the escaped fish for sanctions and remedial measures.

35. Hard parts of fish, that can be used for the chemical analysis, are fin rays, vertebrae, scales, eye lenses and otoliths. They store information in a time-resolved manner from the egg to the capture of the fish. Fin rays, scales and otoliths have all concentric growth but the best studied objects are the otoliths as they show incremental growth usually in distinct concentric rings like tree rings. These “year rings” are created under the influence of different water temperatures of the waterbody or waterbodies and the feeding habits. Mr Zitek then briefly described the growth, function and location of otoliths. He stressed, however, that using otoliths was a lethal method as for the analysis the otoliths had to be extracted from the fish. The extraction of fin rays, or parts thereof, or the use of scales, are non-lethal alternatives but less knowledge is available on the structuring of the zones.

36. Mr Zitek then made reference to the reconstruction of the life history of fish by using Sr isotopes stored in incrementally grown layers of a fish otolith which was for the first time mentioned in a publication4 from 2002. He also described a fish study conducted by himself in Austria that combined genetics and Sr-isotope analysis. The reason for this study was the disliked transfer of fish

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to the River Windbach in Austria where water protection managers of the federal state raised the question whether it was possible to distinguish the transferred fish from the native fish. The study showed that native and transferred fish were genetically different and based on the Sr-isotope analysis and the determination of the Sr/Ca ratios the alien fish could be linked to its original habitat by the chemical information stored in the core, which represents the portion of the otolith grown during the egg phase, and in the adjacent otolith region representing the juvenile fish habitat and developed after hatching and the start of active feeding. Also the season and year of stocking could be determined by the interpretation of the pattern in relation to the year rings. Mr Zitek stressed that up to now not many studies had been accomplished to compare the use of different hard parts for identifying the life history or the population history of fish, e.g. for monitoring invasive species.

37. Being familiar with the fish ecological challenges in the Eg/Selenge river system, in particular with the planned Eg River hydrodam construction, and making due reference to studies carried out in other parts of the world, including Austria, to address similar challenges, Mr Zitek suggested potential research question to be addressed to allow to put basin management decisions on a broader scientific base that includes enhanced knowledge about fish. He said that future work in the Eg/Selenge system could lead to inter alia answering the following questions:

- Are the fish populations of the Eg River and the Selenge River really independent?
- Are the populations of the upper Eg independent of the populations of the lower Eg?
- To which extent do Hucho taimen, lenok and grayling use the tributaries?

38. These questions were not just spontaneous ideas but based on earlier discussions, e.g. on the occasion of the FAO/TCF Workshop on Fish passage design held in Mongolia in April 2014. Apart from the fact that these are very interesting fish ecological questions per se that merit to be looked at for the benefit of stable fish populations, the deeper background is that for example dam proponents challenge the hypothesis that fish from the Selenge River (i.e. the main river) do use, and need to use, the Eg River (i.e. the tributary) to complete their lifecycles as is known for other fish in comparable circumstances in other parts of the world. As regards potential impacts of the planned Eg dam on fish and fish populations, those are easy to deny as long as there is no scientific proof of the contrary, and dam proponents take advantage of this situation. However, there is already ascertained evidence from all over the world that fish from the main river use, and have to use, tributaries, e.g. for spawning and larval growth. This had even become a globally recognized principle in fish ecology. To underline this, Mr Zitek highlighted the importance of small tributaries as spawning places for migratory fish species at the example of the endangered Danube salmon (Hucho hucho). He also cited the FAO/Fishing News Books publication “Rehabilitation of rivers for fish”5 and an Austrian publication6 that showed the problems of the disconnection of tributaries and the consequences for fish populations. Another publication7 was used to highlight the importance of tributaries for Hucho hucho.

39. In the case of the Eg/Selenge system, there is already evidence, as shown by telemetry, that the migration of taimen is more extensive than originally anticipated. Therefore, management of taimen will need to occur at larger spatial and political scales than previously suggested. Recent findings imply that the spring creek Tarvagatai is an important area for reproduction.

40. The suggested questions could be addressed by using radiotelemetry, genetics and isotope/element analysis of hard parts as appropriate. Comprehensive protocols will have to be elaborated and followed. As regards the isotope/element analysis, it is expected that migrations of fish between the Selenge and the Eg can be seen in the otoliths and other hard parts, if the river chemistry is sufficiently different. Similarly, it is also expected that migration between the Eg and its major

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Tributaries can be seen. Answering the afore-mentioned questions will give a clearer picture of the ecology of taimen and other fish in the Eg/Selenge system and their present habitat use, thus help better describe the “status quo” and thus set a baseline as a reference for comparison with future situations. The extended knowledge will also lead to gaining a better understanding of the effects of the proposed Eg dam on the fish and their migrations and inform further discussions. Besides, it will allow for elaborating a population model which is important in biodiversity discussions but also for predicting trends in income-generation, e.g. through recreational fishing and tourism in the fishing camps.

41. Making reference to the behaviour of fish hitting an impassable waterfull and returning downstream, concern was voiced from the audience as to the behaviour of fish when it will hit the Eg River dam. Mr Zitek explained that the dam will – probably severely – change the hydrological characteristics of the Eg, e.g. by water releases at different times of the year and of the day compared to the natural conditions as well as by hydropoeaking to which fish can not adapt. Thus, if no mitigation measures with regard to water release were implemented, fish will not be able to pass the dam, even if a fish pass was constructed. Based on genetic adaptations over evolutionary time periods, fish is, however, bound to spawning in the Eg and if this is not possible fish will return downstream and spawning, if at all triggered, may not be successful. One possibility to reduce the influence of modified discharge conditions is thus to stop hydropoeaking during the migrations or at least to adapt it to the migration cycles. It is extremely important that migrating fish approaching the dam are attracted to the location of the fish pass entrance or entrances. This means that the discharge from the entrance or entrances has to be such that it can be detected by the fish, i.e. it must not be masked by the turbine discharge which is likely to be the case during hydropoeaking. In reply, a member of the Egiin Gol Hydro Power Plant Project Unit pointed out that relevant research was being carried out.

3.2.4 Basic principles of analytical techniques for isotope and multi-element measurements related to applications in ecology with a focus on aquatic ecosystems

42. In setting the scene for a comprehensive knowledge-sharing concerning the strontium isotope analysis, Mr Prohaska started by explaining the basic principles of chemical fingerprinting and provided useful relevant background information. He mentioned *inter alia* that all material on earth was composed of chemical elements and that, strictly speaking, even humans and fish were nothing but a composition of chemical elements. The assumption is that the “decision” to have chemical elements of different properties and eventually the formation of matter “was taken” when the “big bang” happened. The important fact for us today is that all life history is recorded in chemical compositions and if those can be analysed or “read”, the history folds down before our eyes. To underline these statements, Mr Prohaska showed the table of chemical elements, mentioning that everything has a fingerprint and that the information of a fingerprint was translated into a fingerprint. And if the fingerprint can be read, it is possible to backtrack the fingerprint. He explained that this works in the same way as the dermal papillae (finger lines) that act as fingerprint and leave a fingerprint on each item that is touched. In a similar way, the chemical composition of the natural habitat (including the water) is a specific fingerprint which leaves a fingerprint on everything that stems from this habitat, i.e. a fish has this chemical composition (with an element or isotope pattern as fingerprint) incorporated in its hard parts. There, it can be read as fingerprint, which can be directly linked to the habitat.

43. In order to better understand the chemical composition with respect to element and isotope pattern, Mr Prohaska went on by explaining that the atom, i.e. the smallest unit that contains all information about an element, was usually composed of the nucleus with a defined number of protons and a varying number of neutrons, and the electrons which surround the nucleus in a stable flight path. The number of protons determines the property of the atom; the number of neutrons in the nucleus does not change the chemical property but only the mass. In nature, therefore, exist some elements that contain atoms that differ in mass but are chemically identical, i.e. the so-called isotopes. With a neutron, i.e. a mass, added, the atom still belongs to the same chemical element but becomes a different isotope of different mass. Of some elements, more than one isotope can exist. Mr Prohaska
then also mentioned that it was Mr J.J. Thomson in 1913 who first succeeded in separating isotopes by means of a mass spectrometer. The ratio of the amounts of isotopes gives the so-called isotope ratio. Even though in many cases believed as constant in nature, this isotopic composition (and thus the isotope ratio) changes due to different natural chemical, physical and biochemical processes. The knowledge of the change in the isotopic composition can serve as information for these processes. Moreover, these processes result in a variation of the isotopic composition of one element in different regions or zones. This isotopic composition can act as a site-specific fingerprint. This fingerprint can be transferred as fingerprint to any component that belongs to this region or zone either without significant alteration (e.g. the Sr isotopic system) or with a specific and constant shift in the isotopic composition depending on the transfer process (e.g. for sulphur (S)). This is called “intrinsic marking” which is a great feature to be used in environmental studies. The assessment of this isotopic composition requires special analytical tools, in most cases based on mass spectrometry. To study the spatial distribution of an element and isotope pattern in solid materials (e.g. fish hard tissues), a laser ablation system can be directly coupled to an elemental or isotopic mass spectrometer. This allows for the assessment of the chemical composition down to a range of 2 μm. The retrieved chemical data can be transformed to a chemical image of the investigated surface by adequate software tools (e.g. ArcGIS).

44. In making reference to the natural feature of biochronology of organisms, i.e. storage of life history information over time, Mr Prohaska provided detailed insight into the isotopic fingerprinting of carbon (C), oxygen (O), hydrogen (H), nitrogen (N) and sulphur (S), with particular emphasis on the oxygen. He explained that the process of changing the isotopic composition of oxygen was primarily temperature-dependent. Since the incorporation of oxygen into calcifying hard parts (e.g. carbonates) is temperature-dependent, the isotopic composition in samples shows a change in the oxygen isotopes as a function of the ambient temperature. Therefore, oxygen isotopes can be used to trace habitat use (provenance) and habitat change but also temperature changes in water system where the organisms lived. Furthermore, the isotopic composition of carbon and nitrogen contained in organic material are used to provide evidence of the food web of organisms but also to demonstrate (natural or human-induced) changes that happened in such food webs.

