Szent István University

Studies on the reproduction, genetics and habitat use of topmouth gudgeon in Hungarian populations

PhD thesis

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2016

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1. Background and aims of the study

One of the highest threat to water ecosystems is the spreading and establishment of invasive species. Besides naturally appearing species and along with the development of aquaculture, alien fish species were probably introduced to the water catchment area of the Danube River as early as the Middle Ages. The process gained momentum at the end of 19th and beginning of the 20th century when native fish species of North-America were introduced into numerous countries with an economic interest. In the next wave of deliberate introduction in the 1950s and ‘60s, eastern European and Asian herbivorous species were introduced, including the topmouth gudgeon (TEMMINCK & SCHLEGEL, 1846). Some of the introductions resulted in positive economic effect and without considerable environmental burden (rainbow trout), however in some cases economic advantages were accompanied by environmental effects difficult to measure and eliminate (silver carp). Finally, in the case of four species it can be stated that accidental or deliberate introduction of these species resulted in serious economic and environmental damage, namely brown and black bullhead, Prussian carp and the topmouth gudgeon. While the first three species is widely considered as a harmful fish, topmouth gudgeon is still cited as “tolerated and negligible species” in some educational materials. Recent studies changed this judgement. In parallel of the appearance of the species in Western Europe, ecological impact of topmouth gudgeon was widely examined on the territory concerned revealing that these impacts proven to be broader than previously estimated. On the other hand, an appreciation of natural food supply, also consumed by topmouth gudgeon, in Central European pond fish culture happened with an increased economic competition. All these abovementioned reasons has led to the need of the execution of further research in the topic of the biology of the species unrevealed by earlier studies.

Aims of the study

Based on the abovementioned reasons this study aimed:

- to measure the distribution of topmouth gudgeon on one of the most important refugium area of the species: in the Hungarian fish farms, being less studied in previous research,
- to discover the genetic variability of the species in Hungary,
- to examine the temporal development of the ovary in Hungarian populations,
- to measure the rate of yearlings in some populations,
- based on the data gained from Hungarian populations, body length-body weight correlation is going to be described. With the coefficient, condition of each focus population is planned to be examined and obtained results will be compared with reproduction biology results and rate of yearlings,
- to discover the effect of predators on the habitat use of topmouth gudgeon.

All the above information and the previously available data would create a base of knowledge and a scientific background to help to understand the distribution and ecological impact of topmouth gudgeon. With this help a potential is created to fight off the species from natural habitats and fish ponds. The current study also provide a scientific background to the preparation of an action plan, obligatory for Hungary, foreseen by the regulation no. 1143/2014/EU of the European Parliament and of the Council.

2. Material and methods

2.1 Survey

A survey was sent to fish farms of the database of Szent István University, Department of Fish Culture and Fisheries in 2005. Fish farms included in the database covered up to 94% of Hungarian aquaculture surface. Survey was distributed by ordinary mail to 55 fish farms with a reminding phone call if answer did not arrived in time.

34 survey forms arrived back meaning 61% of the total number and representing more than 150 km^2 surface of Hungarian fish farms (66% coverage). Only productive fish farms were interviewed. The survey contained 18 questions: 6 general questions and 4 questions focusing on each harmful invasive fish species (Prussian carp, black and brown bullhead and topmouth gudgeon). Questions were created to be easy-to-answer.

The general questions focused on fish farm size and profile, fish pond structure, quantity and species of produced carnivorous fish and the most harmful invasive fish species. Previous experience and examinations suggested the above factors to be potentially responsible for the presence of invasive fish species. In the questions connected to each invasive fish species quantity, temporal change of population size, management/protection practices were queried. In the current publication data connected to topmouth gudgeon is only introduced and discussed. Data were recorded by using Microsoft Excel program. Answers were recorded on an ordinal scale and an analysis was made based on a data matrix. Statistical analysis of Kurskal-Wallis test was applied run by Graphpad 6.0 for Windows program.

2.2 Genetic analysis

2.2.1 Sampling place and time

The aim of the genetic analysis was to reveal possible differences among Hungarian populations of topmouth gudgeon. Samples were collected from Hungary (5), Czech Republic (2), Poland (1), Slovenia (1) and China (1) between May 2007 and June 2008. Fish were killed by phenoxyethanol and then conserved in ethanol (96%), stored ad 20°C.

2.2.2 DNS-extraction

Fish tissue was placed into Eppendorf tubes with 300 µl of 1xSET puffer (100 nM $NaCl_2$; 20 mM EDTA; 05% SDS; 0.1µg/ml Proteinase K; 50 mM Tris-HCl pH 7.8), then incubated for two hours on 55°C-on. Further 500 µl of phenol was added to the liquid then was shaken and centrifuged (Hettich Rotanta 460R, 14000 rpm) for 15 minutes. In the second phase, supernatant was transferred to a new Eppendorf tube and 500 µl phenol and 500 µl chloroform were added. After 15 minutes of centrifugation at 14 000 rpm, supernatant was transferred to a new Eppendorf tube with500 µl chloroform again. After a 15 minutes of centrifugation at 14 000 rpm, supernatant liquid was removed and tubes were filled by ethanol of -20 °C and 99% concentration. After a 15 minutes of centrifugation at 14 000 rpm, liquid was removed from precipitation and dried at room temperature. In the last step DNA was solubilized in 100 µl distilled water.

