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Ex situ conservation of the thermal rudd (*Scardinius racovitzai*): a general review of work and results

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Abstract. An ex situ conservation project for two critically endangered, endemic species (the thermal rudd *Scardinius racovitzai* and *Melanopsis parreyssii*) was developed. A detailed study on the morphometry and ex situ reproduction biology of the thermal rudd provided detailed information for the morphological characterization of this endemic population and for the requirements for its reproduction in captivity and conservation.

Introduction

This study is a part of a larger research regarding the ex situ conservation of two critically endangered animal species, endemic for the thermal lake in "Peţea Spring" Natural Reserve, near Oradea, northern-western Romania: the thermal rudd *Scardinius racovitzai* Müller 1958 and the snail *Melanopsis parreys-*

sii (Philippi 1847). The project is run during 2013–2014 by three institutions from Romania and Hungary and it is financially supported by the Mohamed bin Zayed Species Conservation Fund. The main goals of the project are: re-enforcement of threatened populations by introduction of new individuals, public awareness and successful enhancement of species conservation strategies (Maitland et al. 2002, SSC Reintroduction Specialist Group 1995). Without a doubt, in situ programs must quickly develop in order to allow the restoration of the severely damaged natural habitat.

The factors presently affecting the thermophilic populations in the lake have been previously studied by various researchers (Crăciun 1997, Ionaşcu 2009, Telcean 2013 etc):

- Over-extraction of thermal water from the deposit feeding the lake, a fact that has a direct influence on its volume and surface, as well as on the variation of abiotic parameters during winter and the occurence of values beyond tolerance limits for the thermophilic species.
- Presence of invasive species.
- Degradation of the habitat.

Under these circumstances, an ex situ conservation project was launched, and the proposed strategy for maintaining the genetical diversity and a viable population is an urgent action to be taken in accordance with the ultimate goal of species, biodiversity and habitat conservation.

Ex situ conservation of species on the brink of extinction is a measure recognized by many authors (Maitland et al. 2002, Kucharczyk 2008, IUCN, Gil 2010, Neufeld et al. 2011 etc.) as a solution for species survival. Still, maintaining of a captive stock on short, medium or long term, captivity breeding and the complementary procedures to preserve the population need to be but an alternative while restoring the natural habitat, and both in situ and ex situ conservation measures should be taken in an integrated manner.

The ex situ conservation program proposed for this study is in accordance with the rules for biodiversity conservation and includes:

- Studying the biological and ecological characteristics relevant for ex situ conservation (estimating the condition status of individuals, immunity to pathogenic agents, synchronic or asynchronic reproduction type, survival of adults after breeding, intraspecific relationships, including cannibalism).
- Temporary salvation by creating and maintaining a captive stock, in accordance with the rules for animal welfare and professional ethics for public aquariums.
- Demographic manipulation by maintaining ex situ the captive stock during

winter, when the critical evolution of the ecosystem close to destruction and the abiotic parameters beyond tolerance limits were noticed.

- Reproduction in captivity and obtaining a descendants stock for restoring and/or strengthening the wild population.
- Reintroduction in the original habitat, a near future objective within the project as a measure for restoring the biodiversity in the lake, depending on the restoration of abiotic and biotic conditions in the natural reserve Pârâul Peţea.
- Public awareness about the endangerment of the species and ecosystem and the importance of their conservation. Public display of captive specimens was restricted due to its ethological characteristics besides its breeding season, while other publicity means were used, such as mass media, educational programs and scientific publications of the results.

As the drastic decrease of water level and temperature affected since Dec. 2011 the entire ecosystem, before any ex situ conservation work we tested the morphological variability of the present thermal rudd population, sampled in 2013, and compared it with available data from literature and a sample of its congener *S. erythrophthalmus,* too. The thermal rudd was considered a species with a Miocene origin (Müller 1958) or as a (sub)species with a more recent derivation from the Central European common rudd (Bănărescu 2002), and the present paper tries to complete the data regarding the morphological features of the present population in the thermal lake. Despite of various references regarding the morphological, physiological and behavioral differences cited to occur between the two species, applying of molecular techniques is still necessary for clarifying the taxonomic status of this population (Bănărescu 2002).

The available data and previous studies on the reproductive biology of thermophilic *Scardinius* are scarce. In an ethological study on the thermal rudd (Crăciun 1997), breeding behavior and immune depression below 25-27 ^{0}C were described, the latter increasing sensibility to parasites and affecting the survival rate of the experimental stock. Another laboratory experiment on thermal rudd breeding was included in an ethological research on the endangered fish species of Romania (Ionaşcu 2009).

According to the published information, a typical breeding season is restricted to February-March in the wild (Bănărescu 1964) or to December-January in captivity (Crăciun 1997). During the breeding season, fish are more sensitive to abiotic and biotic factors in their environment and there are restricting factors related to the triggering of physiological processes needed for reproduction. Therefore, an accurate model with optimal values of environmental parameters simulated in captivity is a critical prerequisite for gonads maturation and for breeding.

Materials and methods

A detailed assessment of the abiotic environment characteristic for the thermal ecosystem was performed based on historical data and our own physico-chemical analysis of water samples, in order to establish the optimum range of variation of parameters. Two collecting trips in the reserve were performed and sampling points for hydro-biological samples were established in correlation with the distance from the emission zone of the thermal spring and of the Glighii cold affluent (Fig. 1).

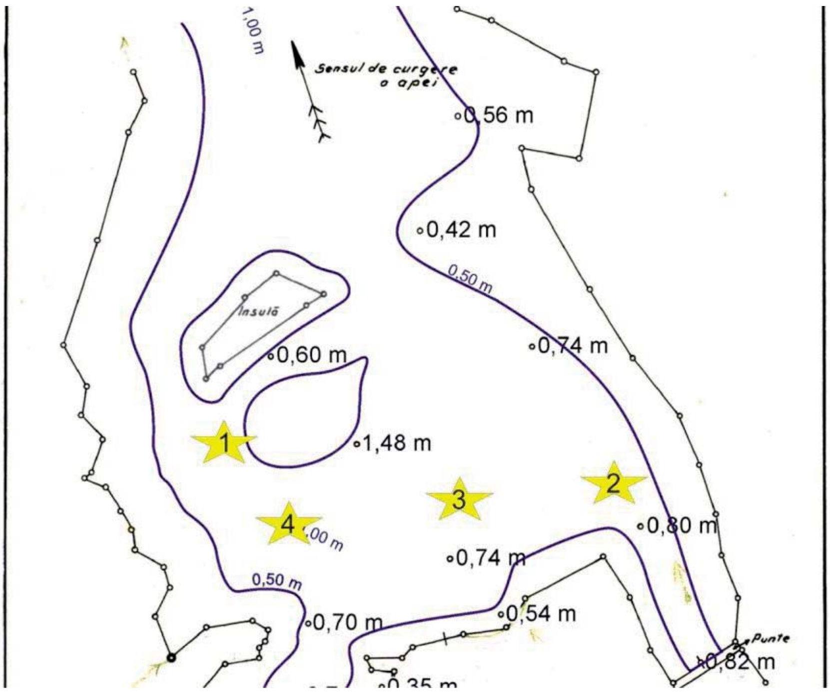

Fig. 1. Sampling points in the „Pârâul Peţea” natural reserve, Ochiul Mare lake (18–20 Aug. 2013 and 27–28 Oct.2013).

A group of 45 thermal rudd specimens was approved for collecting by Order no. 1231/2013 of the Environment and Climate Changes Ministry and was captured by use of a scientific fishing net with 10-16 mm mesh sizes, during the sampling trips in August and October 2013. The sampled fish were transported live, in 50 l plastic bags with 1/3 water/oxygen ratio, to the Aquarium facilities at the Museum Complex of Natural Sciences „Răsvan Angheluţă”, Galaţi, Romania.

Care was taken to establish the transport conditions and norms (animals density in the transport bags, input and maintaining of oxygen and temperature levels within the tolerance limits, the transport medium and duration, and the coefficient of free volume in the transport bags), in order to minimize stress and loss of animals.

Crowding in plastic bags. 5-20 individuals, each with maximum 20 g body weight, were introduced in each bag, and water-oxygen ratio was 1/3. When transportation was accomplished, the fish were acclimated to captivity conditions. Acclimatization lasted 5 hours, for a gradual transfer of fish already stressed during transportation.

A calculation formula for the available oxygen content within the plastic bags at 28 ^{0}C (lake temperature) was used for determining the conditions for safe transportation and it determined the stocking rate of fish in the bags in accordance with trip duration, dissolved oxygen demands, age and health status of the fish.

A thermal isolation foil was used to cover the bags and to maintain a constant water temperature during transport. A total amount of 36 g oxygen introduced into the plastic bags with low fish stocking density was an optimum content for safe animal transport, regardless of the competing reactions to oxygen consumption or dissolved oxygen demands of the thermal rudd.

