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Wetenschappelijke instelling van de Vlaamse overheid

Microsatellite genotyping of Pike (*Esox lucius*) samples to supplement the hatchery stock of the Research Institute for Nature and Forest (INBO)

K. De Gelas, D. De Charleroy, G. Maes, I. Vught, J. Auwerx and F. Volckaert

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KU LEUVEN

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Inhoud

Objective

The objective of this project is to genotype DNA samples of pike (Esox lucius) provided by the customer. In 2 multiplex reactions, 12 microsatellite markers will be analysed.

Introduction

Two batches of samples were taken from adult pike (Esox lucius) individuals. The first batch consisted of 28 samples of pike captured in "De Maten" nature reserve in Genk, Belgium. The second batch consisted of 26 samples taken from pike captured in "Het Wik" and "Munsterbos", Belgium. The adult individuals will be used to supplement the existing hatchery broodstock. The samples are provided as DNA extracts by the customer (Research Institute for Nature and Forest, INBO).

The objective of this study was to genotype the samples and compare them to available data of European pike populations (Maes et al. 2004) in order to detect non-indigenous genotypes, besides assessing the relatedness among potential breeders. Samples were genotyped using a standard multiplex method with 12 microsatellite loci (De Gelas et al. 2008). For comparison with available populations from Maes et al. (2004), not all 12 loci could be used.

Material and Methods

The details of the primers used can be found in Table 1. DNA extracts were used as template for PCR reaction following the protocol described in the tables 2 to 4. PCR was conducted in 10 µl reactions using a Quiagen multiplex reaction kit. Forward primers were fluorescently labelled.

After PCR, fragments were separated on a ABI3130 capillary analyser and scored relative to a LIZ Genescan 500 length standard using GENEMAPPER (Applied Biosystems). Fragments are scored automatically and checked manually

Locus	F-primer	R-primer	Range	Dye
Multiplex 1				
Elu02	5*-GCAGACTTGTTTACTTTCCTT-3*	5*-GTGTGCTTTCCATTTCCAT-3*	147-215	PET
Elu25	5*-CATGGTGGAGTCTTTGGTGAG-3*	5*-CACCAGCCAGACTGAACCAG-3*	146-160	VIC
Elu51	5*-GTGGGCATTCAGCCGATATAGC-3*	5*-CTGTCTCATTACTGCCTGGCTC-3*	111-143	PET
Elu78	5*-CTAGAGGGGGAAAACAAACC-3*	5*-CACTGTCCATCATCACCCCTCTC-3*	129-141	VIC
Elu276	5*-CTGTCACAGTTCAAAGATGGC-3*	5*-TCTTTAAACTGGGGGGGAGGAAAG-3*	119-201	NED
Elu108	5*-TCATCAGAAACATGACTGCTTG-3*	5*-GCACACGGCGATATTATCC-3*	135-143	FAM
Multiplex 2				
Elu19	5*-CATCATGAACATTCAGACGC-3*	5*-GAGATGCTAATTCATCCACTG-3*	116-230	PET
Elu38	5*-TGTCAGTGTGACTTGCTCC-3*	5*-GTATTGTCAGCATTTCAGCTC-3*	170–188	NED
Elu64	5*-GGTAAGACCAGTATCTGGAG-3*	5*-CAAAGGGAGTGATGATTGGCTTC-3*	113-133	NED
Elu76	5’-ACCACATTCCACATCTGATGG-3*	5*-AATCCCTTATTCTGACCCTGC-3*	135-203	FAM
Elu87	5*-AGCACTGCCACACATGACGTG-3*	5*-CCAGCTGCCTCAGATTGCTCCCC-3*	133-159	VIC
Elu252	5*-CACCGACAATATGCGCACACC-3*	5*-GGATTTACCCTGTCATACAG-3*	107-129	FAM

Table 1: Primers used in the microsatellite genotyping of pike samples sorted by multiplex. Locus: Name of primer; F-primer: sequence of the forward primer; R-primer: sequence of the reverse primer; range: expected size range of fragments; dye: color dye for the labelled primer.