DNA concentration was determined by Implen-spectrophotometer (NanoPhotometer Pearl). Absorbance was measured at 260-280 nm. 1.5-2 µg/ml concentration was considered acceptable. Measured values were substituted into the following formula:

$$a \cdot \frac{50}{1000} \cdot b = c$$

where **a**: absorbance measured on 260-280 nm, **1000**: a change between ng-µg, **50**: a constant of absorbance, **b**: dilution rate (in our case, as sample was not diluted, the value was 1), **c**: solution in µg/ml

2.2.3 RAPD-reaction

Composition of the reaction liquid: Final quantity: 25 µl

1x Buffer; 3 mM $MgCl_2$; 0,8 mM dNTP; 1.5 n Taq-polimerase; 0.6pM primer; 15ng DNS-template.

Temperature during PCR:

Pre-denaturation: 85 °C - 2 perc

Cycle: 45x repeated: 94 °C - 20 s, 36 °C - 20 s 72 °C - 1 min. 30 s

Final chain building: 72 °C - 5 min

Storage until electrophoresis: 4 °C.

After preliminary examinations, where 21 primers were tested, the following 2 primers were selected for the analysis:

U 192: GCAAGTCACT

VILA M8: TCTGTTCCCC.

2.2.4 Gel electrophoresis

1.5 g agarose was diluted in 100 ml 1xTBE liquid (89 mM Tris, 89 mM boric acid, 2mM EDTA) then heated until become water-clear. After cooling down to 60 °C, 60 µl ethidium-bromide was added and the mixture was poured into electrophoresis gel box and well combs were added to create holes. PCR product was added to holes and DNA ladder (GeneRuler 100 bp DNA Ladder, Lambda DNA, Fermentase PstI enzyme, producer Fermentas, Thermo Fisher Scientific) was introduced in the last hole to determine fragment length. Gel box was filled with TBE1x. After 30 min of running, photos were taken and analysed.

2.2.5 Evaluation of fragment running

Evaluation of fragments was carried out visually based on a comparison to DNA ladder. Presence of 11 easily identifiable, randomly selected fragments were evaluated as present (1) or not (0). The received binary code was typical for each genotype. For the easier management, binary codes were transformed to decimal codes.

POPGEN 1.32 was used to create dendogram calculating distance based on Nei formule. Dendogram was created based on UPGMA method with 1000 times permutations by NEIGHBOR programme PHYLIP 3.5 version.

2.3 Morphometrical and reproduction biology studies

The data collection of morphometrical and reproduction biology studies is presented together as examinations were carried our parallel on the same individuals. Fish specimens were collected from eight sites of four water systems:

- Babat valley: pond no. I., III. and IX,
- Nagykónyi pond system pond no. II.,
- Pond no. VII. in Paks Nuclear Power Station Fish Organization,
- Fish system of Attala Fish Ltd.: pond no. VII., inlet water channel of the wintering ponds and filter pond of the hatchery.

A total of 1799 individuals were sampled in 29 occasions. Sampling was carried out by using a bait fish net of 1x1m size with 1mm holes. Standard body length was measured by a ruler with an accuracy of 1mm; body weight was measured by Sartorius L310 scale at an accuracy of 0.01 g. After anaesthetic overdosing and dissection, weight of genital organs of each individual was measured by an accuracy of 0.01 g (Sartorius L310). From organs of the fish originated from Babat and Nagykónyi in the year of 2006, tissue samples were taken and sections were prepared if the size of the ovary was over 0.01g.

A total of 77 samples from the preserved 81 ones were included in the examinations. When ovary was proven to be less than 0.01 g, a whole-body dorsoventral section was prepared in 25 individuals from each sampling area (n=11).

Tissue sections was fixed in Bouin-solution and after a fixation of 12-16 hours, were conserved in ethanol. After dehydration and washing with xylene, samples were fixed in paraffin, then cut and dyed with haematoxylin and eosine. Number and rate of oocytes of different phase were counted by using a Nikon Eclipse E600 microscope. Connection between an individual’s GSI and rate of oocytes of different phases (Spearman-test), connection between average GSI of a population and rate of oocytes of different phases (Pearson-test) were searched by correlation analysis.

In the case of morphological analysis, Graphpad Prism 6.0 software was used to calculate correlation between body length and body weight. After excluding outliers with ROUT-method, linear regression model was applied to evaluate correspondence. To simplify the calculation, a transformed formula was used:

$$\log W = \log a + b \cdot \log L$$

where “W” is body weight,
“a” is regression coefficient,
“b” is exhibitor
“L” is body length.
Comparison of different populations were examined by the formula of

$$W_{rm} = W/a_m \cdot L_{bm},$$

where “W_{rm}” is fitness,
“W” is the average body weight,
„a_m” is the average regression coefficient based on literature,
„b_m” is exhibitor based on literature
“L” is average body length in the population.

Finally a calculation on the rate of one-year-old progeny of the total number of individuals and the total biomass were calculated. Examinations were carried out in July and August. A histogram with sections of 5 mm was made based on the standard body length of fish individuals. On the basis of the above mentioned histogram and knowing the body weight, rate of 0+ and older age groups were defined and average standard body length of 0+ age group was determined.

