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RHODEUS SERICEUS AMARUS BLOCH, 1782; MONITORING ELEMENTS IN THE NEW NATURA 2000 CONTEXT IN CROATIA

Doru BĂNĂDUC * and Angela CURTEAN-BĂNĂDUC *

* "*Lucian Blaga*" University of Sibiu, Faculty of Sciences, Dr. Ion Rațiu Street 5-7, Sibiu, Sibiu County, Romania, RO-550012, ad.banaduc@yahoo.com, angela.banaduc@ulbsibiu.ro

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ABSTRACT

The action framework at the European Union level for the conservation of biodiversity was set up based on the Birds Directive (79/409/EEC) and Habitats Directive (92/43/EEC). One principal element of the implementation of these two significant Directives in Croatia is the set up of a Natura 2000 network of protected areas, a network which should be based on a specific monitoring plan at Croatian national level for each species which is considered of community interest. In this general context, this study suggests a set of monitoring elements for Rhodeus sericeus amarus for the Croatian Continental Biogeographical Region. The suggestions are based on eight selected criteria: Croatian national borders proximity sectors overlay; very good quality populations of Rhodeus sericeus amarus in terms of population density and structure (e.g. protected areas) in characteristic good habitats; habitats which need ecological reconstruction to allow this fish species populations structure ameliorate or natural repopulation; key sectors with importance for connectivity (e.g. lotic sectors between different important sectors, rivers confluence areas, etc.); sectors influenced by human impact like: industrial pollution point sources, sectors influenced by agricultural diffuse sources of pollution, sectors influenced by habitats modifications (watercourses remodeling, watercourses regulation, etc.), geographically extreme monitoring sections in the most-upstream and mostdownstream sections of the rivers, in this species range and in the near outer proximities of these extremes.

RÉSUMÉ: *Rhodeus sericeus amarus* Bloch, 1782; des éléments de surveillance dans le nouveau contexte Natura 2000 de la Croatie.

Le cadre des actions pour la conservation de la biodiversité au niveau de l'Union Européenne a été établi en base des Directives Oiseaux (79/409/EEC) et Habitats (92/43/EEC). Un des éléments essentiels de l'implémentation en Croatie des deux directives a été l'établissement du réseau des zones protégées Natura 2000, un réseau qui devrait être basé sur un plan de surveillance adapté aux spécificités nationales et aux caractéristiques des espèces d'intérêt communautaire. Dans ce contexte, la présente étude propose une série d'éléments de surveillance pour *Rhodeus sericeus amarus* dans la région biogéographique continentale croate. Les suggestions ont été faites en fonction de huit critères sélectionnés: la position des secteurs à la proximité des frontières nationales; des populations de *Rhodeus sericeus amarus* de très bonnes qualités en termes de densité et de structure (i.e. des zones protégées) avec des habitats caractéristiques et de qualité; les habitats nécessitant des mesures de reconstruction écologique afin de permettre aux populations de ce poisson de se reconstruire naturellement ou d'améliorer leur structure; des secteurs clés pour la connectivité (i.e. des secteurs lotiques entre des secteurs importants, des confluences de rivières etc.); les secteurs à diverses impactes humains tels que des secteurs influencés par des apports ponctuels de pollution industrielle, par des sources diffuses de pollution agricole, des secteurs dont les habitats ont été modifiés (des systématisations et régularisations des cours d'eau, etc.), des secteurs de monitoring des extrêmes géographiques situés au plus près des deux extrémités des cours d'eau (sources et confluences) sur le territoire de l'espèce ainsi que les secteurs avoisinants.

REZUMAT: *Rhodeus sericeus amarus* Bloch, 1782; elemente de monitorizare în noul context Natura 2000 din Croația.