Morphological analysis. From 19 thermal rudd individuals, randomly chosen, biometrical measurements were collected (Fig. 2). The standard body length of assessed *S. racovitzai* individuals was 83,34 – 108,89 mm, individual weight was

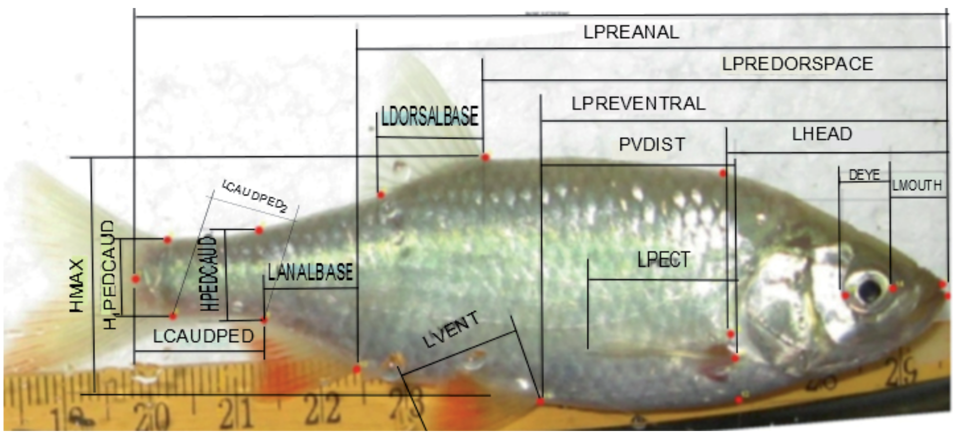

Fig. 2. Determination of morphometric data on thermal rudd individuals sampled in 2013 (n = 19). Explanations of morphometric characters are in Table 4.

9,99 – 20,27 g. Based on the weight/length relationship from a low number of individuals, the condition status of fish was estimated.

The morphometric and geometric data were studied in order to assess the variability of the present critically endangered population. Statistical analysis of morphometrics geometrics was applied in order to expand the degree of confidence of conclusions, having in view the small sample size that was limited by legal permit for capturing of a critically endangered species. The resulting data were compared to those published by Bănărescu (1964) (based on 69 individuals), and to those obtained by Freyhof (2007).

Digital pictures were taken with a CANON A590 IS camera on the left side of each fish and 23 landmarks were defined and recorded as two-dimensional coordinates. Landmarks were selected to provide a homogeneous coverage of the whole shape. The operation was partially taken on live specimens, having in view legal and the project's demands (fish propagation and re-introduction of all adults and juveniles obtained by captive propagation back in their native environment). A total of 14 morphometric characters of the fish were measured and also the body weight of each individual was assessed to an accuracy of 0.1 g. A multivariate approach was applied for the morphometric method using Systat 10.2.

Geometric methods were also applied with MorfoJ 1.05f. The shape information was extracted by Procrustes superimposition, which removes variations in size, position and orientation from data on landmark coordinates. The coordinates of the superimposed landmarks were used in multivariate statistical analysis to address the main question: the current variability of the thermal rudd population, which is strongly pressed by the ecosystem deterioration. As there was a degree of similarity between this species and its congener *S. erythrophtalmus*, the statistical approach helped to analyze the separation between specific groups (species/ecotypes).

Chemical monitoring of water. Chemical parameters of water with a limiting effect on aquatic animals, including by transportation stress, were assessed in the field:

- Total dissolved salts (TDS), with a digital TDS-meter (0 – 9990 ppm, ± 2% accuracy).
- pH, with a digital pH-meter (AF PH1, range 0–14, ± 0.1 accuracy).
- Dissolved oxygen, with a mobile kit (Aquamerck Sauerstoff-Test) and Winkler's titration method.
- Carbonate/total hardness, with a mobile kit (Aquamerck Compact Laboratory) and a titrimetric method.

Laboratory chemical analyses were performed periodically with adequate equipment. Temperature, pH, conductivity, and redox potential were measured continuously by the sensors of an Aquatronica electronic system, which signals the deviations from the set range and keeps the measured data for 7 days. Dissolved oxygen and its saturation were measured daily with an OAKTON pH-oxymeter. Ammonia, ammonium, ammonia nitrogen, nitrites, and nitrite nitrogen were measured with a DR890/HACH photocolorimeter every 3-10 days, depending on the state of the aquarium system and clinical signs in the animals. Alcalinity, GH and KH were measured with a HACH digital titrator every 7-10 days, depending on the state of the aquarium system. Nitrates, phosphates and total iron were measured with a DR890/HACH photocolorimeter every 7-15 days, depending on the development of plant biomass in the aquarium. Total chlorine and chlorides were measured with a DR890/HACH photocolorimeter every 30 days.

Setup of aquariums. The materials for setting up the biotope and simulating an artificial biocenosis characteristic for the thermal ecosystem were received at Aquarium Galaţi in early August 2013. The infrastructure for the aquariums was set up as follows:

Filtration. Closed system, mechanical and biological filtration with a HYDOR PRIME external filter, 900 l/h nominal flow, one for each aquarium. UV-C sterilization, 18 W. Internal filter, 1200 l/h.

Light. Fluorescent tubes T5/JBL, 4500 K, 54 W.

Photoperiod. Gradual increase of artificial lighting duration from 8 to 12 hours a day, as the articial ecosystem stabilized.

Heating. Heaters (300 W each) and a substrate heater (20 W, with controller) to simulate vertical convection through the substrate and to optimize the root system of plants.

Fertilization with carbon dioxide. A 10 l cylinder, with a 60 atm pressure, reductor, macro- and micrometric regulation, electronic control of CO_2 addition with pH controller, electro-valve, bubble counter and preliminary gas dissolvation reactor (Resun pump, flow 350 l/hour).

In the artificial environment, a daily feeding rate of 7–10 % of the fish biomass, consisting of natural food (mainly freezed Chironomids and green, philamentous algae) ensured an optimal condition of the fish during ex situ conservation.

Egg collecting and incubation. Various natural and artificial substrates were used for spawning. The eggs were sticky and adhered to all substrates. They were moved in separate tanks in the same environment, to avoid temperature and chemical shocks. Incubation took place separately from the adults and in two tank types based on age groups, to avoid cannibalism:

- Small tanks (30x30x30 cm) at 26–30 ^{0}C, with thermostated heater and simple, internal filter. During the first days after hatching, frequent partial water changes were applied.
- Large tanks (117x48x43 cm), with LSS (life support system) providing mechanical and biological filtration, UV-C sterilization (18 W), aeration and temperature control.

The eggs were counted with the help of Image J software, for accurately assessing the fertilization and hatching rates.

Rearing the fry and juveniles. Extensive water changes were performed in the first days after egg transfer to avoid pollution due to the decomposition of unfertilized or unhatched eggs. After 45 days from spawning, the fry were transferred in rearing tanks (180 – 220 l). Food granulation was gradually increased and feeding rate was adapted to biomass growth.

Results and discussion

Limiting environmental factors. Water temperature. Water temperature represents the environmental factor that exerts the strongest influence on the biological processes in fishes. In certain zones in the lake, water temperature values are below the minimal tolerance limit for the thermophilic species (20 ^{0}C according to the bibliography) and the survival of those populations depends on the length of exposure to that critical factor. If the annual and winter variation of temperature didn't comply to the amount of degrees-days-temperature needed to trigger reproduction in the thermal rudd, survival of the species and conservation of its gene pool are under threat.

Multi-annual dynamic of water temperature in the Ochiul Mare thermal ecosystem. A comparison of the available data on the temperature regime during 1999–2000 (27.21 ^{0}C, sampling point "mulberry") and during 2003–2011 (Danciu 2006, Fig. 3) reveals a general decrease in annual average values and frequent values below 20^{0}C during winter except the very close area to the thermal spring. In recent decades, the annual average temperature constantly decreased. Historical observations in 1938 mentioned the mean value of 29.1 ^{0}C (April 1938 - March 1939, quoted in Danciu, 2007). The cause of this phenomenon was the decrease of the flow of the thermal spring (from 500 l/sec in the 1960s to 50 l/sec during 1991–2000, according to Danciu (2006).

pH. According to available information from literature (Danciu 2006), we statisti-

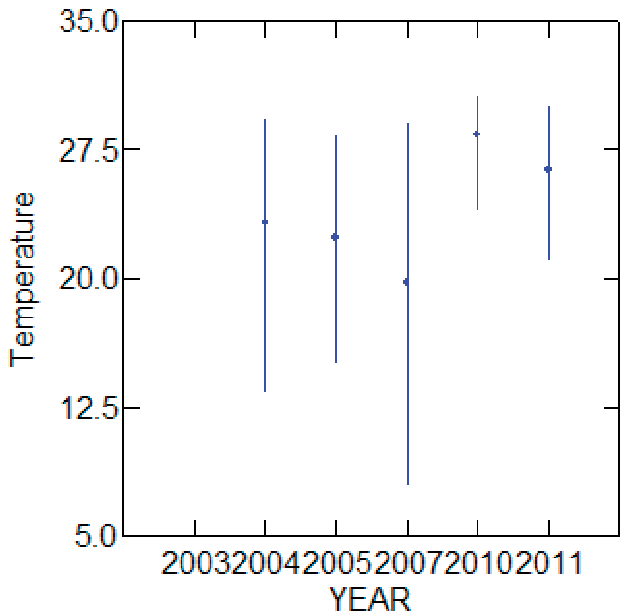

Fig. 3. Multi-annual average, minimal and maximal values of water temperature in Lake Ochiul Mare (Danciu, 2006).

cally analyzed these data and the confidence interval with 95 % confidence level indicates a slightly alkaline domain (7.4-7.8) for the thermal lake in the reserve. The histogram and the frequency of pH measurements shows several isolated values below 6 and above 8. Still, there is no corelation of water pH in various areas of the lake, but they are more influenced by biogenous processes such as respiration. Water samples taken while collecting the fish showed a significantly higher CO_2 concentration in the lake than in the allochtonous source (the brook), having a direct impact on the pH.