45. With particular emphasis, Mr Prohaska then described the strontium (Sr) isotopic system and highlighted its importance in aquatic ecgeochemistry. Strontium is common and omnipresent in nature. Due to the weathering of rocks, Sr is constantly released into the water. The element has four stable isotopes, i.e. $^{84}\text{Sr}$, $^{86}\text{Sr}$, $^{87}\text{Sr}$ and $^{88}\text{Sr}$. As $^{87}\text{Sr}$ originates from a radioactive decay of $^{87}\text{Rb}$, it plays a particular role. Whereas the quantity of $^{84}\text{Sr}$, $^{86}\text{Sr}$ and $^{88}\text{Sr}$ does not vary in nature, the quantity of $^{87}\text{Sr}$ does, dependent on the original geochemical composition and the geological age. As a consequence, the ratio of the radiogenic $^{87}\text{Sr}$ to all other Sr isotopes varies, too. Usually, the natural variation of the ratio of $^{87}/^{86}\text{Sr}$ is used as specific fingermark and geologically different areas have a distinct difference in the $^{87}/^{86}\text{Sr}$ isotope ratio. This fingermark is released through the weathering of rocks and transferred via the water into plants and via the food chain into animals and humans. Sr shows a high chemical similarity to calcium (Ca) and, as a consequence, Sr can be found in significant concentrations especially in Ca-rich structures. In fish, this fingermark of the surrounding habitat, i.e. the water, is incorporated into calcium-rich structures such as hard parts, e.g. otoliths, scales and fin rays. Incrementally grown structures such as otoliths or fin rays incorporate this site-specific fingermark in a time-resolved manner. Therefore, the Sr isotope ratio has a high potential for tracking habitat changes of fish.

46. Mr Prohaska also described other isotopic systems, e.g. lithium (Li), magnesium (Mg), silicon (Si), copper (Cu) and zinc (Zn), which have a distinct potential in ecosystem research but are less frequently applied. A special role plays lead (Pb) which is a good tracer of anthropogenic impact (e.g. pollution).

47. Mr Prohaska continued by introducing the concept of isoscapes, i.e. “isotopic landscapes”, which is the visualization of the geographical distribution of isotopes. Examples of oxygen and strontium isoscapes, the latter from Austria, were shown. Isoscapes can be used to predict the
provenance of a product, an animal or even a human. A special type of isoscape is the aquatic isoscape from rivers and lakes as it can be used to identify the provenance of hard structures of fish with a specific isotopic composition. In addition to the two dimensional picture, the isoscapes can be divided in vertical iso-zones. Mr Prohaska said that samples taken during the practical part of the workshop and already existing samples that could maybe be made available might lead to the creation of a strontium isoscape, the first aquatic isoscape in Mongolia.

48. Finally, the use and application of enriched (natural) stable isotopes for spiking and tracing was explained on the example of fish spiking. In this context, an enriched isotope (spike) is used. The introduction of an enriched isotope alters the natural isotopic composition and leaves therefore a specific fingerprint which can be unique for one sample. As a consequence, this enriched isotope can be used as marker (“extrinsic marker”). As example, a fish spiking study was presented which was conducted under the guidance of Mr. Zitek. A mother fish was administered a solution (which was an isotopic NaCl solution) containing a natural, not radioactive isotope spike of $^{84}$Sr and $^{86}$Sr with distinct isotopic composition. The total Sr concentration corresponded to the Sr concentration which can be found in natural waters. The specific composition of the two isotope spikes results in a unique marker which can be identified. In the particular example, the isotope spikes which were administered to the mother fish got into the eggs and as a consequence ended up in the core of the otolith of the juvenile progeny. Therefore, in the core of the otoliths of these juvenile fish, that is already created during the egg phase, the specific isotopic composition could be retrieved by means of LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry) and after the deconvolution of the isotope pattern the information could be linked directly to the mother fish of this particular individual.

49. At the end of the presentation, various questions were asked by the audience and answered. Some questions centred on the isotope analysis in relation to the issue of habitat use of fish, including habitat use at the larval stage, and the reliability of the results. It was inter alia clarified that the strontium isotope analysis can show that fish migrated but can not give the answer as to why they migrated. Thus the isotope analysis was compared to a passport, i.e. the stamp in the passport says where the bearer has been but does not give the reason for the stay. Also the frequency of migration can be derived from the growth rings of the otoliths. It was further clarified that the information concerning the youngest age, i.e. larval stage, of the fish was recorded in the core of the hard structure, e.g. the core of the rings of a scale.

50. Furthermore, information was provided on the applied instrumentation. The four different isotopes of strontium are distinguished by mass spectroscopy that sorts the resulting ions based on their mass-to-charge ratio. The answer concerning reliability was that the method was reliable but care has to be taken with regard to several points including the need to (i) ensure the use of the appropriate technology; (ii) use a validated technique; (iii) properly evaluate each potential source of uncertainty; (iv) carry out quality controls with certified reference materials; and (v) avoid falling into routine.

51. An additional question targeted the use of the method for the Eg River context, in particular the potential spatial resolution and the required sampling density. In reply, it was explained that the number of sampling sites depended on the geological variation and the resulting change in the isotopic composition. The accuracy of the results depended on the resolution, which in terms depends on the variation of the chemistry, i.e. if significant changes in the chemistry occur at short distances, the distinction of smaller areas is possible. In other words, if there is no change within a river system, no alteration is seen in the isotopic composition of a fish otolith, irrespective of whether the fish did migrate or not. Summing up, the sampling density depends on the variation of the geology in the catchments of the tributaries and on the discharge of the tributaries. In many cases, the position and number of the data sampling points was based on experience. Mr Zitek added that after a first visual assessment it was believed that the differences between the Eg River and its tributaries were sufficiently high so as to distinguish whether fish sojourned in the Eg, in the tributaries or in the Selenge, or migrated between the three. By combining telemetry studies, that allow for a very high spatial resolution (i.e. up to 1 m), and the isotope analysis, a more complete picture of the migration and habitat use behaviour can be gained.
3.2.5 Basic principles of methods for the assessment of freshwater fish migrations by isotopic and elemental patterns in hard parts, making reference to relevant examples

52. To start with, Mr Zitek deepened into the principles of elemental and isotopic distribution in the environment and in fish on the example of studies carried out in the Danube in Austria. He recalled the transfer path of the Sr isotopes from the geology into living organism via the substitution of Ca by Sr and the importance to understand the geology in the catchment. He stressed that it was very smart to use the differences in elements and isotopes for environmental and fish behavioural studies. Thereby, the first step was to take water samples and see if there was a difference in isotope composition that could be exploited. The second step would then be to see if this difference is also reflected in fish hard parts and, if so, utilize it for habitat use and behaviour characterization of the fish. He mentioned that in Austria this type of study had been carried out with one-year old salmonids that had an otolith size of 1 mm. Water and fish samples were taken from the Danube and the tributaries, and the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio determined, underlying available geographical information. Based on the isotope analysis, it had been possible to distinguish the places of sojourn of fish with a time resolution of two months. He pointed out that the water analyses showed that the Danube tributaries were significantly different from the Danube itself and that the signature of the upper Danube was different from the middle Danube (till the confluence of Inn River and Danube).

53. In this context, Mr Zitek also emphasized that a similar study conducted in Eg-Selenge system would certainly likewise show the great importance of tributaries for the migrating fish in Mongolia. In stressing again the need for knowing the geological characteristics of the Eg and Selenge catchments, he encouraged the participants to further support the ongoing assessment by making all efforts to get hold of a geological map of the region.

54. Mr Zitek then continued by lecturing on the assessment of the basic water chemistry and physics, with emphasis on on-site measurements, e.g. conductivity, pH-value, temperature and oxygen content. Conductivity measurements give a good indication of the total amount of dissolved ions whereas pH measurements determine the content of hydronium in water, with the pH-value being an important indicator for the quality of the aquatic environment as habitat for fish. He mentioned that the optimum for fish was pH 7 but that fish can well adapt to some variation of pH in the range of pH 6 to pH 9.

55. Mr Zitek further made reference to the property of water to dissolve substances and explained fishes’ osmoregulation, i.e. the uptake and excretion of ions. The uptake is mainly done via the gills, e.g. about 80 – 90 percent of the calcium taken up by freshwater fish come through the gills. Freshwater fish do not drink but excrete a large amount of water. As fish can take up only dissolved ions, the presence of dissolved ions is an important factor in the living conditions of fish. For the isotope analysis this means, however, that water samples have to be filtered to exclude particulate matter that might have a different elemental/isotopic composition. Even though only part of the strontium is taken up, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio is not influenced significantly. Interestingly, little sodium is stored in the otoliths due to the metabolic filter and thus sodium cannot be used as a direct proxy for the habitat. Heavy metals, e.g. cadmium and lead, are known to accumulate in gills and liver, but are not transferred in significant amounts to the otoliths.

56. To underline the importance of the isotope analysis in environmental, fish-ecological and fish behaviour studies, Mr Zitek presented practical examples, inter alia from the Danube where, based on the Sr isotope ratio technique, the migration of chub from a Danube oxbow into a tributary across a normally unpassable barrier was demonstrated to have occurred during a high flow event. Another practical application was the tracing back to the origins of two charr populations that exhibited different growth rates in a hatchery in Austria. Through the Sr isotope analysis based on material from the cores of the otoliths, that contain the chemical signature of the mother habitat, it was possible to reveal that the faster-growing fish had been imported from Sweden. This is again an example that the fish’s history was recorded in the otoliths.
57. The session ended, as usual, with a round of questions and answers.

3.2.6 Basic technical and analytical principles of the analysis of fish hard parts to assess their elemental and isotopic content by using direct solid sampling techniques (e.g. micro drilling, Laser ablation ICP-MS, SIMS, XRF)

58. Mr Prohaska started his presentation by describing the analytical methods used in analyzing the elemental and isotopic composition and highlighted the difference between solution-based analyses which are performed on water samples and solid-sample analyses. He briefly described the sample preparation where there are basically two options, i.e. (i) the direct analysis of the hard samples by ablating material via a laser beam (laser ablation) or (ii) dissolving the material. In the laser ablation for the analysis of fish hard structures (e.g. otoliths), small parts of the hard structure are introduced into the laser cell where the laser “cuts off” extremely small particles. This is called “ablation”. Then the carrier gas argon takes these small particles to the analyser for the isotope analysis. As regards the second option, the material can be dissolved by digesting the samples using nitric acid (HNO₃), sometimes mixed with hydrogen peroxide (H₂O₂). The raw material is usually pulverised by milling or drilling with extremely small drillers (i.e. the diameter of a couple of micrometres).

59. The major techniques applied for the detection of the elemental composition are atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). These techniques have different limits of detection with ICP-MS being the most sensitive technique. Isotope ratios of Sr are usually assessed by thermal ionization mass spectrometry (TIMS) or ICP-MS. The isotopic composition of C, O, H, N and S is usually assessed by using isotope ratio mass spectrometry (IRMS). Solid samples (e.g. fish hard parts) can be analyzed directly using for example XRF (X-ray fluorescence spectroscopy), the (Nano) secondary ion mass spectrometry ([Nano]-SIMS), LIBS (laser-induced breakdown spectroscopy) or laser ablation ICP-MS (LA-ICP-MS). For determining isotope ratios, the direct analysis of solid samples is accomplished by SIMS and multicollector ICP-MS (MC ICP-MS). Mr Prohaska then mentioned that as regards the cost ICP-MS is the most expensive technique with regard to both purchasing and operating.