Cadrul de acțiune pentru conservarea biodiversității la nivelul Uniunii Europene a fost stabilit în baza Directivei Păsări (79/409/EEC) și a Directivei Habitate (92/43/EEC). Un element principal al implementării în Croația a acestor două Directive semnificative este stabilirea retelei de arii protejate Natura 2000, o retea care ar trebui să fie bazată pe un plan de monitorizare specific la nivel național pentru fiecare specie considerată a fi de interes comunitar. În acest context general, studiul de față sugerează o serie de elemente de monitorizare pentru Rhodeus sericeus amarus în Regiunea Biogeografică Continentală croată. Sugestiile/măsurile de management propuse se bazează pe opt criterii selectate: dispunerea sectoarelor situate în proximitatea frontierelor naționale; populații de Rhodeus sericeus amarus de foarte bună calitate în ceea ce priveste densitatea și structura populațiilor (de ex. zone protejate) cu habitate caracteristice de calitate bună; habitate ce necesită măsuri de reconstrucție ecologică pentru a permite populațiilor acestei specii de pește să se refacă natural sau să își amelioreze structura; sectoare cheie importante pentru conectivitate (de ex. sectoare lotice între diferite sectoare importante, zone de confluență ale râurilor, etc.); sectoare influentate de impactul antropic precum: surse punctuale de poluare industrială, sectoare influențate de surse difuze de poluare agricolă, sectoare influențate de modificări ale habitatului (sistematizări și regularizări ale cursurilor de apă, etc.), secțiuni de monitorizare ale extremelor geografice din secțiunile cele mai din amonte și mai din aval ale râurilor din arealul speciei, precum sectoarele imediat următoare și externe acestora.

INTRODUCTION

Croatia entered in the European Union, which will induce supplementary obligations for this country regarding environment protection and conservation in a similar way with all the older EU countries.

The main objectives of the European Community in the nature field of interest are the conservation, protection and continuous improvement of the environment structure quality for an optimum use of nature resources and services, including that of the aquatic ecosystems. In the last decades, the biodiversity was one of the most important issue in this context.

The European Community action frame to manage the biodiversity was set up and supported mainly by the Birds Directive (79/409/EEC) and the Habitats Directive (92/43/EEC). These two crucial European Directives have essential objectives to preserve the biodiversity in the European Union territory supported by a protected areas network, the Natura 2000 net, to conserve key habitats and species characteristic for all the existent European biogeographic regions: Arctic, Boreal, Atlantic, Continental, Alpine, Pannonian, Mediterranean, Macaronesian, Steppic, Black Sea, and Anatolian (Fig. 1).

The Croatian territory has a relatively good biogeographic diversity in the context of the European Union countries, including four biogeographic regions: Continental, Alpine, Pannonian, and Mediteranean (Fig. 1).

One main element of the implementation of the Directives is the set up of a Natura 2000 network in Croatia as well, a network which should be based on a specific national monitoring plan for each species and habitat of European conservative interest. The joining of this country to EU makes this monitoring proposal a key element for future management related plans in Croatia.

The range of *Rhodeus sericeus amarus* in Eurasia is with a disjunct range of distribution. In Europe, it is present in some parts of the Baltic, North, Black, Caspian, Aegean and Mediterranean seas basins. It also exists in the Croatian national territory. Until now, distribution data about *Rhodeus sericeus amarus* in Croatia was not systematically/completely collected. This species often was not found in some zones but reappeared in nearby zones. The relatively sporadic presence/knowledge of this species in Croatia is known based on the last few decades of studies in Drava, Ilova, Kupa, Sutla and Una and their tributaries watersheds. In some of the Croatian Danube Basin areas this species is common or very common.

Regarding *Rhodeus sericeus amarus* there has been no Croatian national permanent/long term specific monitoring on the distribution of populations and their ecological status until now. Not all potential zones of existence were studied, including those which can be at least theoretically appropriate for establishment of Natura 2000 sites. Despite this situation of knowledge, it is considered by the Croatian ichthyologists as a relatively well spread and common species in the continental biogeographical region. The range and abundance of this species in Croatian continental biogeographical region can be considered as relatively high in the suitable habitats. In other biogeographical regions is an invasive species. That is why it is considered as needing a monitoring program only for the Continental Biogeographical Region.



Figure 1: European biogeographic regions; European Environment Agency - www.eea.eu.in.

Assessments have been done in areas proposed as pSCI sites: rivers Drava (15-30% proportion of the population in relation to the size of the population at the state level), Ilova (2%), Kupa (15-30%), Sutla (2%) and Lonjsko polje (2-15%) and Kopački rit (2-15%) areas.

In the next three years through the Natura 2000 Integration Project (NIP), inventory of freshwater ichthyofauna is expected to be done completely in the areas with present data gaps.