2.4 Habitat use examinations

2.4.1 First set of experiments

Experiments under natural conditions were carried out in the Babat-valley, examining the ethology of topmouth gudgeon in two ponds, Babat I. and Babat III. Both ponds are euthrophic with considerable macrovegetation (reed-segde and submerged vegetation) in the littoral zone and 50% of open water surface. Fish community of Babat I. pond consisted only 6 species at the time of the study. A significant quantity of Prussian carp (*Carassius gibelio*), topmouth gudgeon and perch (*Perca fluviatilis*), less carp (*Cyprinus carpio*), pikeperch (*Sander lucioperca*) and common rudd (*Scardinius erythrophthalmus*) were found in the pond. An important information is that perch was installed in the pond in 2005 and the first successful spawning occurred in 2006. In the lake Babat III., fish fauna was very similar to those of Babat I. pond except that carp and carnivorous species were missing. Topmouth gudgeon population density was similar and considerably high in both lakes, based on preliminary studies, proven by the statistical analysis of the CPU of 60-minute trapping results.
Homemade traps were used for sampling with the following design: a 20 mm-diameter entrance, 1.5 dm^3 capacity, translucent perforated side panels, drain holes and a locking tab, a thin rope, a 200-gram weight and a float comprised on the traps, the latest to allows traps to be lowered in the required water depth. 5 g of fishing bait Maros Mix were placed in each trap. In the experiments, the trap

caught only topmouth gudgeon. The size selectivity of the trap was examined as well. Size rate of 1+ generation fish individuals was examined by comparing the above results with catches of a non-selective trapping method of 1x1x1 m size liftnet with a mesh size of 1mm. Because of the size distribution was not natural, the data analysis was performed using the Mann-Whitney test.

To determine the appropriate exposure time, preliminary trial were carried out. A time scale of 15, 30, and 60 minutes was examined to determine the effect of exposure time on catch probability and number of fish caught. Examinations were carried out in two series and 3-3 traps in each set (analysis: Kruskal-Wallis test).

Finally distance between traps to be independent was examined by mark-and-recapture trials where independence meant that captured animal should only connected to one trap-area. 3 sampling points with a 25 m distance from the neighbouring one were determined. Fish were caught, marked differently and released to the water. Trapping was repeated after 1 day, 1 week and 2 week-time, number of unmarked and marked fish individuals and the original location of the catches were noted. The distance of 25 m was calculated based on preliminary studies and considering natural circumstances by presuming that 25 m would be sufficient to reach independence and still not overlong to be able to mark enough sampling points. Preliminary studies were carried out in 2005.

Based on the above mentioned preliminary studies, a 24-hour sampling series was carried out in July 2006 under sunny weather free of weather fronts. Five independent sampling areas were selected perpendicular to the longitudinal axis of the lake. Within each sampling area three traps were placed as follows: a trap in the coastal vegetation was placed halfway through the water column, one of the open water area 0.5 meters from the surface and one in the open water 0.25 m from the bottom (hereinafter referred to as the littoral, pelagic and benthic samples). During the day four measurements were made with a one-hour exposure time (6:00, 12:00, 18:00, 24:00). Number and size of caught fish, water temperature and oxygen levels in close proximity to the trap were recorded (the measurement was made with WTW Oxi 96 device).

The collected data were recorded in Microsoft Office Excel, and distribution of animals with $\chi 2$ test were analyzed, while correlation analysis was calculated to reveal the effect of temperature and oxygen levels. To perform the analysis Graphpad Prism 6.0 program was used.

3.4.2 Second set of experiments

Based on the results of experiments in natural waters a null hypothesis was set up that the habitat use of topmouth gudgeon was determined by the presence of predators. To prove this hypothesis, a simplified aquarium test system was built in July and August 2009. A free to use space of 1200x600x300 mm was constructed. Aquarium was illuminated for 12 hours a day at the top of the system. Water vegetation was imitated by a 4 x 5 cm wide and 35 cm long

Rashel net, water temperature was 25 °C continuously. Location of the fish was monitored by photographs, made by a Canon EOS 300 camera fixed on rack. Fish originated from two lakes: Kincsem fishing pond from Hatvan and Babat IX. pond. In the first one, besides carps and bream species (*Abramis* spp., *Rutilus rutilus*, *Carassius gibelio*), some large catfish (*Silurus glanis*) are present, which are typically do not feed on topmouth gudgeon. In the Babat IX. pond, carp, Prussian carp and bullhead are present, from which the later regularly feeds on topmouth gudgeon. Specimens were trapped by 1x1 meter lift nets and were randomly chosen for the experiment from the 1+ and older age groups.

Experiments were repeated three times for 4 treatments from fresh individuals of both populations independently. In each setting 35 images were taken at an interval of 2 minutes and evaluated only those where position of each fish in the aquarium was identified. In the first set, only topmouth gudgeon was present in the aquarium. The results were analyzed immediately and experiment was only continued if no statistically verifiable difference in preference ($\chi 2$ test) was found between the use of the left and right side of the aquarium.

Then plastic plants were placed differently (right or left) and after acclimatization (when the fish's behavior showed that become accustomed to the new landmarks) photos were taken.

In the third treatment, a perch of 15 cm total body length was placed in and after a further 24-hour acclimation time shots were made. Perch was placed directly between the examined fish, because according to the literature, the most natural reaction was expected in this way. The perch could hunt or even eat the fish, prompting a more natural response from topmouth gudgeon.

In the last set, perch remained in the aquarium but plant was removed. The resulting photographs were analyzed on computer dividing the aquarium into four parts (center line to the right and left or up and down from the center line) and the number of fish was recorded. The collected data was recorded in Microsoft Office Excel, and then analyzed by $\chi 2$ test. Where $\chi 2$ test is statistically proven that fish location is not random, a Bonnferroni post test was made. To perform the analysis of the program Windows Excel and Graphpad Prism 6.0 was used.

3. Results

3.1 Distribution of topmouth gudgeon in the Hungarian fish farms

3.1.1 General questions

The answers to general questions were representative of the surveyed farms. 13 (38%) and 14 respondents (41%) relied on less than 100 hectares and 101-500 ha surface, respectively. Three farms (9%) fell in the 501-1000 ha category and four farms (12%) produced fish on more than 1001 ha. Based on the first question, the size distribution of farms in the sample was very similar to those of the population (in the population this figure was 47%, 41%, 6% and 5%). More than 5% deviation from the smallest and the largest size category was observed: the smallest farms were slightly underweighted, the largest ones were slightly over-represented in the sample. However, it is important to know that while less than 9% of the share was responsible for the category of less than 0.1 km2 area, more than 37% of the total farm surface in Hungary is managed by farms larger than 1 km^2. The performed statistical test (Kruskal-Wallis test) also confirmed that there were no difference in the share of categories (P = 0.31).