60. Mr Prohaska also explained in detail the general principle of the functioning of a mass spectrometer with (i) the sample introduction, (ii) the ion source used for the ionization of the atoms in the samples, (iii) the mass separator to separate the ions with respect to the mass/charge ratio, and (iv) the detector to detect the number of ions which are directly proportional to the number of atoms introduced into the ion source.

61. There were questions asked concerning the quantity of material needed, the time needed for analysis and the cost. Mr Prohaska answered that only a few millilitres of liquid samples were needed and spot sizes for laser ablation vary between 2 and 200 μm. The time needed varied between some minutes (e.g. for analyzing water) and one day (for imaging a whole otolith) and the costs varied between 30-50 US dollars per sample for the elemental analysis of water samples and approximately 250 US dollars per sample for laser-ablation based Sr analysis (regular price).

3.2.7 Detailed explanation of the use of different hard parts of fish for tracing migration and evaluating the actual habitat status including sampling with an emphasis on non-lethal methods

62. In this presentation, Mr Zitek deepened into the existence of different types of hard structures, e.g. otoliths, backbone, eye lenses, fin rays and scales, in fish and described their shapes, their chemical composition and their suitability for the isotope analysis. Otoliths, consisting of calcium carbonates, are usually growing like a tree trunk, i.e. in rings, and it can be distinguished between non-transparent rings grown in summer and those ones grown in winter, being more or less transparent. The counting of the pairs of rings (usually only the winter rings are counted) allows for the age determination of the fish. Contrary to otoliths, fin rays, vertebrae and scales consist all three of
calcium phosphates and have a metabolism different from the otoliths. Theoretically, they can also be used for the isotope analysis but it has to be taken into consideration that under stress, e.g. if there is not enough food available to the fish, elemental content from these hard structures can, at least partly, be resorbed\(^8\) which means that the information originally stored can be partly lost. In contrast, the otoliths store the information for ever as they do not undergo any degrading changes and thus can be used as life recorder. However, otolith and backbone extraction are lethal methods and fish are lost from the population. Scales and fin rays can be extracted without killing the fish and can therefore be used as a non-lethal sampling alternative. Thereby, one has to be conscious of the potential risk of loss of information as mentioned above and each case should be evaluated for the importance of incomplete information due to the different information storage capacities of the different hard parts. The eye lens, sometimes also explored for its potential to store habitat information, is a very special hard part and was not further dealt with during this presentation.

63. Mr Zitek alluded to the need for strict laboratory protocols for the extraction, preparation and handling of the hard parts before the isotope analysis. He further made due reference to the laboratory equipment needed. A balance for weighing and a scale for measuring the fish as well as extraction tools for extracting the hard parts are basic utensils in each fish otolith laboratory. A microscope with a thousandfold magnification is required. Documenting and imaging all hard parts has to be performed as a routine. He then recalled that there are two methods for determining the length of fish, i.e. measuring the fork length or total length of the fish. Either method is fine but it is critical that the laboratory report mentions which method was used. Weighing and gender determination of the fish are a must.

64. Then Mr Zitek described in detail the extraction of the otoliths, a lethal sampling method. After removal of the gills and gill arcs, the otolith is well visible for extraction from underneath. The extracted otolith is cleaned in water and stored in a properly labelled vial, mentioning the species name, the weight and length of the fish and the name of the river from which the fish was taken. For further investigation by LA-ICP-MS, the otolith is then glued on a glass slide using superglue (“Krazy glue”) or thermoplastic resin (Crystalbond™ 509). After this fixation, the otolith is grinded with a lapping film of 30 μm and 3 μm grain size in order to expose the core of the otolith. Care has to be taken not to miss the core. The otolith is then sonicated, i.e. cleaned in ultrasonic high-quality (HQ) laboratory reagent grade-I water (corresponding to a resistivity of 18 MΩ cm) for 5 minutes and the grinded otolith affixed on the glass slide is transferred onto a bigger glass slide which can carry about 60 small slides and can be put in the laser chamber for subsequent analysis of the otoliths.

65. Another possibility of obtaining testing material is the non-lethal extraction of scales and fin rays. As regards the extraction of scales, it has to be determined from which body location the scales that grow in layers can be taken. The biggest scales should be removed in order to ensure maximum gaining of information. It is noted that in different species the biggest scales are found in different body locations. Scales can be extracted by using tweezers or a knife. Scales are stored in paper envelopes which have to be labelled properly (cf. para. 75). For the LA-ICP-MS measurement, which can be performed directly on the samples, the scales – after cleaning them – are affixed with double-sided tape on the glass slide and pressed to flatten them. The extraction of fin rays is seen as an excellent alternative as the first and second fin ray are supposed to regenerate in the fish. They can be clipped close to the muscles and stored in properly labelled paper envelopes. Before the analysis, they are cleaned and embedded in epoxy resin. The hardened blocks are cut to very thin slides with a low-speed saw. After affixation on a microscope glass slide the growth patterns can then be analyzed by LA-ICP-MS by looking at the fin ray cross section.

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66. Mr Zitek then presented information on the Sr content in different hard parts. As an example for the evaluation of the correlation of the Sr content in otoliths and other hard parts Mr Zitek presented a study on brown trout\(^9\). It can be clearly seen that the Sr concentration is only correlated in otoliths and fin rays; in the scales was found only approximately one third of the Sr content found in otoliths. A study by Clarke and his co-workers\(^{10}\) also showed that the life history as fixed in the otoliths is in good correlation with the information in the fin rays but it was almost impossible to retrieve the same information in the scales. Mr Zitek pointed out that this has been confirmed by his own studies. He also recalled that fish under stress can resorb chemical elements from the scales which can false the results (cf. para. 62). Nonetheless, more work will be accomplished on scales since these prove to be the easiest accessible hard parts and are available through non-lethal extraction. However, it has to be kept in mind that when fish lose scales those are usually regenerated which means that time-bound information can be lost. To avoid missing information, always more than five scales should be taken as there is always the risk that regenerated scales might be taken. In summary, using otoliths is a more trustworthy method but the fish are killed.

67. In addition, the phenomenon of abnormal otoliths was presented. These otoliths are very transparent and shiny, have a high magnesium (Mg) and a low Sr content, do not show distinct growth patterns and are usually found more abundantly in farmed fish. The described type of abnormal otoliths is, however, more frequently seen in fish from conventional fish farms as compared to organic fish farms; it is (almost) not found in natural fish. The stress in fish farms in combination with other hatchery-related factors (e.g. food) is supposed to be the reason for the formation of these abnormal otoliths.

68. Participants asked questions with the aim to deepen the knowledge concerning the cleaning, preparation and fixation of the hard parts and the background information. Mr Zitek answered all the questions providing some more detailed insights. For example, he confirmed that grinding paper could be used but it was better to use a special grinding film as this allows for more precise grinding. Also, normal grinding paper would stick to the otoliths which has to be avoided in order to not contaminate the otoliths. He also further elaborated on the cutting of fin rays, saying that it was standard nowadays to use one to three slices from a location close to the base of the fin ray. For a very young fish this was particularly critical as the life information is best or even only contained in the part close to the base. It is also possible to remove only the upper part of the (pectoral) fin rays in alive adult fish.

69. Answering the question of a participant asking where the information that starving fish can resorb “food” from the scales was taken from, Mr Zitek told that this was published in scientific literature concerning fish metabolism, pointing out again that otoliths, scales and fin rays followed different metabolic processes\(^{11}\). The visible difference between a normal scale and a regenerated scale was explained, i.e. regenerated scales usually have a very diffuse zone in the centre whereas normal scales show a clear structure. In reply to a question concerning the cost of an otolith laboratory, Mr Zitek said that about US dollar 20,000 would have to be budgeted. Practical suggestions for setting up an otolith laboratory in Mongolia were made and discussed.

3.2.8 Standard operation procedures in analytical ecogeochemistry with a focus on trace element and isotopic analysis in aquatic ecosystems including sampling, sample preparation and measurement

70. Mr Prohaska started with the description of a typical set-up of a chemical laboratory and the conditions and prerequisites for operating. Always a key prerequisite is a very clean laboratory environment and pure chemicals. Any contamination has to be avoided. In fact, the chemicals used

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\(^{10}\) Clarke, A. D., K. H. Telmer and M. Shrimpton (2007). Elemental analysis of otoliths, fin rays and scales: a comparison of bony structures to provide population and life history information for the Arctic grayling (Thymallus arcticus). Ecology of Freshwater Fish 16: 354-361.

should be purchased in the purest form commercially available or if this is not possible (also if the purest form is very expensive), they have to be further purified. Special consideration was given to the purification of water and nitric acid. Tap water is purified in several steps. The first step is reversed osmosis (RO) which gives pure water; in the second step this RO-water is purified to high-quality water (HQ water) by using water purification based on membrane filtering and ion exchange. The last step is subboiling by distillation which is the surface evaporation of solvents below the boiling point. During the purification process almost all trace elements are removed or at least their presence is reduced by several orders of magnitude. Mr Prohaska then explained the apparatus used for the subboiling of acids and water which usually consists of ultrapure quartz or perfluoroalkoxy alkanes (PFA). The PFA systems are cheaper but less efficient (in terms of quantity subboiled per unit of time) but both produce the same quality of purified water or acid. He stressed that the grade of cleanness that is needed has always to be well considered.

71. When discussing the reagents, hydrofluoric acid (HF) was mentioned. Hydrofluoric acid is very corrosive and toxic but necessary for the total digestion of soils and rocks, especially when those contain silicates. Special care has to be taken and the handling has to be left to experts.

72. Mr Prohaska continued by explaining the cleaning of tools. He emphasized that also clean pipette tips were important which was not always respected. However, each source of contamination has to be avoided. Glassware should never be used as glass has a surface that absorbs elements via the free Si-O bonds; only plastic (preferably PFA or PTFE (Polytetrafluoroethylene)) but also low density polyethylene (PE), being very clean and resistant, should be used (except for mercury analyses). Attention has, however, to be paid not to use bottles made of recycled polyethylene. If under certain circumstances no appropriate plastic containers are available for taking water samples, then exceptionally also drinking-water bottles may be used (note: chemicals, however, MUST NEVER be stored in food containers!). In case of bottles from uncontrolled sources, always a minimum of three bottles have to be filled and one empty bottle for reference has to be set aside to later test the “background pollution” of the bottles. In any case, it is critical to always mention the exact handling procedure in a report.