Multiannual pH limits in the thermal ecosystem Ochiul Mare. Based on available data from the regional water management board (Apele Române, Administraţia Bazinală Ape Crişuri), including 5-10 measurements each year during the time period 2003-2011, the multiannual variation of the pH in the reserve was determined as being 6.9–8.3 (Fig. 4). There are no available data on the duration of exposure to the extreme values of this range.

A factorial analysis of water chemistry parameters measured during 2005, based on a small amount of historical data (Danciu 2007), provided additional information on the ecological characteristics and the interactions between abiotic

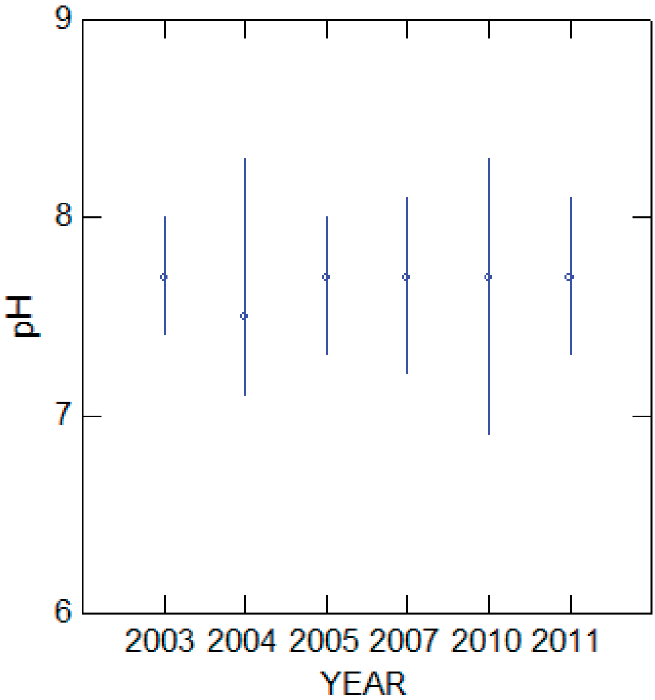

Fig. 4. Multiannual pH variation in the Pârâul Peţea natural reserve.

and biotic environments. The first two factors amount to 71.466 % of the total variance (Table 1). Factor 1 includes the significant and positive contribution of conductivity, hardness, chemical oxygen demand (COD) and phosphates, in contrast to ammonium and nitrites. COD is an indicator of organic build up mostly due to algal growth, implying intense decomposition and the rising of phosphates level (0.843, Table 1) above the rate of their consumption in the processes of photosynthesis and assimilation by superior plants. Mineralization of organic substances produces nutrients, but there is a high rate of nitrification, respectively of their consumption by aerobic nitrifying bacteria: NH_4 - 0.973 and NO_2 - 0.573 (Table 1). Conductivity and GH depend on the ionic concentration in the thermal spring, with a factor of 0.918 (Table 1). Factor 2 (27.066 % of total variance, Table 1) includes the significant and positive contribution of conductivity, dissolved oxygen (DO), nitrites, and phosphates, in contrast to the nitrates. Dissolved oxygen accumulates in the water as a result of photosynthesis, while nitrates are assimilated by plants in the same process. The results of chemical analysis in the field at collecting times and in the laboratory are summarized in Tables 2 and 3.

Table 1. Factorial analysis of Ochiul Mare water quality based on chemical measurements during 2005.

Component loadings

Parameter	Factor			
	1	2	3	4
PH	-0.341	-0.465	-0.606	-0.548
CONDUCTIVITY	0.516	0.832	0.134	-0.156
GH	0.918	0.145	-0.352	0.109
DO	-0.266	0.603	-0.722	0.211
COD	0.956	-0.261	-0.129	-0.042
NH4	-0.973	0.175	0.123	0.083
NO2	-0.573	0.678	0.460	0.026
NO3	0.414	-0.675	0.599	0.120
PO4	0.843	0.500	0.049	0.189
CBO5	0.268	0.388	0.273	-0.838

Variance Explained by Components

1	2	3	4
4.440	2.707	1.710	1.144

Percent of Total Variance Explained

1	2	3	4
44.400	**27.066**	17.097	11.437

Table 2. Hydro-biological sampling points in the reserve.

Crt. nr.	Sta-tion	Sam-ple nr.	Collecting date	Observations
1	1	1	20 Aug.2013	”Ochiul Mare” thermal lake
2	2	2	20 Aug. 2013	Cold, temporary affluent (Glighii Valley)
3	3	3	27 Oct. 2013	”Ochiul Mare” thermal lake
4	4	4	27 Oct. 2013	”Ochiul Mare” thermal lake
5	1	5	27 Oct. 2013	”Ochiul Mare” thermal lake

Collecting, transport and acclimatization of individuals. Two field trips and collecting of hydro-biological and fish samples were performed during 2013. The following species were identified while collecting the biological samples:

- Local species coming from the Crişul Repede River: chub *(Leuciscus cephalus),* carp *(Cyprinus carpio),* bitterling *(Rhodeus amarus),* spined loach (*Cobitis taenia*).

Table 3. Water quality in the Pârâul Peţea natural reserve in 2013

Variable	Sample no.				
	1	**2**	**3**	**4**	**5**
Data	23 Aug	23 Aug	29 Oct	29 Oct	29 Oct
Temperature (^{0}C), 10^{00} o'clock	25	-	20	24	25
16^{00} o'clock	29				
Dissolved oxygen (mg/l)	2	-	1.1		2.5
Saturation (%)					
pH	7.1 – 7.3		7.1	6.9	6.9
TDS (mg/l)			313	338	315
Free chlorine (mg/l)	0.01	0.02	0.03	0.03	0.01
Total chlorine (mg/l)	0.02	0.02	0.03	0.03	0.01
Clorides (mg/l)	60	30	30	29	29
Fe^{2+} (mg/l)	0,04	0.03	0.07	0.05	0.05
CO_2 (mg/l)	160	88	200	160	180
NO_2^- (mg/l)	0.012	0.036	0.012	0.001	0.005
NO_3^- (mg/l)	1.3	0.9	1.3	1.8	1.4
NO_2-N (mg/l)	0.004	0.011	0.004	0	0.001
$NaNO_2$ (mg/l)	0.018	0.054	0.018	0.002	0.007
PO_4^{3-} (mg/l)	0.66	0.27	0.32	0.41	0.47
P (mg/l)	0.21	0.09	0.12	0.13	0.15
P_2O_5 (mg/l)	0.49	0.20	0.28	0.31	0.35
NH3 (mg/l)	0.09	0.28	0.05	0.01	0.01
NH4- (mg/l)	0.09	0.30	0.05	0.01	0.01
NH3-N (mg/l)	0.07	0.23	0.004	0.01	0.01
Total alkalinity (mg/l)	6.0	4.0	5.8	6.0	6.0
Total hardness (0G)	17.41	11.98	18.53	16.68	15.45
Carbonates hardness (mg/l)	12.65	8.9	15.12	13.72	18.44
Acidity (mg/l)	0	80	100	80	90

- Invasive species known to be present in the Romanian fauna as a result of import for aquaculture in recent decades, such as *Pseudorasbora parva* and ornamental, tropical or subtropical species, such as the gold fish *Carrasius auratus auratus.*
- Other allochtonous, tropical species mentioned in literature (Crăciun 1997, Ionaşcu 2009), such as *Poecilia reticulata*, *Xiphophorus hellerii, Macropodus opercularis, Trichogaster trichopterus sumatranus,* and *T. leeri*, were not noticed. This observation may be related to the fishing technique used, but the question remains as to whether the recent evolution of the ecosystem has influenced negatively the survival of these exotic species. Future monitoring of the impact of these allochtonous species on the thermal rudd population remains an important objective for research.

Water temperature during transport varied by 0-2 ^{0}C depending on the weather conditions at that moment and remained within the tolerance limits for the target species (26–28 ^{0}C during the first transport and 23 ^{0}C during the second transport).

Dissolved Oxygen content. Analysis in the field showed a low concentration of dissolved oxygen (1.1–2.5 ppm), a fact in correlation with the stagnant type of ecosystem, the intensity of chemical reactions in the organic mud, and weather conditions. Therefore, atmospheric air was pumped in the transport bags before the introduction of collected animals to compensate those values.

During transportation, filling 2/3 of the transport bags volume with oxygen provided 36 g available oxygen for the animals. Independent of the level of reactions competing with the oxygen consumption or of the degree of oxyphily of a particular species, that amount is sufficient for the duration of transportation of fish in moderately crowded bags.

pH, carbon dioxide and ammonia. At the moment of collecting the biological samples, pH in the pond was neutral or slightly alkaline (7.1 – 7.3), corresponding with the technological rules for transportation. Ammonia content in the transportation bags increased as a result of intense excretion at high temperature, of the increase of stress products, and of the decomposition of organic matter in the water. Rehabilitation of the animals' physiological state was quicker when transportation crowding and duration were lower.

Statistical analysis of morphometrical characters of thermal rudds from the "Pârâul Peţea" natural reserve. A positive allometric growth was noticed (allometric coefficient b = 3.7688), with a high confidence level of weight/length relationship (R^2 coefficient, of weight/length regression, was 0.84). An intermediate estimation after 175 days in captivity showed a 55 % biomass increase (18.78–34.28 g per individual, average individual weight 27.047 g, and standard length 90.0 - 107 mm).