Rhodeus sericeus amarus is a part of the Habitats Directive (92/43/EC) species Annex II. In Central and Eastern Europe, it is rather a common species with a high potential as an umbrella species; a similar situation exists in Croatia as well.

In spite of the fact that no complete data about *Rhodeus sericeus amarus* range in Croatia is acquirable today (a relatively frequent situation in other European countries too), the actual known data represent sure data for the proposal of a medium/long term monitoring elements proposal for this country.

Rhodeus sericeus amarus is a benthopelagic fish species, living in temperate areas, in fresh and standing or slow flowing waters, with aquatic vegetation and sand-silt bottom (canals, slow-flowing rivers, backwaters, oxbows).

This fish species food consists of unicellular filamentous algae and vegetation debris. The sexual maturity is reached at one year. Its reproduction is happening in the end of April until August. The roes are layed down in the *Unio* and/or *Anodonta* molluscs' gills cavity. The larval stage is also taking place in this cavity. It is a species well understood with respect to its biology and partially of its ecology in the studied Croatian areas of interest.

This species is listed in Annex II of the European Union Habitats Directive, in the Annex III of the Berne Convention, and IUCN Red List. In the Croatian territory, it is considered to be a vulnerable (VU) species.

Rhodeus sericeus amarus is threatened directly by human induced pollution and also indirect by pollution effects on freshwater mussels. The aquatic and semi aquatic (riverine) habitats degradation and river regulation, remodelling and flooding control, indirect contamination, influence this species negatively, directly and/or indirectly. Today there are noted important fluctuations in the number of this specie's locations and subpopulations.

Regarding this fish species conservation, protective measures should be done where the local situation require actions for that: preserving and improving the favorable ecological balance of the natural waters inhabited by this species, ichthyological protected areas (reserves) of conservative interest, preventing and avoiding of water and sediments flow regulation as much as possible close to the natural regime, construction of appropriate devices for water recycling, avoiding lotic fragmentations due to different categories of constructions presence in the river bed, etc. These elements cannot be realized on long term without a working monitoring system specific for this fish species.

RESULTS AND DISCUSSIONS

The result of this study is a proposal of elements of monitoring for *Rhodeus sericeus amarus* for the Croatian Continental Biogeographical Region. The proposed monitoring elements are based on the actual distribution data of this fish species and the main human threats. Based on the overlapping of the data of distribution of this fish species on the human disturbed lotic sectors, the scale of a spatial monitoring grid can be proposed and the monitoring frequency in space and time can be revealed. "Theoretical/blind" approach in proposing the temporal and spatial frame of this fish species monitoring can only be an intellectual exercise, which will fail in the future in terms of accuracy of the results, and bring many costly future adjustments of the initial monitoring system elements. Even if the lotic systems are one of the most dynamic ecological ecosystems on Earth, even in the most appropriate approached monitoring elements proposals, sooner or later, they will need adjustments.

The *Rhodeus sericeus amarus* monitoring sectors, at the Croatian national level/Continental Biogeographical Region, were proposed based on eight criteria: **1** Croatian national borders proximity sectors overlay; **2** very good quality populations of *Rhodeus sericeus amarus* in terms of population density and structure (e.g. protected areas) in characteristic good habitats; **3** habitats which need ecologically reconstruction to allow this fish species populations structure ameliorate or for natural repopulation; **4** key sectors with importance for connectivity (e.g. lotic sectors between different important sectors, rivers confluence areas, etc.); sectors influenced by human impact like: **5** industrial pollution point sources, **6** sectors influenced by agricultural diffuse sources of pollution, **6** sectors influenced by abitats modifications (watercourses remodeling, watercourses regulation, etc.), **3** geographically extreme monitoring sections in the most-upstream and most-downstream sections of the rivers, in this species range and in the near outer proximities of these extremes.

The potential future amelioration of the *Rhodeus sericeus amarus* species distribution data regarding Croatia can bring new monitoring proposals. The process of making better the monitoring of this species should be a continuous one.

Spatial monitoring elements

• Croatian national borders proximity areas of interest coverage.

These monitoring sectors were proposed due to their relevance for needed future transboundary European monitoring systems and methods intercalibrations, and for international data checking and exchange. Also, these sectors represent the limits of the Croatian national responsibility for improving this fish species conservation status. These monitoring sectors are proposed to be carried out once every year.