Numerous factors (fish farm size, profile, pond system, produced predator fish species and quantity) were examined to reveal the effect on IAS on the farms but only between farm profile and presence of IAS resulted connection by the applied statistical analysis (Kruskal-Wallis test, P = 0.0215). In all other cases no significant difference was demonstrated (P = 0.2299 to 0.8297).

The last question of the general query focused on which of the three main IAS caused the most problems. Less than 10% of the respondents answered that none of the present IAS caused problems (8.82%), while 2,94% of fish farms pointed on topmouth gudgeon, 5.88% on Prussian carp and 47.06% on bullheads. 5.88% of the respondents named topmouth gudgeon and Prussian carp, while in 29,41%, bullhead and Prussian carp to be the species caused the most serious problem.

3.1.2 Question focusing on the topmouth gudgeon

The first question focused on the presence and amount of topmouth gudgeon in the fish farms. Only 5.88% responded declaring that topmouth gudgeon is not present in the farm. More than 76% reported the presence of the fish with a quantity of less than 1 kg/ha, while 11.76% responded quantities of between 1 and 10 kg/ha. In the case of two farms, representing 5.88% of the total surface, topmouth gudgeon exceeded 10 kg/ha.

In the second question (How the quantity of topmouth gudgeon changed in the last three years?) 58.58% responded unchanged, 29.24% reported decline while 11.67% declared increase in the population.

The third question focused on topmouth gudgeon management. 17.65% of the respondents did not managed the topmouth gudgeon population, 29.24% reported to eliminate topmouth gudgeon at fish harvesting and disinfected pond bed, using different forms of lime. 35.29% of fish farm owners retailed topmouth gudgeon as forage, animal feed or bait and after disinfected fish pond beds. 17.65% of interviewed fish farms had not responded or topmouth gudgeon was not present in the pond.

The aim of the fourth question was to determine the most important elements of the prevention against topmouth gudgeon. 23.53% of the respondents considered topmouth gudgeon as not harmful therefore they did not made any steps against the infection. 26.46% recommended the stocking of carnivorous fish species while filtration and disinfection of inlet waters was mentioned by 11.76%. 35.29% considered that only the combination of the above methods could provide good results.

3.2 Results of the genetic analysis

3.2.1 Results of the RAPD-analysis

In the first set of the trial series 21 primers were tested from which some did not show amplification or polymorphism among individuals. Finally two primers, „U192” and „VILA M8” were chosen to the analysis and samples showed high polymorphism with the above primers. Among the polymorph fragments, 6-6 were selected for further analysis in the case of 201 individuals of 10 populations.

All the selected fragments were present in at least eight populations, however, no sequence was present in all the ten populations. The most frequent fragment was present in 93% of individuals, while the rarest one was found in 11% of the individuals.

3.2.2 Comparison of the populations

Presence (1) or absence (0) of the fragment was tested in each examined individuals. Typical genotype was determined for all the individuals based on one primer and also based on both primers. Comparing the results made with two primers it can be stated that there is no significant difference between the number of genotypes (31 and 36 genotypes), however, when the two primers taken together, a much more detailed resolution is obtained (120 genotypes).

The received results did not show a strong correlation between geographical distance and allele frequencies in the populations. Although the Czech and Slovenian samples proven to be isolated from the samples in Hungary, the Polish and Chinese patterns did not revealed considerable differences. A sample from Poland proven to be similar to a sample from Köröm, while the Chinese sample showed isomorphic patterns from those of Babat Valley. It is important to note that only three individuals from Slovenia were available. For larger number of individuals the genetic distance of this sample would have been different from the rest of the studied populations, as in the case of certain fragments larger number of elements can appear in the sample, thus genetic distance by formula of Nei would have been different.

3.3 Results of reproduction biology and morphometric analysis

3.3.1 Reproduction biology results

An average of GSI was calculated by populations and sampling dates for all the 1799 individuals. The results can be found in Table 1.

Table 1. Average GSI of the examined topmouth gudgeon populations

Sampling site		2006									2007				2008					2009
		4	5	6	7	8	9	10	11	12	5	6	7	8	5	6	7	10	11	3
Babat	IX.															0,61	0,00			
	III.					0,00						0,11	0,00							
	I.	1,75	2,20	0,59	0,00		0,65	1,00	2,65	1,49										
Nagykónyi	2	1,57		0,91		0,00		0,41	1,34		4,98					0,00				
Paks	7					0,00														
Attala	7														6,04	0,55	0,00	0,00		2,08
	Channel											0,00		0,44						
	Filter pond													0,00		0,00	0,00	0,00	0,46	1,33

Furthermore, a table was created with a monthly average of GSI regardless of sampling year or site (Table 2.).

Table 2. Average of all the GSI of a given month, regardless of sampling year or site

March	April	May	June	July	August	September	October	November	December
1,7	1,66	3,33	0,34	0	0,06	0,65	0,37	1,48	1,49

The data clearly showed that GSI value was the highest in May on population level, then started to decline rapidly in June. In July and August measurable ovaries were rarely to be found therefore GSI resulted to be around 0. Then with the development of oocytes from autumn until spring the GSI started to change in accordance.