73. Another important point is that the logistics have to be well planned and prepared, i.e. it has to be defined exactly what is needed in which moment of the process. The whole process has to follow a strict protocol in several steps. The first step is the selection of the tools (e.g. vials, bottles, etc.). Then comes a first cleaning step during which acid-resistant gloves (even in multilayers) have to be worn. All tools which are used in the laboratory should undergo a specific cleaning process consisting of baths of 10% and 1% HNO₃ (i.e. mixtures of concentrated HNO₃ and HQ water), respectively. Bottles have to be soaked and left immersed for 24 hours. The washing solution has to be changed after ten times of usage. He also mentioned acid-steam cleaning of re-usable tools, cautioning the handling of hot acid. Tools are rinsed by HQ water in between and dried under clean conditions, i.e. on a clean bench using purified air. The last step is to store the clean tools in clean zipper bags in a clean room.

74. If possible, trace-element analysis should be conducted under clean-room conditions for which the class of cleanness is given usually by the number of particles per square foot. Mr Prohaska then explained the standards of a clean room and the procedures for its use. The first rules were not to take any contaminants inside and to keep the number of people inside the clean room to a minimum. With the specific clean-room air filter system all particles are pressed to the floor by an airstream from above. Since these particles are migrating on the floor, all extra movements and especially all quick movements should be avoided as much as possible in a clean room. Filtering of air is accomplished by Ultra Low Penetration Air filters (ULPA filters). Personnel have to be trained to gain awareness that a clean room was really a very specific environment. Specific rules of behaviour (e.g. to wear gloves, to protect samples, to avoid moving of particles, to keep bottles closed as long as possible, personal hygiene, etc.) have to be respected. If no clean room is available, measures have to be taken in order to avoid possible contamination during sample handling and the above rules have to
be applied. Building a clean room is certainly expensive, i.e. approximately starting from US dollar 1 million, but pays off in terms of the quality of the results of the analyses.

75. Another crucial issue is sample labelling. All samples have to be carefully and clearly labelled. The labels have to show (i) the name (code) of the sample, (ii) the project name or code, (iii) the matrix (e.g. in 1% HNO₃), if applicable, (iv) the date of sampling, and (v) the name of the person who took the sample. Utmost care has to be taken that the label is not damaged during handling and transport. The following measures have to be taken when sampling water: (i) take utmost care in well closing the sample bottles, (ii) store the bottles in single polyethylene (PE) zipper bags (upright), (iii) store multiple samples of the same kind in larger PE zipper bags, (iv) store samples at 4 °C or in the freezer (note: acidified samples can be stored at room temperature for a limited amount of time).

76. Before the analysis, the samples usually have to be processed, e.g. by milling, drilling or digestion. Some samples, (e.g. scales), can be used directly without any further processing and analysis is done by direct solid analysing techniques. Water samples have to be filtered and maybe even acidified. Then the analysis of the elemental or isotopic composition is accomplished in the appropriate laboratory infrastructure. Liquid samples, i.e. water or aqueous solutions can be analyzed directly. For some analyses solid samples have to be transformed into a liquid form by digestion before the analysis. The analysis itself is done by the elemental or isotopic analytical techniques (which were explained previously, cf. chapter 3.2.5). In addition, it was explained, that liquid samples are separated from their matrix with a Sr specific resin before Sr isotopic analysis, using MC-ICP-MS in order to guarantee an interference-free analysis.

77. Questions were raised to further clarify issues and deepen the understanding. Concerning the maximum time samples can be stored before an analysis, it was explained that there was basically no time limit as along as there was some sampled material left, no new material added and the material protected from sun light. There was affirmation that, contrary to drinking sea water, purified water can be drunk by humans without any harm for the health; it was, however, specified that drinking purified water will, of course, not supply the body with minerals.

3.2.9 Detailed explanation of requirements for sampling strategies, preservation of samples, data treatment and assessing measurement uncertainties

78. Mr Prohaska deepened into the sampling strategies concerning water and fish samples, with a special emphasis on river systems. He again explained how sampling sites are to be selected, making due reference to potentially limiting factors. He mentioned that inter alia (i) the sampling area (length of the river and maybe of the tributaries), (ii) the expected variation (e.g. depending on the geology and the tributaries), (iii) the time potentially required and the time available as well as (iv) the number of bottles available, which determines the number of samples to be taken, must to be considered. Any prior knowledge of geology in the catchment is of great help. Sampling should be done in the target river at defined intervals but in any case following a change in geology and following inflowing tributaries (and if possible, also in those tributaries). If only a limited number of samples can be taken, it has to be ensured that the most relevant sampling locations are defined before the sampling. It has proven helpful to use GPS to speed up sampling by going quickly from one sampling point to the next.

79. Mr Prohaska emphasized that for successful water sampling it was important to wear single-use gloves (as multiple-use gloves can cross-contaminate the samples), use PE bottles, always sample upstream (of yourself or of the boat) to avoid contamination, keep bottle rim lower higher than the bottle bottom and move the bottle quickly down to 20 – 30 cm beneath the surface, rinse bottles three times before taking the water sample, avoid taking particles visibly floating on the surface, and fill at least three bottles per site. Exact protocolling is critical and the protocol needs to mention at least important data such as the “When”, i.e. date and time (with a reference to the time zone); the “Where” (by marking the location on a paper map; recording the location coordinates, e.g. by GPS; using
simple track devices, e.g. a smart phone or a GPS tracker); the “What”, i.e. note down on the spot the most relevant information in relation to the sampling as it might be difficult to later remember all details, and take photos; and the “Who” (including information on who took the sample and who was also present, as well as a record of contact details). Many experienced samplers record even relevant supplemental information. The sampling is then followed by the laboratory analysis.

80. Data treatment was the next step that was described in detail by Mr Prohaska. He stressed that the “blank correction”, the “quantification” based on calibration standards and a resulting calibration curve, and the “correction for dilution” were the crucial steps. The blank correction must always take into consideration all steps of the analytical protocol, i.e. the sampling method, the sampling devices, the storage of the samples and the sample preparation. Expected but also unexpected disturbances must be considered. The person planning and carrying out the sampling must be aware of the potentially expected events and thus must be prepared.

81. The calculation of a proper uncertainty, which includes all possible sources of errors, has to be conducted. Mr Prohaska stressed that a result without a proper uncertainty statement (according to specific protocols such as the guide for the expression of uncertainties in measurements (GUM)) does not have any meaning. The uncertainty is the intrinsic property of the result which consists of the value, the uncertainty and a unit. The uncertainty contains all sources of uncertainty of each single input parameter and is a proof of the metrological quality of a measurement. Even though there should not be any measurement result without uncertainty, Mr. Prohaska explained where uncertainties are definitely required. As uncertainty calculation is part of the ISO norm 17025, any accreditation requires the provision of adequate uncertainties for any measurement result. It is evident that uncertainty calculations are a prerequisite for transparent quality assurance and control. And only the uncertainties allow for the comparability of results on an international scale. As important tool the major contributors to the uncertainty can be assessed and modified accordingly, if a reduction of the uncertainty is required.

82. The method behind the assessment of a combined standard uncertainty was explained in detail. Since all input parameters to the final result have an uncertainty, these uncertainties are propagated to the final result leading to the final combined standard uncertainty. Additional uncertainty contributors, e.g. the heterogeneity of a sample, can be added by summing the squares of the uncertainty. The resulting uncertainty is the square root of the sum of the squares.

83. The calculation of uncertainties can be accomplished following a simple protocol. First, the model equation has to be defined. This is nothing more than the algebraic combination of the input parameters in order to calculate the final result. All input parameters are assigned a value and an uncertainty (as well as a unit). The combined standard uncertainty is then calculated based on different models such as the use of partial derivatives, the use of a Monte Carlo simulation or the Kragten Approach, which is a simple spreadsheet calculation. After the calculation of an expanded uncertainty, the results are reported accordingly. The expanded uncertainty is simply the multiplication of the standard combined uncertainty with a coverage factor (usually $k = 2$, corresponding to a confidence interval of 95%).

84. A discussion of the session content followed. There was also a question as to what was a GPS tracker. Special interest was shown as regards the acquisition of the GPS coordinates. In this respect, smartphone approaches and a simple GPS tracer (i.e. a simple device which records the actual GPS position together with time and which can then be read out by a computer) were discussed.

3.2.10 Examples of the application of methods for the investigation of fish hard parts including own research work

85. Mr Zitek made reference to the relevance of the isotope analysis in environmental and fish behaviour studies in Mongolia. He reminded that the methodology and methods described so far were also appropriate to support a study in the Eg/Selenge watershed in, and upstream of, the area of the
proposed hydrodam. The result of this study could be the Sr/Ca and $^{87}$Sr/$^{86}$Sr isoscapes that would be helpful in learning more about the migration behaviour and habitat use of the fishes. Prior to the practical exercises, the sampling sites had been selected based on the length of the relevant Eg River course and the stretches to be sampled in the Selenge River upstream and downstream of the confluence with the Eg, the tributaries and the amount of money available for the analysis; they were then confirmed during the river descent. A first sample analysis based on samples taken in 2014 showed that the Sr/Ca ratio of water of Eg was relatively homogeneous along the sampled stretch of the Eg but the Sr/Ca ratios in the tributaries were significantly different. The water of the Selenge upstream of the confluence showed a higher Sr/Ca ratio than the water of the Eg. Also the $^{87}$Sr/$^{86}$Sr ratios of the tributaries were significantly different.

86. Also first results concerning lenok sampled in 2014 were presented (note: these results have in the meantime been published in a methodological analytical paper12). The first lenok showed a Sr/Ca elemental ratio and a $^{87}$Sr/$^{86}$Sr isotope ratio which were both corresponding to those detected for the Eg water. It seems therefore obvious that this lenok hatched in the Eg and not in a tributary. The second lenok sample showed a significantly higher Sr/Ca ratio in the centre, clearly pointing to a habitat shift which is also shown by the Sr isotope ratios. This result can undoubtedly be used to confirm a habitat shift from a tributary to the main river (Eg). The third lenok did not show a distinct variation of the Sr isotopes and Sr/Ca ratios. Therefore, it cannot be concluded that it used a tributary; this fish probably always remained in the main river.

87. The significant differences between the tributaries and the main river regarding the Sr isotope ratios and other elemental data underline the potential to demonstrate the use of a tributary by fish. At a first glance, no significant difference between Eg and Selenge could be seen but further elaborate tests will show whether there are discriminators that allow distinguishing between the provenance from the Eg and the Selenge. Then the best discriminator will be determined and analyses conducted. Moreover, more work will be accomplished to elaborate the use of fin rays, a non-lethal method, for provenancing. Mr Zitek reiterated that in order to better understand the chemical differences in the water a geological map of the catchment would be a useful.