Based on this monitoring criterion, ten monitoring sections (Fig. 2, ●) were proposed.

One sampling station should be on Mura River, under once per year monitoring plan, on the Croatian - Slovenian - Hungarian border, in the proximity of Novakovec locality.

One sampling station should be on Drava River, under once per year monitoring, on the Croatian - Hungarian border, at aproximatively 100 km downstream of the Novakovec locality (locality situated on Mura River).

One sampling station should be on Drava River, under once per year monitoring plan, on the Croatian - Serbian border, upstream of the confluence with the Danube River, upstream Aljmaš locality.

Two sampling sections should be on Sutla River, under once per year monitoring, plan on the Croatian - Slovenian border, (section one road number 205 access to Sutla River and bridge from Razvor locality through Razvor Street; section two road number 225 access to Sutla River and bridge from Harmica locality through Ivana Perkovca Street).

Two sampling sections should be on Kupa River, under once per year monitoring plan, on the 118 km long north-west Croatian - south-east Slovenian border, with an around of 50 km among them (section one aproximativelly 50 km downstream of Mandli locality; section two road access to a bridge over Kupa River from Cerje Vivodinsko or Preseka Ozaljska localities).

One sampling station should be on Glina River, under once per year monitoring plan, on the Croatian - Bosnia-Hertzegovina border (road access and bridge near Katinovac locality).

One sampling station in the proximity of the Southern Croatian-Bosnia and Herzegovina border on the Una River, in the Stanic Polje locality.

The second sampling station should be in the proximity of the Southern Croatian-Bosnia and Herzegovina border on the Una River, in the Hrvatska Dubica locality, with road access from the road number 47.

Some human impact from the upstream countries/regions which can induce negatively qualitative (species disappearance) and/or quantitative (relative abundance) changes of the monitored fish populations, will induce a negative future prospects related to this fish species range, habitat quality, long-term viability and conservation status, situation which is recommended to be evaluated once a year.



Figure 2: *Rhodeus sericeus amarus* proposed monitoring sectors (**①**), in relation with the Croatian borders proximity areas of interest coverage criteria; *Rhodeus sericeus amarus* distribution **④**, update situation (Duplić, SNIP, 2012).

• Very good quality populations of *Rhodeus sericeus amarus* in terms of population density and structure in characteristic good habitats.

This second category of monitoring sectors were proposed due to their genetic importance for a healthy status of this species populations on Croatian territory and also in neighbouring states' territories, and for the posibility of natural repopulation of basins sectors where this fish species can live and disperse. Based on this specific monitoring criteria, six monitoring sectors (Fig. 3; O) were identified.

One sampling section should be in the Nature Park Žumberak - Samoborsko gorje, at 30 kilometers south-west of Zagreb, with tributaries for Sava River. Sampling activities are needed once every six years if no extraordinary events should appear (natural and/or human

events which have results of major or significant biocoenosis and/or habitats modifications). One sampling station in the proximity of the Southern Croatia - Bosnia and Herzegovina border on the Una River, downstream of the Stanic Polje locality. One station in the proximity of the Northern Croatian - Hungarian border on the Drava River, near the Donji Miholjac locality, road and bridge access on the road number 53. One sampling section should be on Sutla River, under once per year monitoring plan, on the Croatian - Slovenian border, from the road number 205 access to Sutla River and bridge from Razvor locality through Razvor Street. One station on the middle Česma River course, under once per year monitoring plan. One section should be in the Odransko Polje protected area proximity, in Sava River, samplings are needed once every six years if no extraordinary events should appear (natural and/or human events which have results of major or significant biocoenosis and/or habitats changes).



Figure 3: *Rhodeus sericeus amarus* monitoring sections **2**, based on the good quality populations of *Rhodeus sericeus amarus* in terms of populational structure/ density in characteristic habitats; *Rhodeus sericeus amarus* distribution **•**, update situation (Duplić, SNIP, 2012).

Human impacts in these areas, which can generate qualitative (species disappearance) and quantitative (relative abundance and/or age structure modifications) changes of the assessed populations, will induce bad future prospects associated to the species habitat quality, range and conservation status, medium and/or long-term viability, situation which should be monitored once every six years at national level, if no exceptional events should happen (natural and/or human events which generate significant habitat or/and biocoenosis changings).