A change of GSI in the reproduction period (May and June) was also examined dividing between females non identifiable by the size of the ovary and females with well defined (more than 0.01g) ovary. It was observed that the rate of sexually active individuals with bigger ovary than 0.01g is high: 46% on average, which means that in the case of 50-50% sex ratio all the female individuals should been sexually active. By the time of July, this rate declined to 4%. However, the GSI of females with big ovaries was high in June, meaning that these late spawning individuals were probably in the vitellogenesis phase, being directly prior to spawning.

It was an important goal to prepare histological sections to reveal histological background processes behind quantitative changes. It was observed that in summer, when the weight of the ovaries did not reach 0.01 g, only protoplasmic-stage oocytes could be found in the whole-body intersections, while autumn months (September-October) the number of cells in the ring vacuolization stage increased, moreover, some of the oocytes reach even total vacuolization phase. In spring rate of total vacuolated oocytes raised and the first days of April cells with vitellogenic patterns appeared, still present in the samples until the end of June.

Correlation was also examined between GSI growth and oocyta development stages. The statistical analysis showed that in the samples originated from Babat, GSI values were in strong negative correlation with protoplasmic cell rate (P <0.0001, r = -0.6845), while strong positive correlation was observed with complete vacuolization and vitellogenesis (P <0.0001, r = 0.6422, and r = 0.6806), meanwhile no correlation could be stated with the ring vacuolization (P = 0.6560, r = 0.0293). Samples from Nagykónyi revealed similar results: protoplasmic stage P <0.0001, r = -0.6883; ring vacuolization P = 0.5675, r = 0.1151; complete vacuolization P <0.0001, r = 0.7891; vitellogenesis P = 0.0028, r = 0.5527.

Comparing the population average GSI with the average rate of oocytes of different stages on the sections, a significant correlation was found in the case of protoplasmic (p = 0.034) and total vacuolization stage (p = 0.015), while p value was found to be 0.067 in the vitellogenesis and 0.217 in the case of ring vacuolization.

Furthermore, it can be stated that if ovary did not reach the size of 0.01g, only protoplasmic stage oocytes were found. In the case GSI exceeded that value, 10% of oocytes in the form of vitellogenesis were present. Finally, in the case of spring samples, if ovary weight exceeded 0.01g, ring and total vacuolization phase oocytes were also present, meaning that those individuals were sexually mature. Based on these results in the populations where ovary weight exceeded 0.01g, the lowest standard body weight of sexually mature individuals were measured as follows:

Table 3. The smallest body size of sexually mature fish individuals at the different sampling sites

Sample site		Standard body length (mm)	Body weight (g)
Attala	Pond 7	23	0,20
	Channel	33	0,6
	Filter Pond	22	0,14
Babat	Pond IX	34	0,55
	Pond III	37	0,69
	Pond I	27	0,37
Nagykónyi	Pond II	31	0,44

3.3.3 Analysis of body length-body weight correlation

To calculate the regression coefficient of total body length calculated from TL/SL rate, data of individuals from three populations were considered. One-way ANOVA (significance level 95%) revealed that no difference was detected between the data from the three populations and the mass data of TL/SL rates (p = 0.1740), therefore the coefficient can be used independently from populations. The TL/SL rate for the calculation was 1.208.

Results of the statistical analysis of the samples originated from 8 sample sites are listed in Table 4. In the case of body length-body weight correlation, in the linear regression formula value of the p was proven to be lower than 0.001 in all the cases. It is noticeable that among the samples of ethology experiments a male topmouth gudgeon was caught in Babat III. lake with the following characteristics: standard body length 115 mm, fork length 131 mm, total body length 142 mm and age 4+.

Table 4. Parameters of the formula describing the body length-body weight correlation in the examined 8 populations of topmouth gudgeon

Sampling site		a_{SL}	a_{TL}	b	R^2	n	Number of ouliers	Condition factor
Attala	7	0,0188	0,0321	2,825	0,952	235	4	1
	Channel	0,0176	0,0303	2,888	0,9862	76	3	0,944
	Filter pond	0,0141	0,0252	3,075	0,9214	300	5	0,914
	Total	0,0167	0,029	2,923	0,9505	611	12	0,98
Babat	IX	0,0121	0,0222	3,202	0,9702	100	5	1,32
	III	0,0141	0,0249	3,01	0,9823	248	3	1,161
	I	0,0137	0,0243	3,022	0,9588	468	2	0,836
	Total	0,014	0,0247	3,015	0,9861	816	9	1,039
Nagykónyi	II	0,0171	0,0293	2,865	0,9857	272	9	1,36
Paks	VII	0,0143	0,0257	3,119	0,9466	100	1	0,941
All population total		0,0158	0,0276	2,943	0,9794	1799	28	1,102

3.3.4 Rate of yearlings within the population

The examinations resulted in very different findings considering several aspects. In one case all the population was built up from 0+ age group individuals while in another one this age group was totally absent. Generally 0+ age group resulted the most numerous one with a rate of 70-100% of all the age groups in the population (Table 5.)