88. Mr Zitek’s presentation was followed by a question-and-answer session. A number of questions thematized the potential negative impacts of the planned Eg dam, including lost ecological connectivity and the blockage of upstream and downstream passage for fish. A 60 km long bypass around the reservoir was, however, unrealistic due to the prevailing landscape and the geology. Mr Zitek confirmed that, based on published literature that attributes high importance to tributaries as spawning sites, it can be expected that downstream passage of young fish will be severely impacted at the Eg dam. These questions yet triggered an interesting debate amongst the workshop participants about the approach to EIA in Mongolia in general and a rather controversial discussion about the EIA for the Eg dam in particular. A contribution from the audience suggested that also other biota than fish, e.g. insects and plants, should be taken into due consideration in an EIA before the dam construction as not only fish is threatened. The appropriateness of this request was confirmed by the resource persons; ideally, the whole range of potential impacts on the entire ecosystem and all ecosystem components has to be evaluated in the pre-planning phase. Concerning the loss of genetic exchange, Mr Zitek said that this could certainly be attenuated for lenok, grayling and taimen by transferring upstream a certain number of individuals but needed a well-designed strategy and plan. However, most likely a large number of species will be lost due to the large impoundment.

89. Making reference to the projected energy production figures of the planned dam, Mr Zitek suggested that alternative energy exploitation, e.g. using wind or solar energy, should be considered sparing fish from negative impacts. Experience from all over the world shows that fish species are lost because of dams. Therefore, and as taimen is endangered, from a purely fish-ecological point of view – and without any prejudice to the energy needs and the economic development of Mongolia – this

dam should not be built, the Eg River still hosting one of the last and the largest taimen population in the world. In this context, he made reference to salmon populations lost for example in the USA and to sturgeons lost in Russia. Another aspect to be seriously considered is the filling with sediments of the impoundments created by the dams. Experience shows that in the United States of America, dams are now more often removed when they do no longer produce energy because of filling with sediments. Mr Zitek also reminded that the costs of re-establishing the salmon populations in the USA amounts to millions of US dollar with often limited success. However, should the Eg dam be built, the best management needs to be cared for as of now. There has to be a clear evaluation of what will be gained and what will be lost by the dam construction, and how to manage the impacts. On the positive side, it has to be stated that the Egiin Gol Hydropower Plant Project Unit (EGHPPPU) is committed to do research; this shows that environmental concerns are addressed which is more than has been achieved in some other countries.

90. Regarding a question as to which tributary was used by the fish sampled and analysed in 2014, Mr Zitek responded that the fish was sampled from close to the Tarvagatai River but it could not yet be clearly shown if this fish had used this tributary. The best results are expected by the combination of the Sr isotopes and telemetry methods that would give a clearer picture and it is hoped that the telemetry study using 28 fish will help clarifying.

91. There was then some discussion concerning the height of the planned dam and length of the reservoir as the EGHPPPU mentioned new figures. It seems that the new plans foresee that the height of the dam could be 105 m instead of the originally proposed 90 m and the length of the reservoir be increased to about 75 km. Some uncertainty, however, seem to remain as regards the planned reservoir length, but should the Tarvagatai River, which is a protected area, be impounded, the negative effects for fish will be even more significant.

92. Concerning a question from the audience whether there was any research done regarding the actual status of the fish stocks of the Eg River, and how fish stock assessments in rivers were normally done, it was clarified that no recent information on the fish stock of the Eg was available. Fishing was accomplished at the upper Eg but there are not yet real quantitative data obtained as no quantitative assessment methods (such as electrofishing, point abundance sampling, etc.), that are standard in Europe, are used. However, the need for a comprehensive fish stock assessment was actually obvious. Mr Erdenebat clarified that as regards the upper Eg there were only data available from research carried out between 2003 and 2008 when 2 500 taimen were counted, i.e. 19 taimen per kilometre. Data concerning grayling and lenok were also existing. With reference to fish stock assessments in Austria, Mr Zitek explained how those were generally accomplished. He stressed that a reference site had to be selected where all fish would be caught by electrofishing. Then for each 10-km-stretch of river three sample sites, each having a length of approximately 2 – 3 times the river width, would be sampled by electrofishing. He further said that it was known from experience in Europe, e.g. from the river Danube and related tributaries, that in rivers there are about 600 – 1400 kg fish per hectare whereas in impoundments there are only about 15 – 40 kg fish per hectare.

93. Mr Zitek noted that so far only figures for taimen, lenok and grayling were available to a certain extent but that no data existed as regards the other species present in the Eg and Selenge. Also, the habitat preferences and actual habitats of those other species were not known. He cautioned that this was a real knowledge gap. As most species known for Mongolia will, however, also be present in the Eg and Selenge Rivers their distribution will likewise have to be studied to obtain a complete overview and ultimately to generate a true baseline reference. He stressed that a study of the larval and juvenile fish occurring along the shoreline and in the shallow habitats using electrofishing would be most beneficial to learn more about the species and their distribution, and hence about their habitat use, in the lower Eg down to the Selenge and even in the Selenge. As data so far only existed for the upper Eg, a species inventory with approximate numbers of fish per species and numbers of fish per hectare for the lower Eg should be carried out. By using a drone, available through the cooperation with the US American colleagues, big fish like taimen may be seen from above and counted. For lenok, grayling and all the other species other methods needed to be used. In this respect, Mr Zitek
suggested to combine several methods, e.g. (i) the use of drones for counting big fish, (ii) electrofishing and (iii) snorkelling for counting smaller fish, to identify the species composition of the Eg and the Selenge. Based on these assessments providing data on the total number of fish per hectare for various sites, calculations could be made for example as to how many fish will be lost by a reservoir extending over 75 km. Taking further into account the migrations, i.e. upstream, downstream and into the tributaries, for reproduction and survival, a prediction and even quantified assessment of the negative impacts on the Eg and Selenge fish can be made on which then to base the development of the best possible management plans should the dam be built.

94. It was further clarified that such a study would also give an idea about the size distribution of the fish. This would allow then characterizing the different habitats, e.g. spawning and growing habitats, used by big fish and small fish. With reference to the Danube salmon (*Hucho hucho*), Mr Zitek then explained in general terms the habitat use of different sizes of fish of the same species. Projecting the Austrian results to the situation in the Eg, he developed a scenario whereby small fish might live in the tributaries since there is no big taimen (except for spawning). The taimen in the main river, i.e. the Eg, may migrate upstream and downstream looking for food, and the small fish are in the small rivers, hiding away from the big taimen. He further explained that studying the feeding behaviour was important in order to know whether there was enough food available for adult fish. To illustrate this, he said that Danube salmon needed about 10 kg food to increase its bodyweight by 1 kg. This suggests that it was also important to know how much food taimen needed and how much foodfish was available. Such studies can also help assessing the potential loss of habitat by damming and were nowadays required in EIA's. Mr Zitek reminded the audience that it was important to carry out as soon as possible fish stock mapping in addition to radiotelemetry and genetic studies and isotope analyses. The complexity of the issue shows that all research has to be well coordinated. TCF clarified that they were coordinating all research activities, putting all the data together and reporting to the Egin Gol Hydropower Plant Project Unit.

95. Concerning the questions whether behaviour studies of taimen were carried out and whether there was cannibalism with taimen, Mr Erdenebat clarified that there was evidence that larger taimen cannibalize. Taking this up, Mr Zitek further elaborated on the research of habitat use of *Hucho hucho* by electrofishing and snorkelling in the Danube. He explained that in a big pool had always been seen only one big hucho and no other Danube salmon. The mid-sized hucho do apparently not use the pools but live in a different habitat, i.e. in the deeper running sections or in shallow but flowing parts; the smallest fish live in shallow habitats or riffles or beneath the submerged wood. This clearly shows that it was very important to maintain a good diversity of habitat to support fish populations.

96. In reply to a question concerning the number of dams built on Danube, Mr Zitek explained that there were many dams on the Danube, i.e. ten dams in a cascade solely in Austria and more than twenty dams in Germany, and that the Danube salmon had largely disappeared from the Danube because of these dams. Of the 360 km of the Danube in Austria, only a small section of 30 km in the Wachau region and another one about 30 km east of Vienna were free-flowing between two dams, with the Wachau section been declared a “natural heritage site”. Regrettably, there is no longer any Danube salmon population in the Danube but hucho still live in some tributaries.

97. From the audience was then argued that the situation in the Eg would be different from the one in the Danube because there would only be one dam compared to several tens of dams in the Danube. As a counter-argument, Mr Zitek explained that the Danube dams, despite their average height of about only 10 m, have large impacts but that a higher dam creates even bigger problems, especially when it leads to the formation of an extended impoundment, and the more the bigger the impoundment is. At lower dams it was usually also somewhat easier to provide a fish pass than at very high dams. In explaining the negative impacts of dams and the potential mitigation measures, Mr Zitek made also reference to the FAO publication “Rehabilitation of rivers for fish”\(^\text{13}\) suggesting that upon request further information could be made available.

98. A workshop participant made reference to the request for advice on the fish pass design proposed by Mongolian engineers following the 2014 FAO/TCF Workshop on fish passage design at cross-river obstacles. Mr Zitek replied that after consultation the team would send its comments. Mr Zitek then asked the representatives of EGHPPPU to kindly make available the full EIA report which was consented.

99. Mr Marmulla then intervened to state that he was very pleased with the important discussion that had followed Mr Zitek’s latest presentation and, with reference to issues discussed, made his remarks as follows. First, he stressed that already during the 2014 workshop one of the options suggested for consideration was not to build one high dam but several smaller dams on one or several other rivers for the reason that generally a high dam, especially if as high as the planned Eg dam, imposed bigger problems than several smaller dams. This suggestion was also duly reflected in the published workshop report.

100. Second, Mr Marmulla referred to the discussion at the beginning of this discussion round concerning the environmental impact assessment study for the Eg River dam. Admitting that he ignored at the moment how the responsibility for an EIA was handled in Mongolia, he noted that in many countries the responsibility for the EIA lay with the dam proponent, i.e. in most cases the hydropower company, and that the EIA often was carried out by contractors hired by the dam proponent. However, if the dam proponent was responsible for the EIA, there was the risk that the study was biased. Clearly stating that it was not suggested that this was the case in Mongolia, he stressed that it was, however, always a good idea and certainly important that other stakeholders, e.g. civil society or an NGO, scrutinize or even challenge the results of the proponent-committed EIA for the benefit of a more balanced approach that also takes into due account all potential negative impacts.

101. Making reference to the suggestion by a workshop participant to maybe construct a bypass river around the reservoir to be created by the Eg dam, Mr Marmulla remarked that of course today almost everything was feasible if money was not an issue, but the question was whether this proposed solution was really useful. In fact, as also mentioned in the report of the 2014 workshop, the real challenge namely was to attract the fish to the right point below the dam from where those fish will easily enter the fish passage facility, no matter what type of design the pass itself would be. Therefore, the most important issue to be addressed with highest priority and in the very first step was the design of the fish pass entrance. Furthermore, it has to be kept in mind that any fish passage facility – as well designed as it can be – would only address part of the problem, i.e. fish passage, while the problem of the interruption of the river continuity would not be solved. In fact, taking into consideration the length of the impoundment – now mentioned to be about 75 km of the river course –, the accumulation of sediment to be expected in the reservoir and the hydropeaking, the interruption of fish passage constitutes only a fraction of the impact.