An improvement of the individuals' condition in captivity was noticed on 11 randomly chosen fish, by assessing the Fulton coefficient, which resulted in an absolute range of its values of 2.4–3.4 in spring, compared to 1.7–2.3 as measured after collecting in autumn (Fig. 5). An optimal physiological and health condition is an important prerequisite for successful reproduction. The inferior physiological condition of the fish at collecting time, as expressed by the Fulton factor, is in relation with the poor trophic availability of their natural environment, which was in a state of advanced degradation.

The plastic data obtained through body measurements on thermal rudd are listed in Table 4 (confidence intervals with 95% confidence level, n = 19). The values were expressed as percents of the standard length, with the exception of

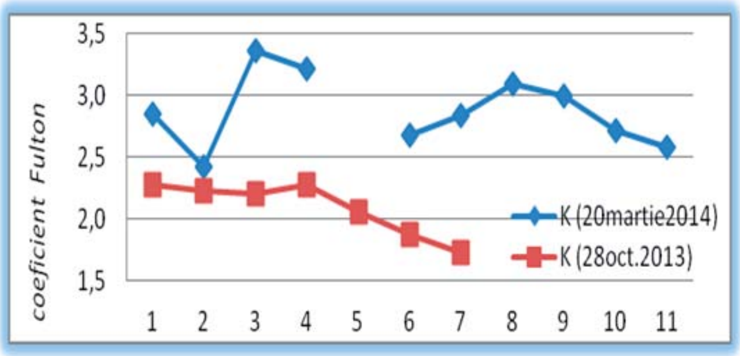

Fig. 5. The Fulton condition coefficient for thermal rudd individuals, at the start of the ex situ conservation program and after 175 of captivity rearing.

$LMOUTH_2$ (length of mouth as percent from head length), $DEYE_2$ (eye diameter as percent from head length), HPEDCAUD (maximum height of caudal peduncle as percent from caudal peduncle length), $LPECT_2$ (length of pectoral fin as percent from distance between pectoral and ventral fins insertions). A lowering of ranges of some morphometric characters was noticed, a fact that can be linked to the phenotypic plasticity of fish. They quickly adapt by modifying their physiology and behavior to environmental changes (Freyhof 2008).

A multivariate test was applied for a comparison between the thermal rudd sample and a sample of its congener, *S. erythrophthalmus* collected from the Danube river (Mm80).

By calculating the Wilks' lambda statistical parameter (Wilks' lambda = 0.0152, prob = 0.0000) the ensemble discrimination is very strong (the more close to zero is the statistical value, the higher the discrimination power will be; the closer to 1 is lambda, the smaller the discrimination power will be), with an advanced degree of affirmation certainty ($P < 0,05$).

The F-to-remove statistics determine the relative importance of variables/ morphometric characters included in the model. A number of 6 morphological characters were found to be the most useful for discriminating among the species (head features, caudal peduncle, and pectoral and ventral fins lengths).

Geometric statistical analysis. The body shape analysis was also applied on 19 thermal rudd individuals that were randomly chosen from the captured batch of fish and a comparative, intraspecific and interspecific study was performed on them and on the closely related *S. erythrophthalmus* respectively (8 individuals). The statistical test was made by use of the MorphoJ software through the analysis of the principal components analysis (PCA) and canonical variate analysis (CVA) of body shape.

Table 4. Statistical parameters of the main morphological features of the thermal rudd (confidence intervals, $P < 0.05$). NOTE. The data were compared to those previously published (Bănărescu 1964, on 9 individuals; Müller 1958, on 60 individuals, Berinkey 1960, on 5 individuals) and to a sample of *S. eyrthrophthalmus* individuals*,* n=8. Legend: * We noted the references of Freyhof (2007). The differences from references are bolded.

Crt. Nr.	Morphometric character		95% CI Upper	95% CI Lower	Comparing results with references (%) (Bănărescu 1964)
1	HMAX	Maximum body heigth (% of standard length)	32.0	**25.3**	28,3-35,7
2	LHEAD	Head length (% of standard length)	26.8	24.6	24,7-30,6
3	LMOUTH	Preorbital length (% of standard length)	6.6	**5.2**	6,7-7,6
4	$LMOUTH_2$	Mouth length (% of head length)	24.9	**21.1**	22,5-25
5	DEYE	Eye diameter (% of standard length)	6.8	6.1	5,5-8,2
6	$DEYE_2$	Eye diameter (% of head length)	26.0	24.2	20,4-27,1
7	LCAUDPED	Length of caudal peduncle (% of standard length)	17.4	16.4	14,7-21
8	$H_1PEDCAUD$	Minimum heigth of caudal peduncle (% of standard length)	10.2	**9.8**	10,3-11,1
9	HPEDCAUD	Maximum heigth of caudal peduncle (% of caudal peduncle length)	**0.79**	**0.84**	1,3-1,7*
10	LPREDORSPACE	Predorsal distance (% of standard length)	58.9	56.7	57-62
11	LPECT	Pectoral fin length (% of standard length)	19.2	**15.8**	18,5-21,3
12	$LPECT_2$	Pectoral fin length (% of the distance between pectoral and ventral fins)	**76.4**	64.3	60,2-69,3
13	LVENT	Ventral fin length (% of standard length)	15.4	**13.1**	15,4-18
14	LPREANAL	Pre-anal distance (% of standard length)	72.9	71.5	70-76
15	LPREVENTRAL	Pre-ventral distance (% of standard length)	50.2	47.4	50-66
16	LDORSALBASE	Length of dorsal fin base (% of standard length)	12.6	11.0	10,6-14
17	LANALBASE	Length of anal fin base (% of standard length)	11.6	10.6	10,5-12,7
18	PVDIST	Distance between pectoral and ventral fins insertions (% of standard length)	25.7	23.9	

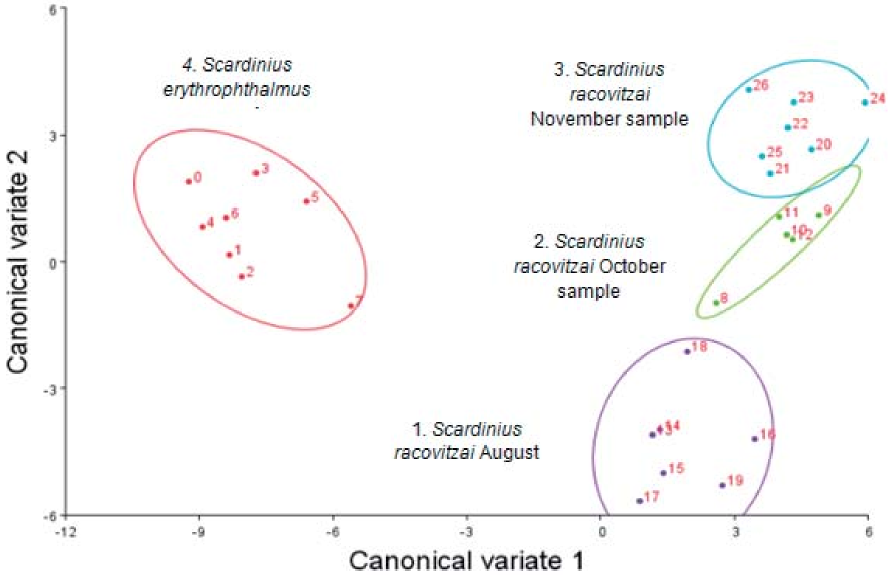

Fig. 6. The scatter plot of the CV scores with equal frequency ellipses (P=0.9) that group the coordinates by sampling stage.

Table 5. Matrix with Mahalanobis distances between groups

Sample	Sample no.4
Sample no.1	**12.2975**
Sample no.3	**12.4924**
Sample no.2	**11.0096**

Canonical variate analysis (CVA) allows a different type of ordination analysis, which maximizes the separation of specified groups (species/ecotypes, Klingenberg 2011). The results provided us the shape features that best distinguish among the fish groups from Peţea Lake and Danube river. The scatter plot of the CV scores displays the species separation (Fig. 6).

CVA generated as a result the matrices of farthest Mahalanobis distances between group no. 4 (*S. erythrophthalmus)* and the other three (representing the observations taken out from Petea lake fish) (Table 5). P-values from permutation tests (10000 permutation rounds) for Mahalanobis distances among groups is statistically significant ($P<0.05$).

The analysis of the geometry of individuals from the two species generated results in accordance with the morphometric discriminant analysis, yet a larger number of observations is needed for greater accuracy of results.

Water quality in rearing tanks. Hydro-chemical monitoring. The monitoring of tanks for ex situ conservation of *S. racovitzai* started with the initialization of the artificial environment. Before animals introduction, evolution of the aquatic environment and stabilization of parameters (temperature, GH/KH, ammonia, pH, conductivity, redox potential) were monitored (Figs. 7, 8). The animals were introduced when hydro-chemical parameters became concordant with the ecological requirements of the target species (Table 6).

Graphic representations of results, as displayed by the electronic monitoring and control system, are shown in Figs. 9-10. The data were collected continuously and gathered weekly by an USB connection in Excel and/or JPEG format.