Table 5. Rate of 0+ and elder age groups of topmouth gudgeon in the eight sampling sites

Sampling place		**Age group 0+**			**Elder age group**			**Sampling date**	**n**
		pcs (%)	**weight (%)**	**Avg. length (mm)**	**pcs (%)**	**weight (%)**	**Avg. length (mm)**		
Attala	**7**	100	100	26,38	0	0	-	2008.07.24	50
	Channel	14	2,73	18,14	86	97,27	42,55	2007.08.15	50
	Filter pond	98	87,21	24,26	2	12,79	46	2007.08.15	50
	Filter pond	100	100	31,7	0	0	-	2008.07.24	50
Babat	**IX**	70	26,58	24,22	30	73,42	43,4	2008.07.23	50
	III	94,17	47,51	24,03	5,83	52,49	61,16	2006.08.16	103
	I	0	0	-	100	100	51,93	2006.07.31	50
Nagykónyi	**II**	80	23,8	22,63	20	76,2	52,3	2006.08.03	50
Paks	**VII**	98	90,81	36,45	2	9,19	61,5	2006.08.24	100

3.4 Results of habitat use experiments

3.4.1 First set of experiments

To prove the non-selectivity of traps 379 individuals were trapped from the age group 1+ or elder from which 329 were trapped by the trap and 50 individuals caught by a lift net, widely approved to be non-selective. The two-way ANOVA revealed that no significant difference between the two method was detectable (p=0.3088). When examining exposition time, two set of trials were carried out catching 224 and 388 individuals, respectively. 6.6 individuals were caught during 15 minutes, 8.9 individuals during 30 minutes and 10.25 individuals during 60 minutes (15 and 30 minutes intervals were corrected to 60 minutes). Statistical analysis proved the 60 minutes interval to catch the highest amount of fish individuals ($p< 0.001$), meaning to be probably the most efficient to describe fish distribution. Fish presence probability in the trap was also been examined resulting in 91.6% in the case of 60 minute-intervals, 79.17% for 30 minute sampling time and 56.25% for the 15-minute period. Difference in fish quantity caught between consecutive trapping in the same place was also checked revealing that no difference was determined ($p > 0.05$). Thus, it can be stated that topmouth gudgeon population density is high enough to offset the effect of few (< 8) trapping in the same place. Finally considering the results of all the 60-minute trapping, no significant difference was found in topmouth gudgeon population density between the two lakes based on catch per unit effort (p=0.1085).

In the mark-and-recapture trial a total of 995 fish individuals were caught (476, 237 and 291 for the threes sampling sites) from which 18 was earlier marked. 17 marked fish was caught on the same place where marked and 1 individual was caught in the neighbouring sampling site. This result confirms that sampling sites being 50 m far from each other can be considered as independent ones.

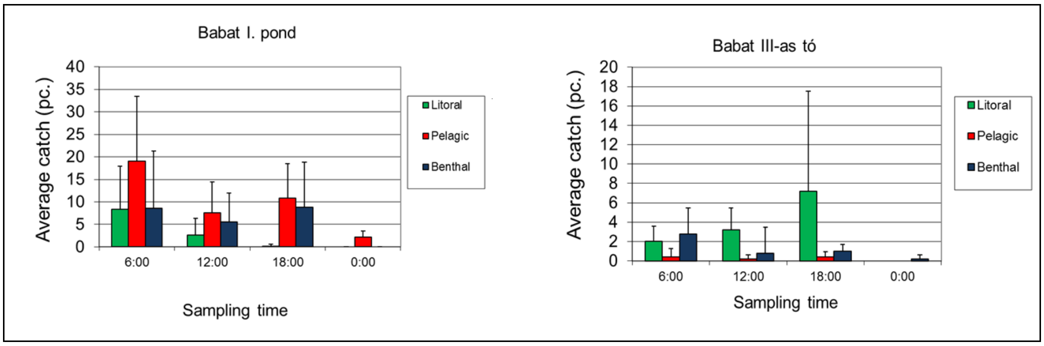

Figure 1. Average trapping results of Babat I. and Babat III. ponds compared to sampling time and site

With the method constructed based on preliminary trials, 461 fish individuals were caught on the two ponds (369 on Babat I. and 91 on Babat III.). Results of the capture is showed on Figure 1. It can be noted that pelagic traps caught the highest number of fish during the whole trial in Babat I. pond while in Babat III. pond capture was divided equally (2-2) between benthal and littoral zone traps. χ^2 showed no significant preference toward benthal zone in Babat I. pond, while littoral zone was preferred and pelagic was avoided by fish individuals ($p < 0.001$). In contrast, littoral zone was preferred and pelagic was avoided in Babat III. with no preference or avoidance toward benthal water zones ($p < 0.001$). In the same figure 24-hours activity of topmouth gudgeon can also be viewed. It is visible that topmouth gudgeon is active during morning and evening time and in fact it is inactive during the night.

Water temperature and oxygen concentration were also measured around traps. Sampling sites differed considerably in temperature and water oxygen level, however, these patterns were not in significant correlation with fish distribution (correlation analysis, Spearman test: Babat I. oxygen concentration $p = 0.91$, $r = 0.0135$; temperature $p = 0.2879$ $r = -0.1407$; Babat III. oxygen concentration $p = 0.7025$, $r = 0.0503$; temperature $p = 0.8437$, $r = -0.0260$).

3.4.2 Second set of experiments

After evaluating 571 photos from the first arrangement (no plant, no carnivore fish), values showed random habitat use of fish individuals. No significant side effect of the aquarium was detected ($p > 0.05$). Water layers was used as follows: lower layer was used in three sets, upper layer was preferred in two cases and no preference toward middle layer was found.

In the second arrangement (plant is present) fish individuals significantly showed preference toward planted side of the aquarium and, except one occasion, bottom layer was also preferred.

Behaviour and habitat use fundamentally changed in the third arrangement, where plant and carnivore fish were also present. In five of six cases individuals chose upper water layer on a statistically verifiable manner and preference toward the dominance of vegetation preference also vanished as follows: plant free area was chosen in one set, vegetation was preferred in two cases and no statistically provable result was found in three cases.

The fourth arrangement (predator presence without plant) also showed a significant difference compared to the first layout: clearly the close-to-surface water layer was preferred by the fish (there was a significant difference in five cases and there was no statistically verifiable difference in one case), and side preference was only proven in one case (table 6).