102. Finally, Mr Marmulla said that the resource persons were all fully aware of the wish and the perceived need of Mongolia to become independent of other countries with respect to energy supply, but they knew that there were more modern approaches available today which would not have such impact on the environment as has a dam and they suggested to seriously consider such other options, e.g. the use of solar and wind energy, in order to avoid the destruction of an important ecosystem as is the Eg River because once this valuable and extraordinary environment is destroyed there was no chance to bring it back later on. Therefore, it is important to assess alternatives and modern options that are today available. He also suggested considering that the 2014 report be translated into Mongolian language to make it available to a wider audience which was widely consented.

3.2.11 Possibilities and potential for the integration into the domestic Mongolian scientific research of the analytical methods presented - expert’s view and advice

103. At the start of this session the resource persons stated that they hoped that this workshop was just the beginning of an intensive cooperation between Mongolian and European experts on fish
ecological issues. In this respect TCF confirmed that they were interested in the setting-up of an otolith laboratory and suggested to explore whether this could be done through the cooperation with the resource persons Mr Zitek and Mr Prohaska.

104. Based on previous discussions with workshop participants, and reflecting on their ideas, Mr Zitek then elaborated on potential and possible subjects that could be considered for future collaboration with BOKU. These were *inter alia*

(i) Setting up an otolith laboratory in Mongolia in collaboration with TCF and universities in Ulaanbaatar as suggested by TCF

(ii) Retrieving and safeguarding fish hard parts (i.e. otoliths, fin rays or scales) from taimen specimen and other species of the Eg and Selenge that are already dead, i.e. that died naturally or were confiscated from people who had fished them illegally; this would be an important boost for the research on taimen without killing additional fish. Standard handling protocols and detailed advice on how to extract these hard parts could be provided by the resource persons along with the appropriate paper envelopes for storing the samples. Logistical details, however, need to be clarified as cooperation with TCF will be critical.

(iii) Taking also scales from taimen during the catch-and-release fishery after, in comparison with otoliths, ensuring that scales are the appropriate material on which to base the analysis, and confirming that the information contained in scales or fin rays was as good a proxy for habitat change as the otoliths.

(iv) Using fish or only scales to monitor pollution in rivers. In case of a polluted river, a fish should be caught and hard parts should be sent to the VIRIS laboratory of BOKU in Vienna in order to have biomonitoring carried out.

(v) Arranging for Mongolian researchers to come to Austria to the BOKU laboratories for a practical course to be trained on the sampling and analysis techniques in order to implement these techniques in the future in the Mongolian science area. There might be funding available for the members of the Mongolian Academy of Sciences to come to Austria in the framework of an Austrian/Mongolian collaboration but this would have to be further investigated to confirm.

(vi) Fostering the exchange of Mongolian and Austrian students in the field of aquatic ecosystem studies by making use of international exchange programmes.

(vii) Exploring possibilities for holding in 2016, and hopefully arranging, a workshop on aquatic ecosystem studies at the example of the Eg/Ur watershed potentially to be held at the eco camp of TCF. Eight students each from Mongolia and Austria would be given the opportunity to deepen knowledge on studying an aquatic ecosystem, including practical exercises on the river.

(viii) Assisting with the impact assessment of the planned Eg hydrodam on the Eg/Selenge watershed, in particular with regard to a fish stock assessment that should be carried out and the mapping of the endangered species (other than fish). The EIA should be discussed with experts in other fields of ecological research to determine whether there are also species in the terrestrial ecosystem that are endangered by the dam construction.

105. Mr Zitek stressed that it will be most important to involve the fishermen along the relevant rivers in the activities pertaining to fish sampling as their participation will have a significant impact with respect to the number of samples. Mr Erdenebat encouraged all participants to collaborate in future sampling of water or dead taimen in order to bring this collaboration to life.

106. Mr Zitek then requested feedback from the participants with regard to the usefulness and completeness of the ideas presented and encouraged them to voice other ideas of potential and possible future collaboration. A number of comments from the audience was received and the general importance and usefulness of this workshop emphasized several times and the resource persons and TCF thanked for their initiative. It was mentioned that this workshop could be seen as an eye-opener with regard to some particular aspects in the field of environmental research. Some participants
expressed deep satisfaction that this workshop broadened their views by drawing attention also to non-aquatic issues.

107. Specifically, inter alia the research interest in non-lethal methods for obtaining hard parts for analysis and the wish to learn more about otolith growth research was underlined. Satisfaction was expressed that, fostered by TCF, isotope research was now carried out for the first time also in Mongolia. Also the necessity to train young scientists and the need for an ecoscience laboratory, maybe in the form of a mobile laboratory, was confirmed. Aquatic ecosystem research that targets both fish and water needed to be more actively promoted and fostered. Fish and water research ought to be combined in order to make use of fish for biomonitoring. Hope was expressed that this would contribute to diminishing the impacts on the environment. Science associations, e.g. the Mongolian Academy of Sciences where many people work in the field of water analysis, should be involved in an ecoscience project targeting fish. As there is not sufficient public-awareness raising so far, the flow of information to the public, including through more videos, newspapers articles and more modern means of communication, should be improved in order to make all the information available to a broader public. Furthermore, it was confirmed that a contract between the Mongolian and the Austrian Academy of Sciences existed already to facilitate the exchange of students.

108. Mr Erdenebat reported that TCF had already conferred about its next year’s plans for research activities and was suggesting exploring the possibility of organizing some skype or WebEx conference to further exchange on their research plans and foster exchange on sampling. Mr Zitek said this would be possible in principle and suggested that discussion of future projects be arranged with the US American colleagues later in the year with the aim to more precisely define the gaps in the research in Mongolia.

109. Also Mr Prohaska underlined the importance of the exchange of students and scientists and welcomed such an initiative. He stressed that it was also important to gather information on the laboratory infrastructure that was already available in Mongolia, even if these laboratories were currently not involved in broad ecosystem research, and to discuss how research efforts could be combined and student exchange streamlined to increase benefit for the ecosystem research in Mongolia. He suggested that this analysis could lead to a sort of gap analysis with the ultimate goal to benefit environmental research for informed decision-making in aquatic-environment management that also takes fisheries into consideration.

110. Mr Zitek made reference to the important discussions on the significance of the unimpaired connectivity of the Eg and Selenge Rivers and reiterated that, in the opinion of the resource persons, this connectivity had to be maintained. He then stressed that, should the Eg dam be built, it was important that during the entire construction phase ecological monitoring be carried out by competent ecologists so as to provide guidance on critical ecological aspects to the engineers to avoid mistakes as slips could usually later not be corrected while during the construction it was still possible to make corrections. Therefore, he urged that specialized and trained ecologists should be following closely the progressing construction. In view of the many negative impacts of hydropower on the aquatic environment, including fish, as demonstrated by experiences from other countries around the world and disappointed about the fact that still today knowledge on fish stocks in the Eg and Selenge Rivers was insufficient, some workshop participant requested a much stronger and pro-active engagement of the hydropower sector of Mongolia still prior to the Eg dam construction for preventing adverse effects. In this respect, issues to be tackled were certainly a comprehensive fish stock assessment and suggestions on how to protect the fish and maintain the important fish populations. If suggested protection measures were then not convincing, the dam should even not be built.

111. Also negative impacts of mining activities in Mongolia were mentioned by workshop participants who claimed that studies of the aquatic environment be also conducted on other Mongolian rivers. The resource persons suggested that Mongolian experts might wish to consider that a mobile environmental laboratory be set up by, and held ready in the hands of, competent national
experts to move to aquatic-environment hotspots in Mongolia in case of need to assess water chemistry. Ways to do will have to be explored through appropriate in-country channels. Further elaborating on the idea of setting up such a mobile environmental laboratory based on field-proven equipment (e.g. a novel spectrometer using a nitrogen plasma was launched by the company Agilent), Mr Prohaska made reference to a similar attempt in Vietnam, mentioning that it might be beneficial that laboratory and instrument manufacturers be contacted by the coordinating Mongolian expert to inquire whether donations, financial assistance or special sales conditions could be obtained.

112. Workshop participants felt it was important that the gained knowledge be shared widely with the objective to increase environmental understanding. From the audience came the suggestion that relevant information material, e.g. small brochures on environmental issues in Mongolian language, be prepared in Mongolia as current dissemination of information was not sufficient to well inform the broad public. This material could be widely distributed *inter alia* by authorities, schools and organizations, with the objective to improve public knowledge of environmental issues and create a better understanding by a broader public. It was also thought that all workshop participants should take an active role in disseminating the knowledge gained during this workshop. It was suggested that TCF could contact schools and universities to present its results and reports. School teachers should then spread the news in biology classes and universities should also relay this information during their courses.

113. Mr Marmulla mentioned that, by chance, he had come across information about a cooperation of the Global Nature Fund, Radolfzell office (Radolfzell on Lake Constance, Germany), and the Mongol Ecology Center (MEC), that apparently established the Lake Khovsgol Conservancy in 2012. It seems that the purpose of this cooperation was apparently improving sustainable tourism and national park management as well as public-relations in the Lake Khovsgol area. TCF not being aware of this initiative, Mr Marmulla suggested that it might be opportune that a designated official or maybe TCF make contact with MEC to see if there could be some synergies in the field of sustainable fisheries management. It might be interesting to discuss whether there could be some cooperation with the Lake Constance fisheries authorities for exchange of knowledge. In any case, combining efforts, also with other ongoing projects in the region, should be taken into consideration for mutual benefit.

4. FEEDBACK ON THE USEFULNESS OF THE WORKSHOP BY THE PARTICIPANTS

114. Mr Marmulla thanked all participants for their valuable contributions and the good discussions and asked them to fill in a questionnaire to evaluate this workshop. The participants expressed their appreciation to the organizers for conducting the workshop, and to the resource persons for sharing their knowledge on fish ecological aspects, fish behaviour, water chemistry and methods, including the isotope analysis, to assess fish migration and feeding behaviour in view of improved management. Participants were highly satisfied with the workshop content, the logistics and the documentation provided and rated the overall usefulness of the workshop with an average of 1.4 as “very high”. All questions asked in the questionnaire received on average very high or high marks. Details of the workshop evaluation by the participants can be found in Appendix 4.

5. CLOSING OF THE WORKSHOP

115. After calling the participants to order, Mr Erdenebat expressed his sincere appreciation to all participants for their contributions, active participation and great cooperation during the workshop. On behalf of the workshop participants he also thanked Mr Zitek, Mr Prohaska and Mr Marmulla for their excellent contributions.