Product	1 reaction	End concentration
Quiagen MM (2x)	5 µl	1x
Elu02	0,1	1 µM
Elu25*	0,1	0,5 µM
Elu51	0,1	1 µM
Elu78	0,1	1 µM
Elu108	0,1	1 µM
Elu276	0,1	1 µM
DNA	1 µl	
mQ water	3,4	

Table 2 Reaction mix for multiplex 1 (10µl reactions)

Note: Working solution primers 10µM, except * 5µM

Product	1 reaction	End concentration
Quiagen MM (2x)	5 µl	1x
Elu19	0,1	1 µM
Elu38	0,1	1 µM
Elu64	0,1	1 µM
Elu76	0,1	1 µM
Elu87	0,05	0,5 µM
Elu252	0,4	4 µM
DNA	1 µl	
mQ water	3,15	

Table 3 Reaction mix for multiplex 2 (10µl reactions)

Note: Working solution primers 10µM

Step	Temperature	Time
Activation	95°C	15 min
Denaturation	95°C	30 sec
Annealing	59 (multi1) °C 58 (multi2) °C	1 min 30 sec
Elongation	72°C	1 min
Polymerisation	60°C	30 min
Preservation	4°C	

Table 4 PCR reaction steps and temperature

Note: Step 2 to 4 are repeated 23 times

Comparison with data from Maes et al. (2004)

Maes et al. (2004) studied a wide range of populations in Europe using a LICOR electrophoresis system. To be able to compare with the results obtained with the LICOR system by Maes et al. (2004), we have to correct the fragment lengths obtained on the ABI. The correction is based on the analysis of reference samples on both systems. The correction method used is summarized in Table 5. Not all loci used on the ABI system were used by

Maes et al (2004), therefore all analysis comparing our data to the data of Maes et al. (2004) is based on only 9 loci instead of 12.

	ABI	LICOR	Correction		ABI	LICOR	Correction
ELU2	191	187	-4	**ELU76**	141	141	0
	195	191	-4		159	157	-2
	199	No data	-4		163	161	-2
	215	211	-4		178	No data	-1
	219	215	-4		182	No data	-1
ELU19	117	116	-1				
	125	124	-1	**Elu78**	135	135	0
	127	126	-1		137	No data	0
	129	128	-1				
Elu25	148	146	-2	**ELU252**	109	111	+2
	151	148	-3		111	113	+2
	153	150	-3		113	115	+2
	155	152	-3		115	117	+2
	157	154	-3				
ELU51	112	111	-1	**ELU276**	119	119	0
	114	113	-1		124	No data	0
					158	155	-3
ELU64	116	117	+1		171	167	-4
	118	119	+1		187	183	-4
	122	123	+1		191	No data	-4

Table 5 Correction table for fragment lengths resulting from ABI analysis. The correction is needed to be able to compare with the data from the LICOR system from Maes et al. (2004). In case of 'no data' we had to extrapolate the correction

Results

Exploratory data analysis

All 54 samples could be genotyped for all 12 loci. In order to explore the data before analysing diversity parameters, a Factorial Correspondence Analysis (FCA) was conducted using GENETIX. Reference populations from Ireland, the Netherlands, France, Norway and Poland are included in the analysis together with the newly analysed samples in this study. For reasons of clarity of the figure, the reference populations of Romania and Hungary are omitted from the analysis. However, the new samples analysed in this study do not show genetic affinities with these two reference populations (see further, assignment test). The result of the FCA analysis is depicted in Figure 1. Samples clustering together show similar allele composition, samples separated in space show a more differentiated allele composition.

Several distinct groups are visible in Figure 1. The samples from Munsterbos, Poland and Norway form three separate clusters and clearly deviate from the rest of the samples from France, Ireland, The Netherlands and the rest of the newly analysed samples.