• Lotic habitats/sectors which need reconstruction to permit the *Rhodeus sericeus amarus* populations structure ameliorating for natural repopulation. This is an exclusive case, if the lack of data did not induce some false breaches in the distribution data/knowledge.

This third category of monitoring sectors was proposed due to the discontinuous distribution of this species, maybe as a consequence of human activities pressure, and also due to some breaches in the present knowledge. Based on this proposed monitoring criteria, three monitoring sections (Fig. 4; $\textcircled{\bullet}$) were selected.

One sampling section should be on Sutla River, under once per six years period monitoring plan, on the Croatian - Slovenian border, access on the road and bridge to Sutla River from Kraj Donji locality to the road number 676. One sampling section should be on Mura River, under once per six years period monitoring plan, on the Croatian - Hungarian border, in the proximity of the Hungarian locality Muraratka. One sampling section should be on Sava River, under once per six years period monitoring plan, downstream of Sisak, in the proximity of Sunja locality, road access from the road number 224.



Figure 4: *Rhodeus sericeus amarus* selected monitoring sections (€), based on the lotic sections which should be reconstructed, to allow *Rhodeus* sp. population structure amelioration or natural repopulation criteria, potential sectors to be proposed for ecological reconstruction. *Rhodeus sericeus amarus* distribution €, update situation (Duplić, SNIP, 2012).

• Key sections with relevant importance for ichthyofauna connectivity (e.g. intermediate river sectors between diverse important fish populations' areas; rivers confluences).

This fourth category of monitoring sectors was proposed due to their potential positive role as connectivity coridors with relevant importance in the continuity of this species distribution, but they can also represent breaches in the present knowledge. If all these sectors prove to be only breaches in the actual knowledge, which will be covered with information in the future, they can be removed from the selected list of monitoring sectors.

Based on this monitoring criteria, five monitoring sections (Fig. 5; \bullet) were proposed here.

One sampling section should be on Sutla River, under once per six years period monitoring plan, on the Croatian - Slovenian border, access on the road and bridge to Sutla River from Kraj Donji locality to the road number 676. One sampling section should be on Mura River, under once per six years period monitoring plan, on the Croatian - Hungarian border, in the proximity of the Hungarian locality Muraratka. One sampling section should be on the lower Česna Sava River, its "export of biodiversity" regarding Bitterling species too, being obvious. One sampling section should be on Sava River, under once per six years period monitoring plan, downstream of Sisak, in the proximity of Sunja locality, road access from the road number 224. The second sampling station in the proximity of the Southern Croatia -Bosnia and Herzegovina border on the Una River, in the Hrvatska Dubica locality, with road access from the road number 47.



Figure 5: *Rhodeus sericeus amarus* selected monitoring sectors **4**, based on key sectors with relevant importance for connectivity; *Rhodeus sericeus amarus* distribution **5**, update situation (Duplić, SNIP, 2012).

Any human activities pressure in these river sectors which can induce the lacking of at least accidental presence of *Rhodeus amarus* individuals, generate negative future prospects associated with this fish species conservation status and range, a situation which should be monitored once in every six years at the Croatian national level.

S industrial and waste water pollution point sources areas (Fig. 6).

The Drava and Kupa rivers were approached regarding the industrial pollution point sources hot spots, which need once per year period monitoring sectors.

Drava River needs a once per year period of monitoring section downstream the Osijek locality, with road access from the road number 213.

Kupa River needs a once per year period of monitoring section downstream the Karlovac locality, which releases partially treated waste water.

Also in the confluence area of Kupa with Sava at Sisak locality (chemical, metal, leather, textile and food) where the industry negative impact brings a supplementary reason for monitoring this area.



Figure 6: *Rhodeus sericeus amarus* selected monitoring sections **9**, based on the industrial and waste water pollution point sources data criteria; *Rhodeus sericeus amarus*, update situation (Duplić, SNIP, 2012).

6 sectors influenced by agricultural pollution diffuse sources (Fig. 7).

The following Sava River tributary was approached in relation with the agricultural pollution diffuse sources, which needs monitoring sections.