116. In his closing remarks, Mr Marmulla underlined the importance of this workshop and welcomed that there had been the opportunity to learn from each other. This exchange of knowledge
was very important and he expressed the hope that this process could be continued and even intensified. Some aspects will hopefully be further addressed in more detail in future research and opportunities for further cooperation should be seized whenever possible. At the end of this successful workshop during which a lot had been said about environmental issues, potential human-induced environmental threats, actual and potential impacts on fish and fisheries in the Eg/Selenge catchment and potential assistance of science in supporting requests for change in environmental management and in designing solutions, Mr Marmulla mentioned that it seemed to be time that the issue of environmental and fish protection be taken to a higher level, i.e. the concerns have to be brought to the attention of decision makers. By now, Mongolian authorities would probably be aware of the status of the environmental assessment in the Eg/Selenge, recognize the gaps that still exist and enter into a dialogue with other sectors or politicians to seek solutions. While it had to be noted with respect that Mongolian scientist, in cooperation with foreign experts, are currently addressing issues in relation to fish, fisheries and the aquatic environment in the Eg River, other groups, including the politicians and the general public – as mentioned by workshop participants – , had now to become involved, should a change be really wished. In this context, he also welcomed the suggestion by workshop participants to seek more efficient ways to better spread the news about environmental research and its results. Mr Marmulla then suggested that the next step should probably be that the scientists hand the baton over to politicians to seek a political decision. In this respect he said that this initiative should be started and pursued by dedicated Mongolian citizens or groups because they know best whom to approach and by which means. TCF might play an important role in this process. Mr Marmulla suggested that, upon request by the Mongolian authorities, FAO might be of help in organizing another workshop to present to a well selected target audience the results of this workshop and the workshop on fish passage issues held in April 2014 and even try to show – from a fish-ecological perspective – potential ways forward for alternative electric energy production, e.g. the use of solar or wind energy, which would take away pressure from the aquatic environment. In connection with such a workshop, an information or briefing session for parliament members could be envisaged.

117. In concluding, Mr Marmulla again expressed his thanks to TCF, especially Mr Purevdorj and Mr Erdenebat, for the great support in preparing and holding this workshop. He also thanked again his colleagues Mr Zitek and Mr Prohaska for the excellent collaboration and expressed the hope that the fruitful cooperation with all stakeholders might continue for the benefit of the aquatic environment, fish and fisheries in Mongolia. Furthermore, he thanked the interpreter for her hard and efficient work.

118. In his concluding remarks Mr Purevdorj stated that this workshop had been very successful and thanked everybody for their excellent collaboration and contributions. Further possible steps will have to be explored in future and contact with FAO and the resource persons in particular will be maintained.

119. Mr Erdenebat then declared the workshop closed on 13 October at 12:00.
Appendix 1

Agenda

Tuesday 6 October

Arrival in the Emt Resort of those participants and resource persons who take part in the field training on Eg and Selenge Rivers

Wednesday 7 October through Friday 9 October

Field training on Eg and Selenge Rivers (with three nights in the TCF field research camp)

- Planning for habitat assessment and selection of sites for fish and water sampling
- Application of fish and water sampling strategies in the field under natural conditions
- Stratified sampling in the Eg-Selenge tributary system
- Handling of samples and sample preservation under field conditions
- Sample preparation (filtration) in the field laboratory and assessment of basic parameter

Saturday 10 October

Return to the Emt Resort for those participants and resource persons who take part in the field training on Eg and Selenge Rivers

Arrival in the Emt Resort of those participants who take part only in the classroom sessions

Sunday 11 October

9:00  
Official opening of the Workshop
- Taimen Conservation Fund
- FAO

Basic biological and ecological principles relevant in fish migrations (A. Zitek)

General introduction to the analysis of hard structures of fish to assess their migration and feeding behaviour in view of improved management (A. Zitek)

12:30 – 13:30  
Lunch break

13:30

Basic principles of analytical techniques for isotope and multi-element measurements related to applications in ecology with a focus on aquatic ecosystems (Th. Prohaska)

Basic principles of methods for the assessment of freshwater fish migrations by isotopic and elemental patterns in hard parts, making reference to relevant examples (A. Zitek)
Basic technical and analytical principles of the analysis of fish hard parts to assess their elemental and isotopic content by using direct solid sampling techniques (e.g. micro drilling, Laser ablation ICP-MS, SIMS, XRF) (Th. Prohaska)

Monday 12 October

8:30
Detailed explanation of the use of different hard parts of fish for tracing migration and evaluating the actual habitat status including sampling with an emphasis on non-lethal methods (A. Zitek)

Standard operation procedures in analytical ecgeochemistry with a focus on trace element and isotopic analysis in aquatic ecosystems including sampling, sample preparation and measurement (Th. Prohaska)

12:30 – 13:30 Lunch break

13:30
Detailed explanation of requirements for sampling strategies, preservation of samples, data treatment and assessing measurement uncertainties (Th. Prohaska)

Examples of the application of methods for the investigation of fish hard parts including own research work (A. Zitek)

Possibilities and potential for the integration into the domestic Mongolian scientific research of the analytical methods presented - expert’s view and advice (Th. Prohaska and A. Zitek)

Tuesday 13 October

8:30
Feedback on the usefulness of the Workshop by the participants (Sh. Purevdorj/G. Marmulla)

Closing of the Workshop (Sh. Purevdorj)

12:00 – 13:00 Lunch break

13:00
Departure of the participants and resource persons
Appendix 2

List of participants

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Opening remarks

Opening remarks by Mr Shirendev Purevdorj
Executive Director, Taimen Conservation Fund (TCF)

Ladies and Gentlemen,
Dear Participants,

First of all, on behalf of the Taimen Conservation Fund, I would like to express thanks to FAO of the United Nations for providing funding for organizing this very important Workshop on “Advanced methods for the analysis of hard structures of fish to assess fish migration and feeding behaviour in view of improved management”. Also, I would like to express many thanks to Mr. Gerd Marmulla, Fishery Resources Officer, Marine and Inland Fisheries Branch, FAO, for his guidance and valuable advice during the preparation stage of the workshop and to Dr. Andreas Zitek and Dr. Thomas Prohaska, experts in fish ecological studies and chemistry, for their participation.

As some of the participants of this workshop know, FAO and TCF have successfully conducted the workshop “Fish passage design at cross-river obstacles – experiences from different countries, with potential relevance to Mongolia” at the Selenge Resort, Mongolia, in April 2014. One of the recommendations of that workshop, that all participants had agreed to, was that in case of building the Egiin Gol Hydro Power Plant a fish passage facility, which previously had not been included in the construction design, must be constructed. This recommendation or outcome came as a result of the discussions of the workshop participants which were guided by the experienced FAO resource persons. Now we are going for a next step.

In today’s workshop, representatives from the Ministry of Nature, Green Development and Tourism, the Institutes of the Mongolian Academy of Sciences and universities as well as other government and non-government organizations participate.

The Taimen Conservation Fund is conducting a whole range of research studies of fish, and especially Taimen, in the Eg and Uur rivers since 2003 and based on this TCF has been selected to carry out fish studies in connection with the construction of Egiin Gol Hydro Power Plant. The main goal of our study is to find out the impact of the planned EGHPP on the fish community.

This workshop consists of two parts, i.e. a theoretical part and a practical part. Participants of this workshop will familiarize with new methods for carrying out water and isotope analyses and improve knowledge on conducting field research studies pertaining to fish ecology.

I hope that this workshop will provide the participants with detailed knowledge and I am wishing this workshop the best success.

Thank you very much for your kind cooperation.
Opening remarks by Mr Gerd Marmulla
Food and Agriculture Organization of the United Nations (FAO)

Distinguished Mr Byambabayar,
Distinguished Mr Purevdorj,
Honourable Workshop participants,
Dear colleagues and friends,
Ladies and Gentlemen,

It is a great pleasure and honour for me to welcome you on behalf of FAO to the Opening Ceremony of this Workshop. I am asked to convey to you the greetings and best wishes for successful deliberations of Mr Gallagher, Deputy FAO Representative in Mongolia and Head of the FAO Office in Ulan Bator, as, unfortunately, neither Mr Gallagher nor his colleagues are able to attend this Workshop due to other urgent commitments.

I am truly pleased to be again in Mongolia – it must be my sixth time or so, and I always very much enjoyed it. It is now the second time that FAO and the Taimen Conservation Fund are jointly organizing and holding an important workshop and I like to take this opportunity to express my deepest appreciation and my thanks to Mr Purevdorj, Executive Director of TCF, and his colleagues, in particular Mr Erdenebat, for all the efforts made to make this workshop happen. Sincere thanks also to Mr Purevdorj for his kind words of welcome. It is a great feeling to be so warmly welcomed to Mongolia. Again, the Taimen Conservation Fund eagerly engaged in an Agreement with FAO for organizing and holding another important Workshop. We are, indeed, grateful that TCF suggested holding this Workshop here in the Emt Resort, in the absence of possibilities for accommodation in the Selenge Resort, as this resort is directly located on the Eg River where an important part of the field training sessions of this Workshop took place during the last three days. And again, Mr Purevdorj, Mr Erdenebat and all the TCF team took so well care of all the local arrangements. However, I would not like to finish this part of my opening remarks without also thanking my FAO colleagues in Ulaan Baatar who provided valuable assistance to me while I was preparing this mission.

Indeed, I take great pleasure in welcoming here participants from the Ministry of Nature, Green Development and Tourism, the Mongolian Academy of Sciences, different universities, different academic institutes and institutions, NGOs and the private sector. We strongly believe that managers and scientists, such as biologists, ecologist and chemists, should be informed about the options and possibilities for assessing migration and feeding behaviour of fish in view of improved management of fish and water resources. We note with great satisfaction that so many representatives from the different stakeholders followed the invitation to participate in this Workshop and hope that this Workshop can be an eye-opener.

Mongolia, especially in the upstream reaches of the watersheds of its northern and western parts, still has rivers that are essentially in such ecological conditions that can be defined as “pristine” or “close to pristine” or at least as “natural”. Many of these rivers have so far been largely spared from anthropogenic influences such as industrial or agricultural development, water development projects or urban discharges that cause water pollution. Yet, these intact environments are under increasing threat from human activities including hydropower development. There is for example a plan to construct a large hyrodam on the lower Eg River – one of the World’s cleanest rivers – which dewateres into the Selenge River, a tributary to Lake Baikal which is the oldest freshwater lake in the world. The Selenge River, which is formed by the Ider River and the Delgermurun River, and the Eg River, which flows out of Khovsgol Lake, are of great importance for sensitive fish species such as Hucho taimen and Lenok. These species are of high value, e.g. in the sport fishery but also for biodiversity. However, they are very vulnerable to human-induced habitat modifications.