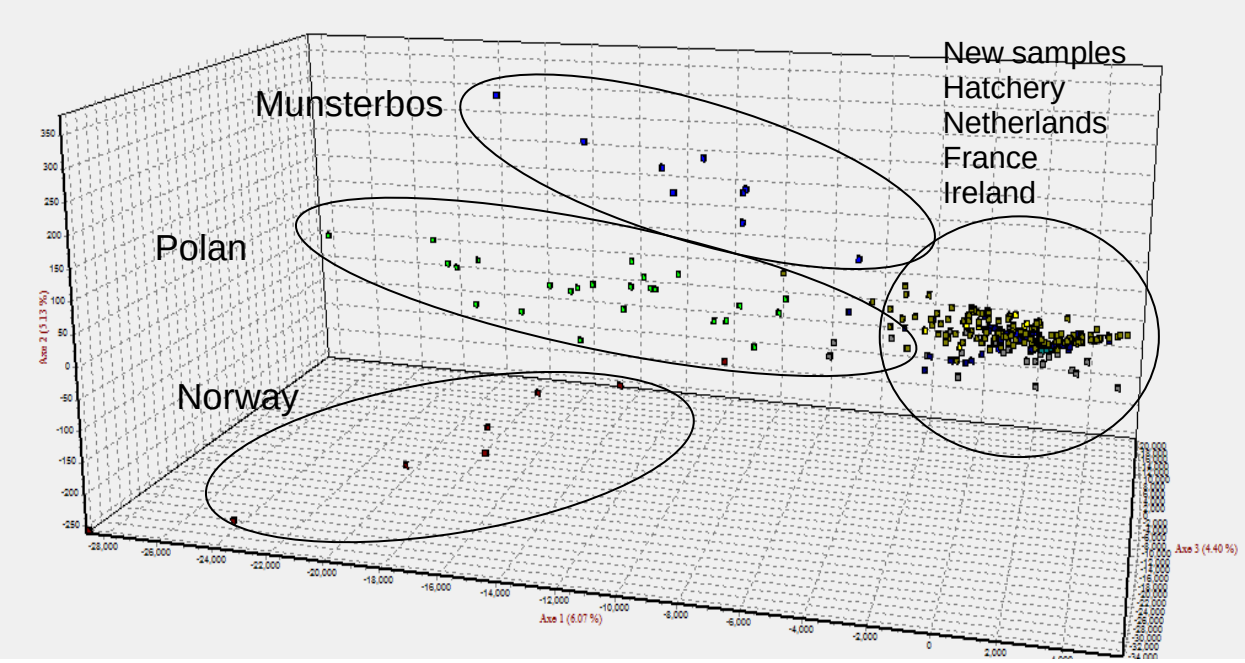

Figure 1: FCA plot based on the microsatellite genotypes.

Diversity parameters

The summary of genetic diversity parameters for all individuals and all loci is given in Table 6. We also made a comparison with the hatchery population of 2008. Because of the high genetic differentiation of the samples originating from Munsterbos compared to the rest of the (western European) samples, the samples from Munsterbos will be treated as a separate group in the calculations of the diversity parameters.

Sample	N	Hexp.	Hobs.	P(0.95)	P(0.99)	MNA
Maten	28	0.2873	0.2708	0.6667	0.7500	3.25
Wik	17	0.2176	0.2500	0.4167	0.5000	1.83
Maten + Wik	45	0.2826	0.2630	0.6667	0.7500	3.33
Munsterbos	9	0.3663	0.3519	0.8333	0.8333	2.50
Hatchery 2008	134	0.3685	0.3346	0.6667	0.9167	3.91

Table 6 Diversity parameters (12 loci) for the analysed samples and for the hatchery population sampled in 2008 (De Gelas et al. 2009). N: number of analysed individuals; Hexp: expected heterozygosity; Hobs.: observed heterozygosity; P(0.95): proportion of polymorphic loci for which the most abundant allele has a frequency lower than 95%; P(0.99): proportion of polymorphic loci for which the most abundant allele has a frequency lower than 99%; MNA: mean number of alleles per locus.

Geneclass assignment test

The analysed samples were conducted to an assignment test in GENECLASS2. We used reference populations from Denmark, France, Hungary, Ireland, the Netherlands, Norway, Poland and Romania from Maes et al. (2004). The results of the assignment test are provided in table 7