Sutla, due to the proximity of large corn fields cultivation that has high heavy metals concentrations values in the water due to K_2O , Co, Cu sulphate and Ti used in chemicals fertilizer, needs a monitoring section in this river between the localities Ključ Brdovečki and Drenje Brdovečko. In this section, high values of enterococs numbers (coming from the farms situated in this basin), N total, P total, humic substances (including U complexes) from chemical fertilizers we also found.

Drava is one of the main collectors for agricultural waste waters, including organoclorurates, a monitoring section before the confluence with Danube River being necessary in these circumstances.

Sava is the main collector for agricultural waste waters in Croatia, including the organoclorurates, a monitoring section downstream the lower sector where *Rhodeus sericeus amarus* that was found on this river in Croatia (in the Una and Sava rivers confluence proximity) and is a necessity.



Figure 7: *Rhodeus sericeus amarus* selected monitoring sections **③**, based on areas influenced by agricultural pollution diffuse sources data criteria; *Rhodeus sericeus amarus* **④**, update situation (Duplić, SNIP, 2012).

• changed sectors influenced by modifications of habitat (channeling, remodeling, dams, watercourses regulation, etc.) (Fig. 8).

On Sava River, downstream of the Trebez Dam, location from which *Rhodeus sericeus amarus* has not been present for over 50 km, a monitoring station for this species should be settled in the proximity of Trebez locality (road and bridge access).

On Drava River, upstream of Donja Dubrava locality, where hydroelectric power plant dams were built (Varaždin, Čakovec, Medmurje and Dubrava). Downstream Donja Dubrava locality, before the confluence with Mura River a monitoring section for this fish species should be settled, with access from the road number 20.



Figure 8: *Rhodeus sericeus amarus* selected monitoring sections (2), based on the modification of habitats criteria.
 Rhodeus sericeus amarus distribution , update situation (Duplić, SNIP, 2012).



• geographically extreme monitoring sections (Fig. 9) in the most-downstream and upstream sectors, in this fish species range and in the near outer proximities of these extremes.

Figure 9: *Rhodeus sericeus amarus* selected monitoring sectors (③), based on the geographically extreme monitoring sectors;

Rhodeus sericeus amarus distribution 🗢, update situation (Duplić, SNIP, 2012).

Evaluation of the conservation status

First a systematic survey of this fish species distribution on the Crotian territory should be realised and the range of this species should be continuously compared by the future monitoring data.

Rhodeus sericeus amarus population units qualitative and quantitative elements, units proposed in the upper monitoring sections can rely on some fish biotic index criteria. The proposed combination of metrics was designed to reflect insights of communities and population comparable perspectives. Each selected metric value should be compared with the value estimated from other similar sites. It should be considered the fact that as the biotic integrity (found on the following selected metrics) decreases, the lotic ecosystem quality decreases too.

The selected categories of metrics are: I fish species richness and composition (with the following metrics: 1. total number of species; 2. proportion of benthic species; 3. proportion of water column species; 4. proportion of individuals of intolerant species; 5. proportion of individuals of typically tolerant species); II trophic composition (1. proportion of individuals as omnivores feeders; 2. proportion of individuals as insectivores feeders); III fish abundance and condition (1. numbers of individuals in sample; 2. introduced species will be assigned to each metric species, on zoogeographic basis).

Ratings of one to five should be assigned to every metric according to whether its assessed value approximates deviates little or much from the expected value of the best expert judgement at a comparable site that is relatively similar but also relatively undisturbed.

The total score for every assessed site should represent the all nine-metrics sum and the scores can be understood based on the next comparison intervals: 45-43-excellent, reflects comparable to natural conditions, exceptional communities of species; 42-36-very good, shows a decrease in species richness, in particular intolerant species, present sensitive species; 35-31-good, fair intolerant and sensitive species absent, skewed trophic structure; 30-24-fair, some expected species are rare or absent, dominant omnivores and tolerant species; 23-17-fairly poor, score which reveals few species and individuals, dominant tolerant species; 16-10-poor, very few individuals and species present, tolerant species or fish absence.

An assessment of any fish species population conservative status can be done in the ichthyocenosys assessment context. Any other assessment approaches will have a low quality.

Using these fish metrics, it permits the possibility to assess the conservative status of the target populations in the local specific ichthyologic assemblage context and also of the habitat.