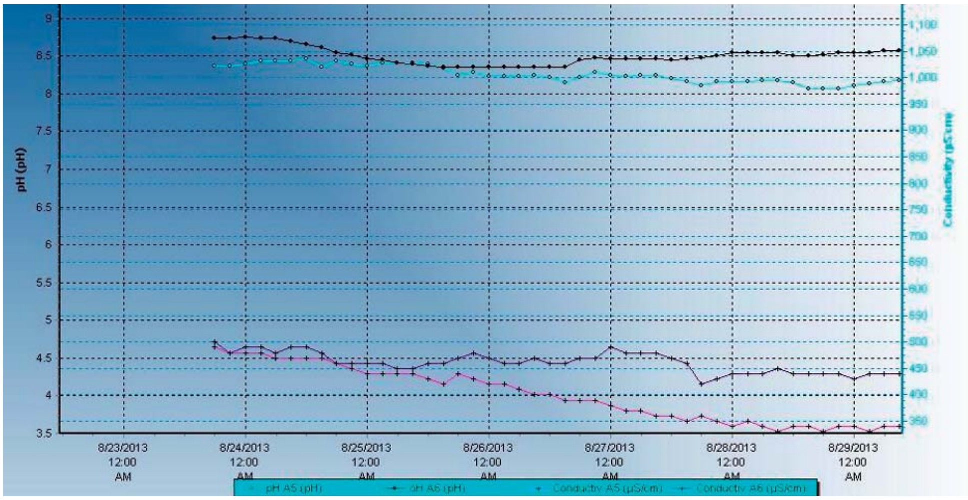

Fig. 7. Dynamics of pH and conductivity in quarantine tanks for S. racovitzai, module 2, aquariums aq_5 and aq_6, 23–29 August 2013.

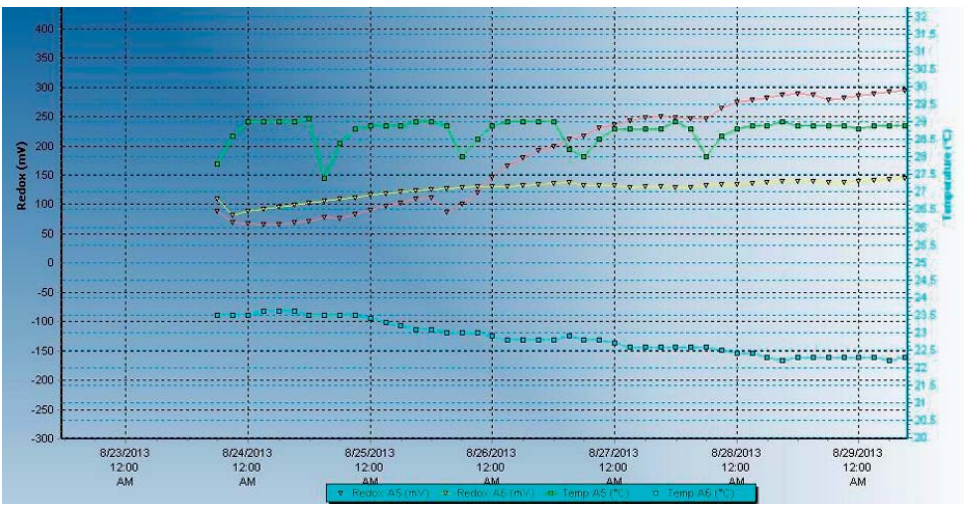

Fig. 8. Dynamics of temperature and redox potential (ORP) in quarantine tanks for S. racovitzai, module 2, aquariums aq_5 and aq_6, 23–29 August 2013.

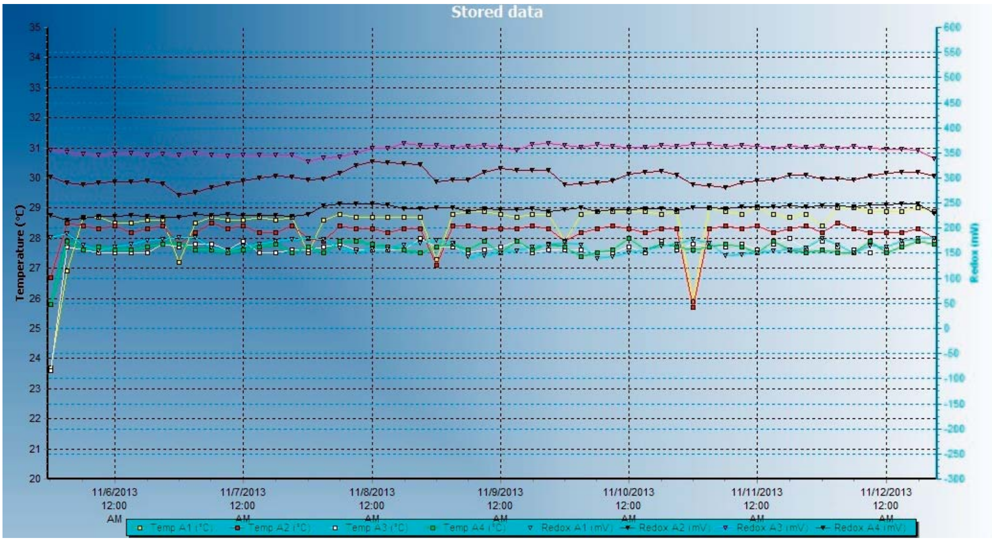

Fig. 9. Dynamics of temperature and conductivity in quarantine (aq_1) and rearing aquariums (aq_2: ex situ conservation of *Melanopsis parreyssii*); aq_3 and 4: rearing of *S. racovitzai* (module 3, 5–12 Nov. 2013)

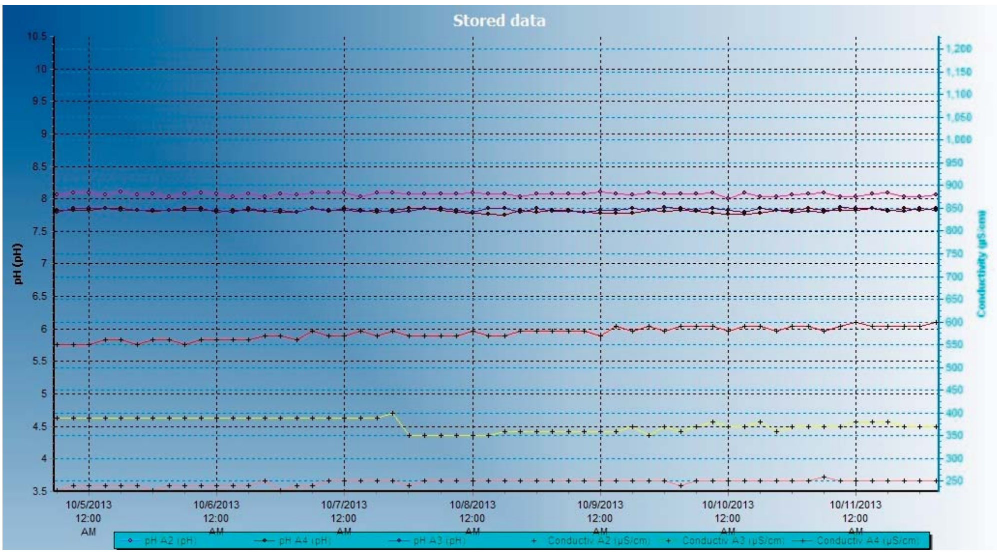

Fig. 10. Dynamics of pH and redox potential (ORP) in quarantine (aq_1) and rearing aquariums (aq_2: ex situ conservation of *M. parreyssii*); aq_3 and aq_4: rearing of *S. racovitzai* (module 3, 05–12 Nov. 2013)

An optimal environment in the period before the reproductive season is an essential requirement for sexual maturation in fishes.

Table 6. Dissolved oxygen (ppm) in tanks for rearing thermal rudd larvae and fry.

Aquarium	Interval of measurements	Nr. of tests	Confidence interval (95%)	Min.	Max.
Maternity 1	10 March – 04 April	8	6.64 ± 0.3	6.1	7
Maternity 2	10 March – 04 April	7	6.63 ± 0.3	6.1	7,07
Maternity 3	13 March – 09 May	8	6.64 ± 0.31	6.01	7
Maternity 4	17 March – 09 May	6	6.47 ± 0.63	5.8	7.52
Maternity 5	30 March – 04 April	2		4.63	6.6
Maternity 6	30 March – 04 April	2		5.42	6.7

Table 7. Temperature regime in rearing tanks for thermal rudd adults.

Parameter	Experimental variant (aquarium)			
	1	**2**	**3**	**4**
	A1	**A4**	**A5**	**A6**
Temperature (^{0}C, confidence interval, 95% significance)	28,502±0,23	27,646±0,08	27,279±0,16	28,014±0,16
Minimal temperature (^{0}C)	27,5	27,3	27,1	28,2
Maximal temperature (^{0}C)	29,0	28,0	27,9	28,8

Water temperature is a limiting factor influencing the maturation of gonads and triggering reproduction. Before the reproductive season, this factor was maintained constant in captivity and in accordance with the ecological requirements of the species (Table 7).

The chemical reaction of water was influenced by the pH of the primary source of water and the chemical and biochemical reaction in the aquarium and the external filter, excepting experimental variant 2 (aquarium „A4", heavily planted with thermophilic aquatic plants, where carbon dioxide was added by electronically controlled means as a nutrient for plant growth, and pH was maintained at 8.1); circadian variation and lower values in the night than the set level were due to plant metabolism.