	EL-DEN	EL-FRA	EL-HUN	EL-HUN2	EL-IR01	EL-IR02	EL-NET	EL-NOR	EL-POL	EL-ROM	hatchery
KW2010-01	0.250	0.335	0.004	0.000	0.000	0.006	0.294	0.000	0.017	0.000	**0.631**
KW2010-02	0.002	0.298	0.089	0.006	0.000	0.118	0.463	0.003	0.069	0.000	**0.871**
KW2010-03	0.260	**1**	0.141	0.012	1	1	0.991	0.119	0.622	0.000	0.966
KW2010-04	0.006	**0.909**	0.038	0.004	0.000	0.300	0.297	0.020	0.529	0.000	0.043
KW2010-05	0.004	**0.715**	0.013	0.001	0.000	0.265	0.261	0.058	0.516	0.000	0.094
KW2010-06	0.003	0.357	0.087	0.008	0.000	0.118	0.168	0.004	0.061	0.000	**0.556**
KW2010-07	0.260	**1**	0.141	0.012	1	1	0.991	0.119	0.622	0.000	0.966
KW2010-08	0.004	**0.715**	0.013	0.001	0.000	0.265	0.261	0.058	0.516	0.000	0.094
KW2010-09	0.052	**0.996**	0.052	0.004	0.043	0.688	0.772	0.192	0.850	0.000	0.966
KW2010-10	0.000	**0.123**	0.043	0.001	0.000	0.023	0.084	0.003	0.003	0.000	0.006
KW2010-11	0.000	0.034	0.003	0.000	0.000	0.000	0.175	0.000	0.066	0.000	**0.704**
KW2010-12	0.000	**0.183**	0.005	0.001	0.000	0.023	0.123	0.046	0.474	0.005	0.056
KW2010-13	0.000	**0.229**	0.004	0.000	0.000	0.023	0.037	0.000	0.077	0.001	0.031
KW2010-14	0.000	0.105	0.003	0.000	0.000	0.000	0.230	0.000	0.113	0.002	**0.827**
KW2010-15	0.000	0.048	0.003	0.000	0.000	0.000	0.230	0.000	0.090	0.000	**0.775**
KW2010-16	0.000	0.269	0.003	0.000	0.000	0.046	0.104	0.068	**0.641**	0.001	0.003
KW2010-17	0.004	**0.715**	0.013	0.001	0.000	0.265	0.261	0.058	0.516	0.000	0.094
KW2010-18	0.004	**0.715**	0.013	0.001	0.000	0.265	0.261	0.058	0.516	0.000	0.094
KW2010-19	0.000	**0.313**	0.013	0.001	0.000	0.065	0.185	0.007	0.172	0.001	0.098
KW2010-20	0.202	**0.642**	0.081	0.003	0.000	0.023	0.070	0.000	0.175	0.000	0.043
KW2010-21	0.000	0.064	0.003	0.000	0.000	0.006	0.033	0.014	**0.260**	0.003	0.004
KW2010-22	0.000	0.183	0.005	0.001	0.000	0.023	0.123	0.046	**0.474**	0.005	0.056
KW2010-23	0.000	0.269	0.003	0.000	0.000	0.046	0.104	0.068	**0.641**	0.001	0.003
KW2010-24	0.061	**0.978**	0.120	0.012	0.043	0.761	0.663	0.246	0.665	0.000	0.644
KW2010-25	0.000	**0.073**	0.004	0.000	0.000	0.000	0.029	0.000	0.014	0.000	0.001
KW2010-26	0.075	**0.661**	0.013	0.001	0.000	0.118	0.929	0.001	0.058	0.000	0.204
KW2010-27	0.000	0.155	0.004	0.001	0.000	0.023	0.045	0.039	**0.683**	0.000	0.006
KW2010-28	0.000	**0.313**	0.013	0.001	0.000	0.065	0.185	0.007	0.172	0.001	0.098
feb11-01	0.075	**0.894**	0.123	0.004	0.043	0.863	0.703	0.046	0.314	0.000	0.769
feb11-02	0.004	**0.715**	0.052	0.007	0.000	0.265	0.261	0.058	0.716	0.000	0.390