In every six years, supplementary sampling sectors should be done in all the downstream and/or upstream extreme (geographically speaking) areas to highlight the possible territorial extension of this species.

The reduction in range can be highlighted through the presence or absence of the species in the monitoring stations.



Evaluation Grid A 50/50 km grid was used in the Danube Basin map of Croatia (Fig. 10).

Figure 10: 50/50 km grid, used as a base for *Rhodeus sericeus amarus* monitoring areas; *Rhodeus sericeus amarus* distribution , update situation (Duplić, SNIP, 2012).

The minimum number of monitoring areas - 15, for *Rhodeus sericeus amarus* should be at least one monitoring sector in each 50/50 km plot (*); these plots were proposed based on the previous eight selected criteria (Fig. 11).



Figure 11: The minimum 15 sampling stations for *Rhodeus sericeus amarus* should be in the marked (≉) 50/50 km plots; *Rhodeus sericeus amarus* distribution , update situation (Duplić, SNIP, 2012).

The 15 minimum sampling monitoring sectors, one in every 50/50 km plots, were proposed based on the eight criteria overlapping; thus each of these 15 sampling areas correspond to as many criteria as possible, including the lowest possible effort, cost and time.

Depending on the available financial support, time and local/national working team potential, the number of the monitoring stations can be multiplied with 2, 3, 4, 5, etc., for every 50/50 km plot.

1. From the **qualitative** point of view, the presence of the *Rhodeus sericeus amarus* individuals in each of the 15 selected plots offer a first level of information in relation with its conservation status in Croatian Danube Basin, in terms of suitable habitats, future prospects, populations and range. The identification of this fish species in all selected 15 plots reveal an excellent conservation status in Croatia, in 11 plots a very good conservation status, in 10 plots a good conservation status, in nine plots a fair conservation status, in eight plots a fairly poor conservation status, in seven plots a poor conservation status, and in six plots or less a very poor conservation status.

2. The second proposed level is also a **qualitative** approach, in relation with: age structure, absence/presence of 0+ age fish individuals, absence/presence of 1+ age fish individuals, absence/presence of 3+ age fish individuals, absence/presence of 3+ age fish individuals, absence/presence of 5+ age fish individuals. Each of the 15 proposed plots is evaluated based on the absence/presence of the proposed age classes. Any plot with all six age classes will be assessed in an excellent conservation status; five age classes present will highlight a very good conservation status, four classes represent a fairly poor conservation status, one class represent a poor conservation status. This approach should be made independently for each 50/50 km plot and in the end, an average for all the plots should be done, which represent the mean national conservation status.

3. The third needed level is also a **qualitative** approach, in respect of species composition; *Rhodeus sericeus amarus* presence reveal a poor conservation status; *Rhodeus sericeus amarus* + another native fish species highlight a fairly poor status of conservation; *Rhodeus sericeus amarus* + two native fish species highlight a fair conservation status; *Rhodeus sericeus amarus* + three native fish species highlight a good conservation status; *Rhodeus sericeus amarus* + three native fish species highlight a very good conservation status; *Rhodeus sericeus amarus* + four native fish species highlight a very good conservation species; *Rhodeus sericeus amarus* + five or more native fish species highlight an excellent conservation status. This specific approach should be made independently for each 50/50 km plot and finally an average for all the plots should be done, resulting the mean national conservation status for *Rhodeus sericeus amarus*.

4. The fourth proposed level is an **integrated** approach. That is why, for every monitoring sector should be obtained results in terms of: fish biotic criteria score (45-43-excelent, 42-36-very good, 35-31-good, 30-24-fair, 23-17-fairly poor, 16-10-poor, 9-1-very poor), which reveal at quantitative level the conservation status for the *Rhodeus sericeus amarus* species population in the ichthyocenosys assessment context. This specific approach is made independently for each 50/50 km plot and in the end an average for all the plots, which means a national conservation status.

5. Finally an average among the previous four steps at the Croatian national level should be made, which is the final value for the national conservation status for *Rhodeus sericeus amarus*, as a result of the proposed monitoring activities programme.