In the other tanks, pH was slightly alkaline, with a sinusoidal circadian rhythm. The highest values were measured in the morning at 6-9 o'clock and pH values decreased until 18 o'clock as a result of biomass activity intensification, metabolic processes, respiration and addition of food.

Oxidation reduction potential (ORP) of water is correlated with the dissolved oxygen content, among other factors. The intensity and level of redox processes in water are correlated with the conditions for a balanced and healthy environment. Monitoring the redox potential during the experiment was performed in order to keep it within the slightly oxidative range (200 – 300 mV) and to allow its gradual evolution, without sudden variations. Its values during the experiment showed a consistent level of redox processes, and a low content of reducing substances and pollutants (mainly organic, highly reducing), and its maximum level was recorded in variant 4 (densely planted aquarium), as a consequence of photosynthesis that involves both oxidation and reduction reactions.

Water conductivity while rearing adults was similar to the thermal pond in two experimental variants (aquariums A4 and A5, conductivity > 600µS) and lower in variants A1 and A6 (250 – 500 µS).

Other chemical measurements were performed on nutrients content, limiting parameters such as dissolved oxygen, nitrites, ammonia, and water hardness through periodical analyses in the laboratory. The limiting factors such as dissolved oxygen concentration and water saturation with dissolved oxygen (Tables 6, 11), nitrites and ammonia/ammonium (Table 15) were kept within acceptable limits. Biological filters functioning well ensured a low nitrites and ammonia content, reducing water conditioning through technological means and also the interventions and stress on the fish.

Nutrients content was within tolerance limits according to the ecological requirements for freshwater Cyprinids: nitrates max. 50 ppm and inorganic phosphates max. 0.6–1.0 ppm (Munteanu 2003). Highest values were measured in the heavily planted aquarium, where nutrients (potassium nitrate and phosphate) were supplemented in a controlled manner to preserve its phytocenosis and to enrich the behavior of the breeding stock.

Water hardness was lower than in the thermal pond because of using water filtered through reverse osmosis in order to reduce the chlorine content in the primary source. The range of hardness values was in accordance with the rules for animal welfare. Although the thermal ecosystem has a high concentration of salts due to the origin or its water, the results of this ex situ conservation experiment prove that water hardness was not a limiting factor for reproduction in captivity at medium hardness values (10 –12 DG).

Chlorine concentration was 86–220 mg/l, with lower values in aquariums where water filtered through reverse osmosis was partially used. Free chlorine was less than 0.02 mg/l.

Average rate of water changes was 3.571 % daily.

Experimental variants to induce naturally directed reproduction in thermal rudd. Feeding of adults. Triggering gonads maturation by controlling temperature and photoperiod. The reproductive season of the thermal rudd is typically February-March (Müller 1958, Bănărescu, 1964), yet exceptions were mentioned about early spawning in captivity in December-January (Crăciun, 1997). In the experimental conditions at Aquarium Galaţi, no reproductive behavior was noticed in the aquariums initially set up. Modification and control of environmental stimuli started on Febr. 25 and triggered the reproductive response, differentiated depending on the degree of complexity of triggering factors and on inherent factors such as sex ratio.

Experimental variants during the reproductive season had in view an assessment of the reproductive response as triggered by environmental factors, providing an optimal physiological and condition state and environmental quality, and reducing stress caused by external stimuli (noise, human impact). A synoptic presentation of the modifications in the main factors triggering reproduction is shown in Table 8. Although the different experimental variants in this project have shown the factors influencing the reproduction of the thermal rudd, further research is needed to define the degree and significance of that influence on the results.

Controlling environmental factors such as temperature and/or photoperiod is a strategy for inducing reproduction in captivity, included also in technologies based on stimulation with hormonal extracts (Watanabe 2006, Abraham 2007, Vlaming 2006, Gil et al. 2010, Neufeld et al. 2011, Targonska et al. 2012, etc). Thermal and photoperiod determinism of reproductive cycle in temperate bony fishes which reproduce in spring are connected with the seasonal rhythm of those factors and the impact of temperature and photoperiod variations on the maturation of gonads.

The aim of the experimental model within the present study was to trigger reproduction by inducing natural stimuli in a way similar to phenomena in the natural environment of the species: increase of daylight duration (11 hours on Febr., 20) and decrease of temperature during winter (according to historical data for the thermal pond Ochiul Mare). Stimuli variations were gradually induced and reproductive response occurred spontaneously in aquariums A1 and A6 after different durations since the start of environmental stimuli modification (4 and 8 days, respectively). (Figs. 11, 12).

Average temperature during spawning in captivity was with 2.5 ^{0}C lower than the average temperature during rearing (Tables 7, 8).

Reproductive parameters: period and duration, estimation of prolificacy, fertilization and hatching rates, non-invasive collecting procedures, durations of hatching and yolk sac resorption. Duration of reproduction was 51 days in variant 1 (aquarium A1, 1 March – 20 April 2014) and 35 days in variant 4 (aquarium A6, 4 March – 8 April). Oocytes maturation in the thermal rudd belongs to the

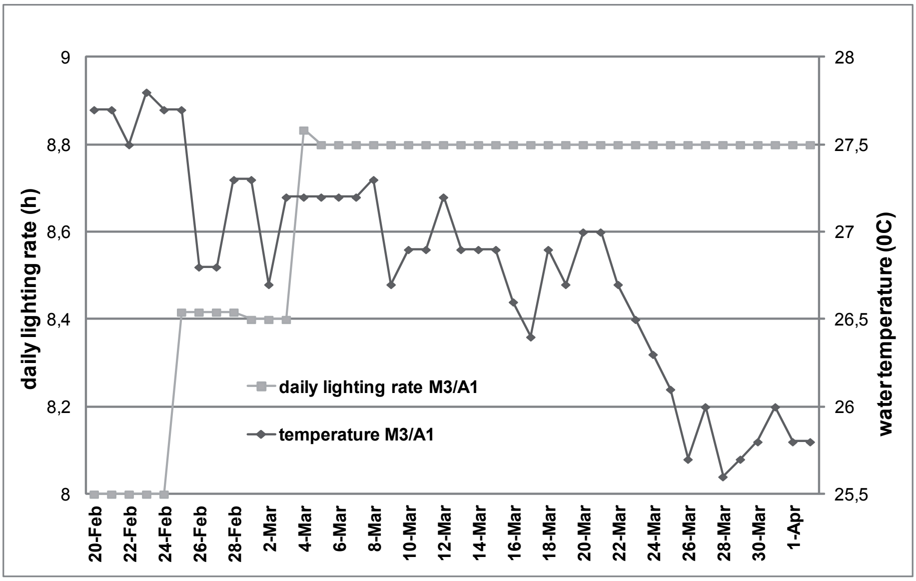

Fig. 11. Modification of temperature and light stimuli in variant 1 (aquarium A1) and reproductive response.

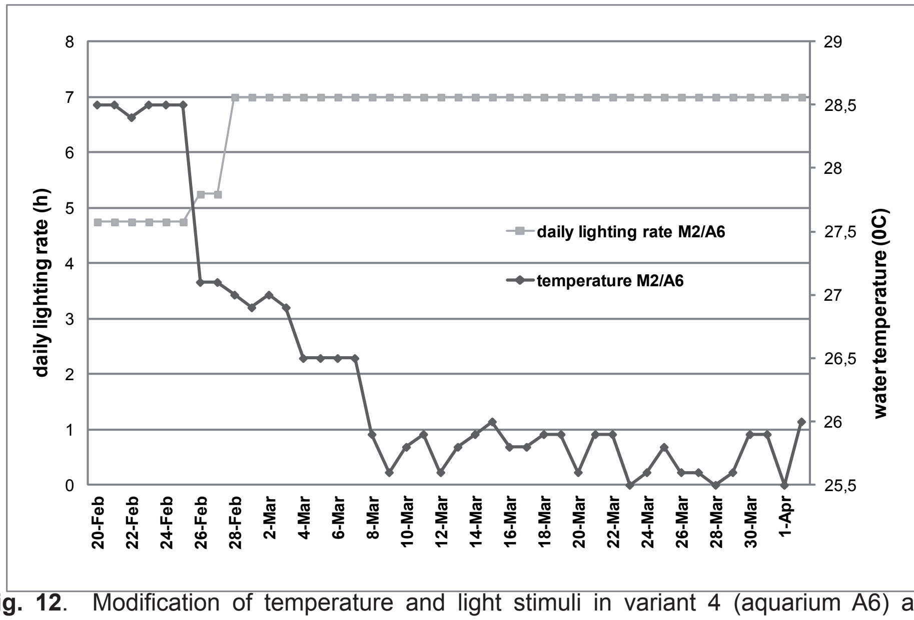

Fig. 12. Modification of temperature and light stimuli in variant 4 (aquarium A6) and reproductive response.

heterochronous type (with spawning in portions and adults surviving reproduction), yet more study is needed to complete the information on life cycle. Egg laying frequency constantly decreased during the reproductive season (Fig. 13).