feb11-03	0.033	0.661	0.009	0.000	0.000	0.118	0.772	0.001	0.058	0.000	**0.858**
feb11-04	0.000	**0.177**	0.036	0.001	0.000	0.026	0.123	0.004	**0.178**	0.000	0.061
feb11-05	0.004	0.421	0.009	0.000	0.000	0.023	0.701	0.000	0.032	0.000	**0.710**
feb11-06	0.033	0.661	0.009	0.000	0.000	0.118	0.772	0.001	0.058	0.000	**0.858**
feb11-07	0.000	0.145	0.003	0.000	0.000	0.000	0.184	0.000	0.115	0.001	**0.165**
feb11-08	0.000	**0.366**	0.003	0.000	0.000	0.006	0.243	0.005	0.346	0.001	0.307
feb11-09	0.000	**0.177**	0.036	0.001	0.000	0.026	0.123	0.004	**0.178**	0.000	0.061
feb11-10	0.121	0.952	0.013	0.001	0.000	0.265	1	0.011	0.174	0.000	**0.997**
feb11-11	0.052	0.541	0.072	0.001	0.000	0.437	0.255	0.083	0.337	0.000	**0.569**
feb11-12	0.121	0.952	0.013	0.001	0.000	0.265	1	0.011	0.174	0.000	**0.997**
feb11-13	0.004	0.402	0.003	0.000	0.000	0.006	**0.908**	0.000	0.100	0.000	0.720
feb11-14	0.033	0.799	0.013	0.001	0.000	0.118	1	0.001	0.126	0.000	**0.984**
feb11-15	0.000	0.376	0.044	0.003	0.000	0.118	0.190	0.013	0.414	0.000	0.223
feb11-16	0.260	**1**	0.141	0.012	**1**	**1**	0.991	0.119	0.622	0.000	0.966
feb11-17	0.004	**0.715**	0.052	0.007	0.000	0.265	0.261	0.058	0.716	0.000	0.390
feb11-18	0.000	0.003	0.008	0.001	0.000	0.000	0.014	0.000	**0.076**	0.000	0.000
feb11-19	0.000	0.008	0.016	0.003	0.000	0.000	0.004	0.000	**0.017**	0.000	0.000
feb11-20	0.000	0.015	0.008	0.001	0.000	0.000	0.016	0.007	**0.119**	0.000	0.000
feb11-21	0.000	0.045	0.016	0.001	0.000	0.000	0.020	0.000	**0.070**	0.000	0.000
feb11-22	0.000	0.000	**0.008**	0.001	0.000	0.000	0.006	0.000	0.004	0.000	0.000
feb11-23	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.001**	0.000	0.000
feb11-24	0.000	0.000	0.003	0.001	0.000	0.000	0.000	0.000	**0.009**	0.000	0.000
feb11-25	0.000	0.008	0.013	0.001	0.000	0.000	0.002	0.000	**0.017**	0.000	0.000
feb11-26	0.000	0.001	**0.004**	0.000	0.000	0.000	0.001	0.000	0.003	0.000	0.000

Table 7 Probability results of assignment test of the analysed individuals to reference populations from Denmark, France, Hungary, Ireland, the Netherlands, Norway, Poland and Romania from Maes et al. (2004). Highest probabilities are highlighted.

Diversity parameters of the samples compared to other European populations

In order to be able to situate the diversity relative to other populations, we list the main diversity parameters of the newly analysed samples together with the data of the reference populations (Table 8).

Population	N	Hexp.	Hn.b.	Hobs.	P(0.95)	P(0.99)	MNA	MNA N=9
Maten	28	0.2550	0.2597	0.2341	0.6667	0.7778	3.22	2.34
Wik	17	0.2045	0.2107	0.2353	0.3333	0.4444	1.77	1.71
Maten + Wik	45	0.2520	0.2548	0.2346	0.6667	0.7778	3.33	2.30
Munsterbos	9	0.3532	0.3740	0.3210	0.8889	0.8889	2.44	2.44
EL-DEN	3	0.1790	0.2148	0.2593	0.5556	0.5556	1.56	
EL-FRA	32	0.3209	0.3266	0.2974	1	1	4.44	2.98
EL-HUN	36	0.6047	0.6144	0.5007	1	1	8.89	5.45
EL-HUN2	41	0.6679	0.6762	0.5911	1	1	9.67	5.96
EL-IR01	44	0.0000	0.0000	0.0000	0.0000	0.0000	1.00	1
EL-NET2	59	0.2606	0.2629	0.2645	0.6667	1	3.67	2.26
EL-NOR	9	0.2614	0.2785	0.2108	0.5556	0.5556	1.67	1.67
EL-POL	25	0.4576	0.4684	0.4329	0.8889	1	4.00	3.31
EL-ROM	54	0.6256	0.6315	0.5908	1	1	7.22	4.77
Hatchery 2008	134	0.3641	0.3655	0.3284	0.6667	0.8889	3.67	2.46

Table 8 Diversity parameters for the analysed population and 11 European populations for 9 loci. N: number of analysed individuals; Hexp.: expected heterozygosity; Hn.b.: non-biased heterozygosity; Hobs.: observed heterozygosity; P(0.95): proportion of polymorphic loci for which the most abundant allele has a frequency lower than 95%; P(0.99): proportion of polymorphic loci for which the most abundant allele has a frequency lower than 99%. MNA: mean number of alleles; MNA N=9: simulated number of alleles when N=9 individuals

Conclusions

Genotyping

All samples were successfully genotyped at 12 loci and data are available in appendix 1. The major weak point in this study is the complete absence of suitable genetic reference data on natural populations of pike in Belgium. All current populations have been heavily stocked with hatchery brood or brood from unknown or foreign origin. Therefore a clear assessment of hatchery genetic diversity is hard to make, until such data is available. Nevertheless, we made a comparison relative to neighbouring populations to detect possible foreign origin in hatchery broodstock and to assess genetic diversity.