CONCLUSIONS

The *Rhodeus sericeus amarus* monitoring sections, were selected and proposed based on the following eight criteria: ① Croatian national borders proximity sectors overlay; ② very good quality populations of *Rhodeus sericeus amarus* in terms of population density and structure (e.g. protected areas) in characteristic good habitats; ③ habitats which need ecological reconstruction to allow this fish species populations structure ameliorate or for natural repopulation; ④ key sectors with importance for connectivity (e.g. lotic sectors between different important sectors, rivers confluence areas, etc.); sectors influenced by human impact like: ⑤ industrial pollution point sources, ⑤ sectors influenced by agricultural diffuse sources of pollution, ⑦ sectors influenced by habitats modifications (watercourses remodeling, watercourses regulation, etc.), ③ geographically extreme monitoring sections in the most-upstream and most-downstream sections of the rivers, in this species range and in the near outer proximities of these extremes. All these criteria based monitoring sector selection is a relevant sum of influences which can negatively affect this fish species distribution, survival and abundance of its populations, and conservation status. It was considered that all these proposed criteria elements can influence the future conservation status of this fish species in Croatia. The monitoring sectors selection was based on these specific criteria and the monitoring sectors were identified one by one on the maps, based on the existent fish related bibliography and data.

It should be stated the fact that the potential improvement of *Rhodeus sericeus amarus* distribution data on the Croatian territory in the future, can improve the monitoring sectors situation, the process of improving this proposed monitoring system being a flexible one.

The ecological and biological monitoring in this context cannot be replaced by the physico-chemical monitoring, not even in the monitoring sites selected for the human impact analysis; but some physico-chemical criteria of the fish species habitat quality should be included in the monitoring, if the fish monitoring sectors will overlap with the national Croatian integrated monitoring sectors in the future.

Rhodeus sericeus amarus conservation status elements

The *future prospects* as one of the four components of *Rhodeus sericeus amarus* conservation status are revealed using the following criteria for monitoring sector selection: national border proximity; habitats which should be ecologically reconstructed; and areas/sectors negatively influenced by human impact. Thus, also the trends regarding the human induced pressures and threats towards this species can be revealed.

The *habitat* of *Rhodeus sericeus amarus* is the second element of its conservation status, related to the area and quality of the suitable habitats. Thus, also the trends considering the occurrence areas of this fish species, increasing versus decreasing areas situations, increasing versus decreasing habitat quality situations can be revealed. For these aims, monitoring sectors criteria based on selection was done, including the following criteria: Croatian national borders proximity sectors overlay; very good quality populations of *Rhodeus* sericeus amarus in terms of population density and structure (e.g. protected areas) in characteristic good habitats; habitats which need ecological reconstruction to allow this fish species populations structure ameliorate or natural repopulation; key sectors with importance for connectivity (e.g. lotic sectors between different important sectors, rivers confluence areas, etc.); sectors influenced by human impact like: industrial pollution point sources, sectors influenced by agricultural diffuse sources of pollution, sectors influenced by habitat modifications (watercourses remodeling, watercourses regulation, etc.), geographically extreme monitoring sections in the most-upstream and most-downstream sections of the rivers, in this species range and in the near outer proximities of these extremes. In this context also the following metrics were proposed: I absence/presence; II age structure, absence/presence of 0+ age individuals, absence/presence of 1+ age individuals, absence/presence of 2+ age individuals, absence/presence of 3+ age individuals, absence/presence of 4+ age individuals, absence/presence of 5+ age individuals; III species composition; IV relative abundance.

The *population* is the third element of the conservation status for *Rhodeus sericeus amarus*. It is evaluated based on population size and structure in terms of age structure and reproduction. To cover this element, namely the favorable reference populations which are considered as appropriate to ensure the long-term viability of *Rhodeus sericeus amarus*, some metrics were proposed: I absence/presence; II age structure, absence/presence of 0+ age individuals, absence/presence of 1+ age individuals, absence/presence of 2+ age individuals, absence/presence of 5+ age individuals; II species composition; IV relative abundance in the local ichthyofauna.

The fourth component of *Rhodeus sericeus amarus* conservation status is the *range*, which correspond to the spatial limits within which this fish species occurs. The trend of this species range increasing or decreasing can be revealed based on some criteria, which were proposed for the choice of some monitoring sectors: Croatian borders proximity sectors coverage; sectors with significant importance for fish populations connectivity; geographically extreme monitoring sections in the downstream-most and upstream-most sectors of rivers, in this fish species range and in the near outer proximities of these extremes.

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