Table 6. Habitat use of topmouth gudgeon originated from different areas under diverse aquarium arrangements (NS: non significant)

	Arrangement	Without plant	With plant	With plant and predator	Predator only
Hatvan	Upper zone	NS.	NS.	avoidance	preference
	Upper zone with plant (if plant is present)	avoidance	NS.	preference	preference
	Bottom	NS.	NS.	avoidance	avoidance
	Bottom with plant (if plant is present)	preference	preference	avoidance	avoidance
Babat	Upper zone	NS.	avoidance	preference	preference
	Upper zone with plant (if plant is present)	NS.	NS.	preference	preference
	Bottom	NS.	NS.	NS.	avoidance
	Bottom with plant (if plant is present)	NS.	preference	avoidance	avoidance

3.5 New scientific results

1. Distribution analysis of topmouth gudgeon in Hungarian fish farms was carried out and it was found that in 93.67% of the fish farms topmouth gudgeon was present. Furthermore, 17.64% of fish farms reported considerable amount of topmouth gudgeon (>1 kg/ha). This fact can lead to the conclusion that management of topmouth gudgeon population in Hungary can only be carried with the inclusion of fish farms.
2. High genetic variability of Hungarian population of topmouth gudgeon was proven by RAPD method: 120 genotypes were found in 201 individuals.
3. Oocyte development of topmouth gudgeon was described in several Hungarian populations. It was demonstrated that spawning period was shorter and average GSI of topmouth gudgeon individuals was lower than those of Japanese, Slovakian and Polish specimens cited by the literature. Sexual maturity of females was reached early (at a body length of 22mm); this size was only reported from one Polish sample before.
4. Body length and body weight of fish individuals were examined in numerous Hungarian topmouth gudgeon populations. Based on the results of 1799 individuals, regression coefficient for standard body length of topmouth gudgeon was declared (a): 0.0158, while the value of exhibitor was calculated (b): 2.943. Based on the calculated values fitness of Hungarian topmouth gudgeon individuals from different populations was tested and a generally better fitness was stated compared to the average of data from the literature.
5. For the first time, rate of 0+ age group (yearlings) in Hungarian topmouth gudgeon populations was examined with the following results: high rate of young age group was found, indicating that the species is sensitive to the success of each reproduction process in a season.
6. Ethology trials proved variable habitat use of topmouth gudgeon under close-to-nature and artificial environment. Based on the gained data it is probably that the presence of carnivorous fish in an environment can affect the habitat use of topmouth gudgeon. The current experiments proved to be a good basis for further studies in this topic.

4. Discussions and recommendations

4.1 Conclusions of the survey

Answer given to subjective („Which species causes the most serious problem?") and objective questions (quantity of IAS in the fish ponds) verified that invasive fish species meant a serious problem to fish farms. Survey revealed that all of the fish farms was infected by at least two invasive fish species and only 20% of the farms reported less than 1kg/ha quantity of the examined invasive fish species on its premises. It is notable that even the above mentioned quantity was considered to be problematic by almost 10% of respondents based on their subjective answers. Moreover, quantity of at least one of the examined species exceeded 10 kg/ha in 44% of the fish farms.

Questions focused on topmouth gudgeon revealed that 94% of the fish farms were infected by the species and 17% reported considerable quantities. Based on the above data it can be stated that fish farms are salient refugium of topmouth gudgeon therefore management of the species should happen with the inclusion of fish farms.

Recommendations

Based on the results of the study it is evident that attention should be paid to mitigate the extent of damages caused by the presence of topmouth gudgeon. The attention of fish farmers should be drawn to the above fact. Besides, public authorities should propose an aim to reduce the quantity of topmouth gudgeon and to help it with administrative tools (grants and funds). Examinations should be repeated as changing market trends favour the production of carnivorous fish species and this process, together with more strict environmental regulations have an effect on the distribution of topmouth gudgeon.

4.2 Genetic analysis

If the results of the current study is compared to the findings of micro-satellite analysis it can be stated that comparison is difficult but show similar results considering general conclusions. Both set of experiments proved that the European, including Hungarian topmouth gudgeon populations show great variability (120 genotypes were found in 201 individuals in the current study) and no genetically defined populations with segregate genotypes were found.

Recommendations

Based on the current study Hungarian populations of topmouth gudgeon are heterogeneous, no separation among population can be found. Therefore when planning management systems, there is no need to differentiate among population management methods. The above results are likely to lead to the fact that the outcome of further examinations (reproduction biology, growth, ethology) would be highly affected by environmental patterns as genetic variability does not show as great differences as to explain considerable differences. Finally an attention should be paid to the high genetic variability in a context of unintended introductions and that extension and a gene flow among populations is a current process.

4.3 Reproduction biology and morphometric study

4.3.1 Reproduction biology examinations

When comparing the current data with previous findings it can be stated that GSI of the Hungarian topmouth gudgeon populations is considerably lower than those of Japanese, Slovakian of Polish results. Only the data from the most disturbed Slovakian habitat were similar to the current findings. However, low GSI could not be explained by disturbance as results from close-to natural, undisturbed habitats showed similar results. Further examinations should be made to explain differences.

In spite that Japanese topmouth gudgeon population differed considerably from the Hungarian populations, the rate of oocytes in different developmental stages and the results of cytological analysis revealing the different processes in the ovary showed great similarity. As a further result of the current study it can be stated that some cytological processes in the gonads can be detected using simple GSI measurements.