The taimen, the largest salmonid fish in the world, depends on specific habitats for reproduction, feeding and larval growth which can still be found in the upstream reaches of a few rivers. To reach
the appropriate spawning habitat, adult taimen undertakes upstream migrations. Young taimen and spent adult taimen then migrate back downstream. Long-distance migrations or shorter movements are also critical lifecycle features of other fish in Mongolia. In general, longitudinal and lateral fish passage in the form of movements or migrations is very important for many species to satisfy their physiological needs and successfully complete their lifecycles.

Migrations and movements are important to maintain genetic diversity. In some parts of the world, migrations even directly contribute to the influx and exchange of nutrients, e.g. in salmon rivers where decaying salmonid carcasses lead to nutrient enrichments; and this is particularly well-studied in North America. Hence, the diversity of communities of living aquatic organisms (including fish) is very important for the productivity, stability in terms of resistance and resilience, and aesthetics of inland water ecosystems. It is well recognized that biodiversity in fish communities is important for fisheries as only diverse fish communities and populations can form the basis of a thriving and sustainable fishery.

For the management of migratory fish species and their protection, if under threat, it is very important to know their behaviour, including the migration behaviour. However, on a global scale the migration behaviour of most of the migratory species is not yet well known. This is in particular true for Mongolia where only recently – namely with the increase of human-induced environmental risks – the importance of the protection of the aquatic habitat and the living aquatic resources, including the fishery resources, is acknowledged. This has led to the initiation of studies and knowledge exchange with the aim of gaining behavioural knowledge on which to base informed management decisions. One of the key players in this process is the Taimen Conservation Fund of Mongolia which, together with specialists from the Mongolian Geo-Ecology Institute, foreign universities and FAO, is collecting information useful in protecting the very valuable fishery resources of Mongolia. In this connection, FAO, in cooperation with the TCF, has successfully organized and held the workshop “Fish passage design at cross-river obstacles – experiences from different countries, with potential relevance to Mongolia” in 2014. Hardcopies of the report of this Workshop are available from FAO, the electronic version is available from the web.

In order to better characterize fish movements over longer distances and larger geographical areas, but also for an improved management of fish populations, a new sophisticated technique, that provides more precise evidence of the migration behaviour than other methods, has been developed and put in place by renowned scientists in many parts of the world: the analysis of isotopes in hard structures of fish. Isotopes are the various atoms of different weight of the same chemical element. As the ratio of these isotopes can vary in the water of different regions as a result of the different composition of the rocks and their weathering over hundred thousands of years, each river section can have a different pattern of isotopes, and this is then reflected in the isotope pattern of the hard structures of fish, e.g. in the otoliths or scales, where the isotopes accumulate. The sojourn of a fish in different regions then generates zones with different isotopes content in these hard structures. In analysing the isotope characteristics of hard structures, the geographical sequence of sojourns of a fish, that are interconnected by the upstream and/or downstream migrations in the river system, can be proven if the stay in different regions was sufficiently long. It also supports the analysis of seasonal habitat uses of different age classes of a sub-population, and can hereby provide essential information for an improved management of fishery resources.

In Mongolia, the conditions are ideal for the use of the “isotopes technique” as pristine conditions and free migration routes are expected to have left different characteristic “fingerprints” on the hard structures. Even effects of mining activities could be potentially “recorded” in hard parts and used as additional site-specific indicators. Most importantly, the data gained by the isotope analysis can readily be used to define more precisely the migration behaviour, e.g. of taimen, which is useful in showing the importance of the Selenge/Eg system for the spawning migrations of these fish. An “atlas of fish movements” might then be created which could help to better manage and possibly protect this and other species. Furthermore, these “baseline” data (e.g. concerning fish movements) can be combined with complementary data e.g. from radio telemetry, and can later be compared with other
data, e.g. to demonstrate that ecological conditions have changed, and in which way, and to prove negative effects on fish.

In order to carry out the isotopes analysis, hard structures of fish and water samples have to be taken and preserved and then treated in a specialized laboratory. Thereby it is important that the relevant hard structures of fish, e.g. fin rays or scales, can be removed without damaging the fish so that the fish are not lost for further reproduction and population dynamics. Sampling otoliths, however, represents a lethal alternative. Some of the Workshop participants have heard and seen this already during the field training sessions of this Workshop, but all participants will learn about the theory behind, the methods and possible conclusions to be drawn from such analyses during the classroom part of this Workshop that starts today.

This Workshop is organized for Mongolian scientists, including fish/fisheries biologists, river ecologists, geo-ecologists and chemists, as well as for administrators (responsible for watershed planning and management). Ideally, a wide audience, including different categories of professionals, should get familiarized with this advanced methodology and technique to stimulate discussions about fish migrations, protection of fish, aquatic habitat and living aquatic resources management, and related issues. The Workshop is especially important for Mongolia as various human activities (including intensified industrialization, intensified agriculture, and increased hydropower development) are known to have negative impacts on fish and fisheries. Therefore, the Workshop intends to provide the participants with a broad overview of the theoretical and practical aspects of the isotope analysis and included, in the first days, some practical work in the field pertaining to sampling of river fish and extracting, preserving and stocking of the hard structures under field conditions; as well as water sampling and preservation and stocking of samples. It is now important that both biological and chemical questions in relation to all steps of the isotope analysis are addressed, discussed and understood for further successful follow-up in the participants’ home institutions. Most importantly, the Workshop will also briefly touch upon the use of the isotope data in characterizing the fish behaviour and the consequences for management decisions.

As a result of this Workshop, the participants shall have gained sufficient knowledge to understand the background and potential of isotope analysis in fishery management, and be able to develop and support the application of the isotope method in other river systems of Mongolia independently.

This is the end of my opening remarks and I shall leave the floor to our renowned specialists, Dr Andreas Zitek and Dr Thomas Prohaska, who will now present and discuss all the technical and scientific details of the analysis of hard structures of fish. Please be, however, reminded that the true success of this Workshop will lie in your participation. Therefore, I encourage you not to be shy and ask all the questions you might have concerning the topic! In concluding I take this opportunity to herewith express my sincere gratitude to my colleagues Dr Zitek and Dr Prohaska for having readily accepted to serve as resource persons for this important Workshop which I wish best success.

Thank you.
### Appendix 4

**Workshop evaluation by the workshop participants**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Percentage of respondents [%]</th>
<th>Average score</th>
<th>Number of participants answering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>very high (1)</td>
<td>high (2)</td>
<td>medium (3)</td>
</tr>
<tr>
<td>1. Usefulness of workshop</td>
<td>68.2</td>
<td>22.7</td>
<td>9.1</td>
</tr>
<tr>
<td>2. General course content</td>
<td>50.0</td>
<td>22.7</td>
<td>22.7</td>
</tr>
<tr>
<td>3. Course content during field training*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Course content during classroom sessions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Course content fish ecology</td>
<td>40.9</td>
<td>36.4</td>
<td>13.6</td>
</tr>
<tr>
<td>6. Course content chemical aspects</td>
<td>63.6</td>
<td>18.2</td>
<td>18.2</td>
</tr>
<tr>
<td>7. Documentation and materials provided</td>
<td>45.5</td>
<td>36.4</td>
<td>13.6</td>
</tr>
<tr>
<td>8. Experienced expertise of resource persons</td>
<td>63.6</td>
<td>31.8</td>
<td>4.5</td>
</tr>
<tr>
<td>9. Capability to convey content</td>
<td>50.0</td>
<td>31.8</td>
<td>18.2</td>
</tr>
<tr>
<td>10. Course duration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>too short (1)</td>
<td>a bit too short (2)</td>
<td>optimum (3)</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>90.9</td>
</tr>
<tr>
<td>Comments</td>
<td>no additional comments received</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* results not considered as more replies received than participants attending the field exercises; however, all responses showed ratings of "very high" or "high"
The workshop “Advanced methods for the analysis of hard structures of fish to assess fish migration and feeding behaviour in view of improved management” was organized by the Food and Agriculture Organization of the United Nations (FAO) in collaboration with the Taimen Conservation Fund (TCF) of Mongolia, and held in the Emt camp, Bulgan Province, Mongolia, from 7 to 13 October 2015. Workshop participants included representatives of the Ministry of Nature, Green Development and Tourism of Mongolia, the Environment Protection Department of Khovsgol Province, the Watershed Management Authority of the Delgermurun river, the Watershed Management Authority of Khovsgol Lake and Eg River, the Institute of Chemistry and Chemical Technology of the Mongolian Academy of Sciences (MAS), the Institute of Biology of MAS, the Mongolian National University, the Agricultural University of Mongolia, the Mongolian Educational University, the Egiin Gol Hydro Power Plant Project Unit (EGHPPPU), the private sector and the Taimen Conservation Fund (TCF).

The workshop consisted of two parts, i.e. (i) classroom sessions to provide all participants with a broad overview of the background and theoretical aspects of the isotope analysis and (ii) some practical work in the field pertaining to identifying and characterizing relevant habitat structures as sampling sites, sampling of river fish and extracting, preserving and stocking of the hard structures under field conditions as well as water sampling and preservation and stocking of samples. The resource persons presented state-of-the-art knowledge on important aspects of the isotope analysis in fish hard parts with the aim to raise awareness of the power and the benefits of this advanced technique for the characterization of fish behaviour, including migration and feeding behaviour, and to stimulate informed discussions about fish migrations, fish protection, aquatic habitat and living aquatic resources management, and related issues in view of an improved integrated basin management. The ecological objectives underlying the use of migratory information are the protection and restoration of fish populations and, if required in this respect, the request for protection or restoration of the aquatic habitat or access to it, e.g. through the construction of fish passage facilities. The basics of fish ecology, the fish hard parts composition and ecogeochemistry were presented followed by a general introduction to the isotope and elemental analysis of hard structures of fish. The workshop then deepened into the principles and the methodology of analytical techniques for isotope and multi-element measurements related to applications in ecology with a specific focus on aquatic ecosystems and particular emphasis on the Eg/Selenge catchment in Mongolia. Examples of successful application of the isotope analysis in fish ecology in Europe, and implications of the resulting findings for management, were presented and discussed and the usefulness of, and the need for, integrated aquatic ecosystem research in the context of basin management in Mongolia examined. Especially during discussions in the last phase the workshop scrutinised local aquatic environmental issues, potential human-induced threats to the aquatic environment, and especially actual and potential impacts on fish and fisheries in the Eg/Selenge catchment, as well as potential assistance of science in supporting requests for change in environmental management and in designing improved solutions. There was agreement that further aquatic environmental studies would be beneficial to better understand river-related processes and potential implications of planned measures in the Eg/Selenge basin.