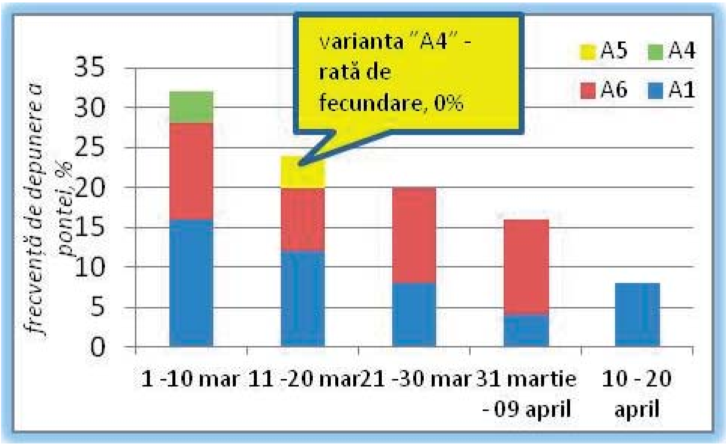

Fig. 13. Spawning period and frequency in the 4 experimental variants.

Spawns were estimated, photographed and counted using the ImageJ software. Incubation took place in separate tanks and in groups based on age, to reduce cannibalism, except variants MAT.3 and MAT.4 where successive spawns occurring at 24-72 hours interval were hosted.

Eggs were incubated at 26–28 ^{0}C for 48–72 hours, with a high hatching rate towards the end of that interval. Active feeding occurred after 24–48 hours. Six tanks for incubation and rearing the fry were used in order to separate the spawning, in accordance to the available space in aquarium.

The rate of fertilization/hatching/larvae survival up to 14–18 days of age was 44.2–69.2%. Populating incubation tanks with successive spawns obtained at 24-72 hours interval (variants MAT3 and MAT4) did not lead to significant differences in the rates of fertilization/hatching/larvae survival up to 14-18 days of age. After the fry entered the free swimming stage, survival percentage was nearly 100%, as losses were due mainly to the fertilization/hatching rate.

Rearing the fry. Water quality in the aquariums for rearing larvae and fry. After the larvae began feeding actively, they were fed every 3 hours in the interval 8 to 20 o'clock with *Artemia* nauplii from a culture in water with 25 ppm salinity and at 25 ^{0}C after 20-24 hours from hatching. After ten days from hatching, commercial flakes of freezed plankton and aquatic invertebrates (Rotifera and Cyclops) were added to this intake. No prompt reaction to artificial food (STARTER) was noticed, thus adaptability to that type of food was not tested in order to avoid quantitative and qualitative losses and to ensure an optimal growth rate in the first life stages, which is a critical condition for obtaining viable biological material for reintroduction in the natural habitat.

Table 8. Synoptic table of environmental and biotic factors controlled in order to trigger naturally directed reproduction in *S. racovitzai* in 4 experimental variants (I – IV)

Factor		I **A1**	II **A4**	III **A5**	IV **A6**
Biotope type		”Sterile” (no substrate), natural and artificial support for spawning	Densely planted ecosystem. Environmental enrichment	Natural substrate (sand), spawning support	Partly decorated with natural elements (wood, shells, epiphytic plants)
Rate of introduction (individuals per tank): minimal number of individuals to ensure the optimal sex ratio for reproduction		11 1,65 kg/m^3	12 1,8 kg/m^3	11 1,35 kg/m^3	14 1,72 kg/m^3
Tank volume and characteristics (rectangular, ”raceway” tanks)		180 l (116x36x43 cm)	180 l (116x36x43 cm)	220 l (117x48x43 cm)	220 l (117x48x43 cm)
Filtration: continuous in all 4 variants		External filter (900 l/h)			
Support for spawning		Natural and artifficial	Natural	Natural and artifficial	Natural and artifficial
Temperature (during 25 Febr.–2 April 2014, confidence interval interval). Gradual decrease.		26.74 ± 0.08 ^{0}C	26.41 ± 0.04 ^{0}C	26.04 ± 0.05 ^{0}C	26.00 ± 0.07 ^{0}C
Lighting during reproductive season: gradual increase of duration and/or intensity.	Type	Low, artificial lighting **	High, artificial lighting ****	Ambiental *	Medium, artificial lighting ***
		Solar Tropica Ultra JBL, daylight equivalent, 4000 K, CRI 90+, 54 W (2 tubes), 3750 lumen/lamp (0.56uE/s/W)			
	Intensity of lighting: ambiental (*) or applied (lux)	15420	17024	482 (*)	26900
	Intensity at water surface (lux)	143 – 253	4977 – 6725	67 – 82	300 – 463
Other characteristics of light as stimulus	Seasonal rhythm of light intensity	Not applied. Constant during ex situ conservation	Not applied. Constant during ex situ conservation	Not applied. Constant during ex situ conservation	Applied. Increased light intensity for trigering reproduction (<67 lux, initial intensity)
	Light duration	Increased during reproductive season	Increased during reproductive season	Constant during ex situ conservation.	Increased during reproductive season.
Feeding: constant intake in all variants, stimulation of breeding stock condition		Freezed Chironomids, 75 – 90 g/day (10–7 % of fish mass). Filamentous, freshwater green algae from own culture.			
Results of freproduction (spawnings)		12 spawnings (1 March – 20 Apr.)	One spawning (6 March).	One spawning (15 March). Fertile eggs percentage – zero.	11 spawnings (5 March – 9 Apr.)

Table 9. Water temperature in rearing tanks for thermal rudd larvae and fry (^0C)

Aquarium	Interval of measure-ments	Nr. of tests	Confidence interval (95%)	Min.	Max.
Maternity 1	10 March – 25 April	12	25.95 ± 0.3	25.1	26.5
Maternity 2	10 March – 25 April	10	26.18 ± 0.61	25.0	27.5
Maternity 3	09 March – 25 April	263	26.25 ± 0.06	25.6	27.4
Maternity 4	15 March – 02 April	10	26.22 ± 0.62	24.5	27.3
Maternity 5	02 – 25 April	2		26.3	26.6
Maternity 6	02 – 25 April	2		25.3	26.5

Table 10. Water pH in the tanks for rearing larvae and fry

Parameter	Interval of measure-ments	Nr. of tests	Confidence interval (95%)	Min.	Max.
Maternity 1	10 March – 25 April	12	8.11 ± 0.13	7.67	8.54
Maternity 2	10 March – 25 April	10	8.14 ± 0.09	7.89	8.29
Maternity 3	09 March – 25 April	263	7.72 ± 0.05	6.75	8.41
Maternity 4	15 March – 02 April	10	8.26 ± 0.15	8.11	8.83
Maternity 5	02 – 25 April	2		7.85	8.09
Maternity 6	02 – 25 April	2		8.12	8.18

Table 12. Water conductivity (μS) in tanks for rearing thermal rudd larvae and fry

Aquarium	Interval of measure-ments	Nr. of tests	Confidence interval (95%)	Min.	Max.
Maternity 1	10 March – 25 April	12	330.83 ± 45.53	270	500
Maternity 2	10 March – 25 April	10	344 ± 67.9	230	510
Maternity 3	09 March – 25 April	263	321.94 ± 19.31	160	700
Maternity 4	15 March – 02 April	10	386 ± 41.74	270	470
Maternity 5	02 – 25 April	2		260	360
Maternity 6	02 – 25 April	2		430	470

Table 13. Redox potential (mV) in tanks for rearing thermal rudd larvae and fry

Aquarium	Interval of measure-ments	Nr. of tests	Confidence interval (95%)	Min.	Max.
Maternity 1	10 March – 25 April	12	156.83 ± 36.81	75	239
Maternity 2	10 March – 25 April	10	151.7 ± 36.68	77	221
Maternity 3	09 March – 25 April	263	166.03 ± 4.85	89	243
Maternity 4	15 March – 02 April	10	193.5 ± 53.36	105	335
Maternity 5	02 – 25 April	2		151	169
Maternity 6	02 – 25 April	2		194	200

Table 14. Concentrations of nutrients (nitrates, phosphates) in tanks for rearing thermal rudd larvae and fry

Parameter	Concentration (ppm)
NO_3^- (mg/l)	14.9 ÷ 15.1
PO_4^{3-} (mg/l)	1 ÷ 1.01
P (mg/l)	0.33
P_2O_5 (mg/l)	0.75

Table 15. Concentration of nitrogen compounds (nitrites, ammonium, ammonia nitrogen) in tanks for rearing thermal rudd adults

Parameter	Concentration (ppm)
NO_2^- (mg/l)	0 ÷ 0.136
NO_2-N (mg/l)	0 ÷ 0.041
$NaNO_2$ (mg/l)	0 ÷ 0.203
NH_3 (mg/l)	0 ÷ 0.11
NH_4^- (mg/l)	0 ÷ 0.12
NH_3-N (mg/l)	0 ÷ 0.09

At 45 days after spawning, the fry were transferred in rearing tanks (180–220 l), at densities of 3.65–4.31 individuals per liter. The size of food and the ration (10–12 % of body weight) were gradually increased in accordance with biomass growth. The early life stages are pictured in Figs. 14-17).

Maintaining an optimal abiotic environment during the development of the fry is very important for obtaining good biological material. Physical and chemical parameters in the tanks for rearing larvae and fry were monitored and kept within optimal limits (Tables 8-15).

Intense partial changes of water were performed in the first days after transferring the spawns, to avoid environmental degradation due to the decay of unfertilized or unhatched eggs. Water temperature was controlled by a thermostated heater. Water pH was above 8, but lower than in the tanks for adults. In the MAT 3 variant, lower values were recorded before populating the tank.