Finally, comparing the standard body length of mature females of our study with the literature it was found that very small (22 and 23 mm), but sexually mature individuals occurred in two sampling sites. These small size was only reported from Poland (22.96), from a cooling pond of a heat plant where significantly different temperature circumstances were present.

4.3.2 Examination of body length- body weight correlation

The aim of the study was basically not only to obtain data from Hungarian populations but also to reveal connections on body length and body weight and to valorise the condition of Hungarian topmouth gudgeon populations.

Results of the current study showed that both “a” and “b” values fell within the limits of earlier studies in all the examined populations. “a” values showed higher than literature while value “b” fell close to the average. This fact could be explained by early sexual maturation, showing that shorter body linked with higher body weight, but this connection was not proven by the statistical analysis (Spearman-test, $r = -0.23$, $p = 0.6615$).

Four populations from the examined eight one showed average fitness while more than 10% difference was found in four cases. Correlation between fitness and different parameters (average body weight, reproduction processes) could not been proved statistically on a significance level of 95%. An idea for the explanation of differences in the fitness was the effect of weather. Although statistical analysis was not carried out due to the lack of sufficient data, sampling year of the two most extreme values (Babat I and Nagykónyi) was the same.

An extra information should be mentioned that a 142 mm long (total body length) topmouth gudgeon was caught during ethological studies proving that in contrary to literature data, individuals of the species can reach bigger size.

4.3.3 Examination of the rate of yearlings in the population

Less attention was paid to the rate of different topmouth gudgeon age groups within a population so far. Japanese researchers examined the rate of yearlings in an irrigation channel declaring that the lifetime of topmouth gudgeon could be one or two years. Although the publication did not share detailed information on the age groups, figures of the article revealed over 50% share of yearlings in the population during the months of June and July. When tracking different cohorts it became evident that presence of 1+ age group was detectable but elder age groups were only present sporadically.

The current study showed that in seven populations of nine, rate of yearlings was more than 70% and only two showed different values. In the ponds included in the study presence of the species was guaranteed by yearlings which means that one unsuccessful spawning can lead to the collapse of population if no external input (e.g. immigration, introduction) occurs.

Recommendations

Based on the dataset of the current study, a few key data, like the GSI in April, May and June and the rate of yearlings in the population would be sufficient from a habitat to create management techniques in the future. The later dataset was proven to be very important as our research showed that the age pyramid of Hungarian topmouth gudgeon populations was flat and wide, meaning that the damage caused by the species is highly dependent on the rate of yearlings. This fact means that the key element of the prevention against the species is the elimination of spawns.

4.4 Habitat use studies

Habitat use of topmouth gudgeon was examined by several research groups. Polish researchers found that topmouth gudgeon definitely preferred water vegetation areas during summer period, while Chinese and English researchers found that topmouth gudgeon did not preferred special areas in a lake and a river habitat, respectively. Similar results were also published by Japanese scientist examining irrigation channels. The above results are interesting in the light of the fact that topmouth gudgeon showed definite habitat preference under a laboratory experiment imitating a river habitat, which was fundamentally changed when a predator species arrived and individuals avoided the previously preferred zones.

In the current experiment a non-selective sampling method was developed for the trapping of age 1+ or elder generation. Results obtained with the catch-per-unit effort method showed considerable difference between two lakes of very similar environmental conditions (abiotic patterns, vegetation and food availability). The only significant difference between the two lake was the presence of perch in Babat I. pond while fish predators were totally absent from Babat III. pond. Of course other predators like birds were presumably present equally in both lakes due to the small distance between the ponds. While in the first habitat topmouth gudgeon avoided littoral zones filled with vegetation, in the second pond definite preference was observed. A statistically justifiable observation was also made that in the presence of predatory fish topmouth gudgeon was active all day long, while in the pond without the perch topmouth gudgeon preferred morning and sunset periods, being inactive during the night as only one fish was caught from ten traps.

Based on own results gained from close-to nature environment and literature cited data originated from an artificial river environment a null hypothesis was created that presence of predators could have an effect on topmouth gudgeon habitat use. The hypothesis was definitely confirmed under artificial conditions imitating lake environment. Independently of the sampling place and of having previous experience with predators, topmouth gudgeon individuals reacted alike: they changed the zone of living. In contrary to previous findings the hypothesis could be proved both under artificial conditions and close-to nature environment.

Recommendations

The above mentioned statement is very interesting. On one hand, a predator using only one habitat type is not sufficient to control topmouth gudgeon populations, as the topmouth gudgeon is able to avoid the predator because of its wide food spectrum and rapid adaptation ability. On the other hand, predators introduced to control the species can force topmouth gudgeon to an environment where it was not occurred before or only was present in low density. But appearing in huge populations in a new habitat natural fish communities of a given habitat can be threated and forced to competition.

Finally the results of the current experiments can provide a reliable basis for a comprehensive study, examining the above described reaction of topmouth gudgeon in different habitats. If topmouth gudgeon can show very high adaptive capacity, new predator species or a combination of predators of different feeding strategy should be applied to control topmouth gudgeon populations.

Publication connected to the topic of the thesis:

First author impact factor articles:

Csorbai, B., , Pereszlényi, Á., Kovács, R., Urbányi, B. & Horváth L. (2014) The habitat use and selectivity by topmouth gudgeon (*Pseudorasbora parva*) Acta Zoologica Academiae Scientiarum Hungaricae 60 (4), pp. 389–400

Book on Hungarian language:

Horváth, L**., Csorbai, B.** & Urbányi B. (2007) A tájidegen gyomhalak visszaszorítása őshonos ragadozó halfajokkal Gödöllő, Szent István Egyetem, ISBN 9789639483989 pp. 1-128

Part of book on Hungarian language:

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