Dissolved oxygen concentration was below the saturation limit. Due to high density, temperature and respiration rate in larvae and fry, the water in their rearing tanks was intensely aerated. Water conductivity was lower than in the tanks for breeding stock, due to the usage of reverse osmosis filtration to decrease chlorine/chlorides content, as fishes are sensitive in their early stages. Accidental exposure

Fig. 14.Thermal rudd embryo, age 30 hours. CMSN Aquarium Galaţi.

Fig. 15. Thermal rudd fry after hatching. Yolk sac present. CMSN Aquarium Galaţi.

Fig. 16. Thermal rudd larva with the yolk sac resorbed, before free swimming. CMSN Aquarium Galaţi.

Fig. 17. Thermal rudd immobile larvae, before active feeding. CMSN Aquarium Galaţi.

to extreme concentrations of nitrogen compounds (nitrites, ammonia/ammonium) was short in variant MAT 6, where density of individuals was maximal (highest concentrations: nitrites 0.541 mg/l, ammonia 0.72 mg/l, ammonium 0.77 mg/l, and ammonia nitrogen 0.6 mg/l, at pH 8.0).

Conclusions

Water temperature represents one of the restricting environment factors that strongly influences on the biological processes in fishes. The latest thermal conditions in „Ochiul Mare" lake have dramatically deteriorated as a result of over-exploitation and a drastic decrease in the thermal spring flow, water temperature and level. The winter water temperature values are below the minimal tolerance limit for the thermophilic species (20 ^{0}C according to the bibliography) except in the lake's central area that is influenced by the flow of the geothermal underwater spring. The survival of warm-water populations from Petea Lake is threatened by extreme temperature and length of exposure to this critical factor.

Water temperature is a critical factor for the survival of the thermophilic, endemic species in Lake Peţea, and more studies for identifying and eliminating the causes affecting the optimal water input in the lake (spring clogging, over-extraction of thermal water in the area), as well as a co-operation of all responsible organizations must be main objectives for managing the situation, for species survival and for ecosystem restoration.

Maintaining the temperature values in the natural ecosystem close to the tolerance range for the thermophilic species depends on the balance between hydrologic factors (thermal flow, Peţea rivulet flow), climatic factors (air temperature, rainfall regime), and lake volume and evaporation intensity, respectively.

Following the deterioration of thermal ecosystem, the testing of morphological variability of the present thermal rudd population *(S. racovitzai)* sampled during 2013 and comparison to its congener *S. erythrophthalmus* represented a first objective of any ex situ conservation work. The geometric morphometric analyses revealed statistically significant differences between present population of thermal rudd and *S. erythrophthalmus.* We noted the lowering of the ranges of some morphometric characters that can be linked to the phenotypic plasticity of fish. They quickly adapt by modifying their physiology and behavior to environmental changes. A total number of 6 morphological characters are most useful for discriminating among the species (head features, caudal peduncle, pectoral and ventral fins lengths). Despite of various references regarding the morphological, physiological and behavioral differences between the two species, molecular techniques are necessary for clarifying the specific status of thermal rudd community form thermal lake.

Successful naturally controlled reproduction in captivity was correlated with: broodstock capture; providing of a good physiological status of the breeding stock; presence of both sexes; identification and control of environmental factors that regulate the timing of sexual maturation of fish; providing conditions for fertilization, hatching and growth of larvae and fry; preparation of captive reared/bred individuals for (re)introduction in their natural habitat.

The analysis of wild adults' condition showed a positive allometric growth, yet inferior to their condition after 175 days in captivity, possibly indicating a poor trophic availability of the habitat, which does not constantly provide qualitative and/or quantitative optimal nutrient resources.

Restricting factors for fish growth were kept within optimal limits: temperature 27.3–28.5 °C, dissolved oxygen 7.3–7.6 ppm (saturation 84–96 %), ammonia/ammonium < 0.1 ppm, nitrites < 0.04 ppm; nitrates < 32 ppm; hardness is not a limiting factor for animal welfare, as the thermal rudd accepted medium hardness and conductivity (10-12 ^{0}G, 250-700 µS); also, pH values of 7.81–8.50, in accordance to the tolerance of this species to a wide pH range, ensured an optimal physiological state in captivity, including an optimal reproductive response.

The experimental model applied had in view the triggering of reproduction by gradual control of natural stimuli: increase of photoperiod (with 10.41–47.36 %) and light (143–463 lux) and decrease of temperature (0.5 ^{0}C daily, down to a minimum of 26 ^{0}C).

The reproductive season was 1 March – 20 April. Reproduction belongs to the heterochronous type, with multiple spawnings during a short season of 35-51 days. The experiments confirm the recent researches regarding a longer life cycle than 2 years and the breeding stock was still viable at 90 days since the start of reproduction.

The frequency of spawning was highest at the start of the reproductive season and gradually decreased. The preferred level for spawning was a natural or artificial support for the sticky eggs to adhere, at medium or superior depth and the fish did not avoid the highly oxygenated and filtered flow from the external filter. A nocturnal rate of fish ovulation was noted, with the highest probability between 0 and 6 hours.

An at least satisfying level of ecological plasticity to captivity, as noticed during the present study (e. g. tolerance to space, pH, salt content and dissolved oxygen), proved no justification for the scarcity of research on this fish. The preliminary results of the present study justify the need for the continuation of studies on the thermophilic populations and habitat, and they complete the knowledge of life cycle, reproductive biology, circadian rhythm of breeding, sex ratio, and tolerance to abiotic factors.

Suggested measures for conservation. Maintaining and reproduction in captivity and complementary procedures for conservation of the thermal rudd population have to be but an alternative while restoring the natural habitat should be achieved, thus in situ and ex situ conservation should be taken in an integrated manner. Through its objectives, the strategy for ex situ conservation should provide conditions for an efficient management in order to (re)introduce adults and/or their offspring in the natural habitat, having a positive impact on biodiversity conservation in the thermal lake in the reserve.

Table 16. Threats and suggested management measures for in situ conservation of the themal rudd

Risk factor	Effect on fish	Measures for better conservation. Methods and actions.
Frequent, sudden and critical decrease of water surface	Increased competitivity for trophic resources. Decreased living space. Increased vulnerability of adults and eggs to predators and cannibalism. Decreased distribution of thermal water lily, a shelter for fish schools (the species is shy and has an avoidance behavior) and possibly preferred support for spawning at low water depth.	Restoration and conservation of natural habitat. Restriction of over-extracting thermal water, including adapted rules. Suggestions for new legal rules to protect and conserve the habitat during reproductive season. Conservation of thermal water lily by conserving the habitat by restriction of thermal water over-extraction.
Interruption of thermal water flow in the lake because of excessive extraction and decrease of spring flow/ pressure	Indirect. The entire microbiological complex in the substrate is deprived of favorable effect of ascending water circulation. Increased risk for an anoxic environment in the substrate, unfit to the constant development of benthic communities and affecting quantitatively and qualitatively the trophic base and condition of fish. Lower physiological and condition of fish because of diminished trophic resources. Decreased water temperature below tolerance limits for thermophilic species, endangering sexual maturation, ovulation, ontogenetic development of offspring, and the survival of the fry, which are more sensitive to environmental factors and their variations.	Restriction of excessive extraction of thermal water, including adapted legal rules, with a positive impact on the ascending circulation and constant inflow of thermal water, stimulating ecological mechanisms in lake sediments, and density and diversity of benthic trophic components. Quantitative and qualitative conservation of water and sediments under natural conditions.
Presence of invasive species	In addition to the occurrence of invasive species, already known and mentioned in the management plan of the reserve, there is a risk of egg consumption, noticed in captivity, by invasive Gastropods such as *Physa/Physella*, with a short life cycle and highly prolific.	Quantitative and qualitative conservation of thermal water and sediments under natural conditions. Ex situ and in situ conservations, by measures suggested here, of the thermal rudd population, so that its reproductive potential ensures species survival and compensates predators' influence at different life stages of the fish.
Past, excessive growth of plant biomass, mainly the invasive and opportunist *Ceratopteris sp.*	Dead plants biomass affected sediments structure. Excessive thickening of organic mud layer changed the regime of gases in the substrate, influencing the quality and quantity of benthic communities (source of food for fish).	Continued control of excessive growth of invasive plants. Control of nutrients by dilution with constant inflow of allochtonous water (the thermal spring and the temporary Glighii brook). Ensuring ascending circulation and a constant inflow of thermal water, with a positive impact on ecological mechanisms in sediments and decomposers activity. Conservation of thermal water lily, by all the measures suggested here.
Pollution of the Glighii tributary. Eutrophization.	Organic and inorganic pollutants transported by the tributary influence the vital functions of fishes. Accumulation of nutrients favors explosive development of aquatic macrophytes, limiting open water areas (which are adequate to schooling behavior in fishes, especially during breeding, when they are extremely sensitive), and generating a hypoxic environment in hot days.	Control of garbage disposal and any pollutans near the reserve and its Glighii tributary. Control of nutrients by dillution with constant inflow of allochtonous water (the thermal spring and the temporary Glighii brook).

Cumulative stressing factors affect the thermal ecosystem, surpassing the benign level and consuming the energy of the fish to avoid dangers, which adds to the extinction risk for an already small and isolated population.

Ex situ studies on the biology, ecology and behavior of the thermal rudd at CMSN Aquarium Galaţi allowed a detailed assessment of threats and management measures needed for a viable conservation of this population (Table 16).

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