Munsterbos samples

Nine samples originating from Munsterbos showed a relatively high genetic differentiation compared to the other analysed samples and compared to the available reference populations. These samples could not be assigned to any of the reference populations with a high probability, suggesting that they originate from a population not included in our dataset. Some of the samples showed the highest genetic affinity with the reference population from Poland but even here, probabilities of assignment were rather low. The relatively high genetic diversity contained in these nine samples suggests a central-European, rather than a west- or north-European origin. However, this is speculative and further research is needed to confirm or reject this hypothesis. As a matter of precaution it is advisable not to use the individuals from Munsterbos as hatchery broodstock to supplement or stock populations in Belgium.

Assignment to reference populations

Most samples are either assigned to the hatchery population analysed in 2008 or to the reference population of France. Some samples however show the highest probability of assignment to the Polish reference population. This result is not uncommon and has been observed earlier (De Gelas et al, 2008). It is probably the result of introgression of "Polish alleles" due to hybridization of stocked pike of Polish origin with indigenous pike and subsequent backcrossing in the hatchery population and in natural populations. Genetic screening of putative hatchery broodstock can prevent this phenomenon to happen again, as the use of broodstock with indigenous genotypes will further dilute foreign alleles in stocked populations.

Although the assignment test is informative, the results still have to be interpreted with caution because of the limited number of reference populations. Chances are high that the true reference populations or regions where the individuals come from are not included in our test. Nevertheless, the results can point out "genetic affinities" among the samples and the reference populations.

Diversity parameters compared to other European populations

The diversity parameters are in general low in northern pike populations and higher in central-European pike populations, see also Maes et al. (2004). Unfortunately no reference data are available for truly indigenous Belgian pike populations. Contemporary populations have historically been stocked with hatchery brood or brood from an unknown or foreign origin. It is only in the most recent years that an interest was taken not to stock with non-indigenous brood.

In our dataset, we are able to compare the newly analysed samples with two neighbouring populations: one from The Netherlands and one from France. The diversity parameters such as heterozygosity and mean number of alleles of the hatchery population (2008) and for the combined samples of De Maten and Het Wik are comparable to the population sampled in The Netherlands and slightly lower than the population sampled in France. The values P(0.95) and P(0.99) are however lower for the combined samples of De Maten and Het Wik compared to the neighbouring populations. This means that some alleles are present in very low frequencies. The risk of losing these alleles by random drift in future generations or by adverse events (dying of individual fish) is high and will lower the genetic diversity of the (future) hatchery population.

De Maten samples

For the samples from "De Maten" it is known that they were stocked in the ponds in 1996 and possibly in 1999 and 2000 and that the brood was supplied by the hatchery of INBO. Therefore, using these individuals to supplement the current broodstock at the hatchery can not be viewed as a genetic enrichment with unrelated individuals and chances are high that the samples from "De Maten" and the current broodstock at the hatchery share a common ancestor in the recent past. This could increase the relatedness among the broodstock fish and hence the inbreeding in the hatchery population.

Advice for further research

Reference material

Two major lacks in reference data are compromising the assignment of individuals intended as hatchery broodstock: No reference data of truly indigenous populations are available making it impossible to estimate diversity parameters of natural populations in Belgium. Second, only a limited set of European reference populations is available making it difficult to assign non-indigenous individuals to a reference population.

An effort could be made to try to sample indigenous museum material (if available) or collections of pike predating the intensive stocking of populations. If these samples can be genetically analysed they would provide a baseline for the diversity parameters of indigenous populations.

If needed more European reference populations can be studied to complement the dataset and further refine assignment analysis.

Marker assisted breeding programs

Apart from the assignment of hatchery broodstock as indigenous or non-indigenous, other problems arise. The breeding population of the INBO hatchery in Linkebeek is rather small and the risk of losing genetic diversity trough genetic drift is significant (see also De Gelas et al 2008). Moreover, the small population size increases the risk of a high relatedness and inbreeding among individuals especially when consecutive generations are used in the breeding program. Molecular markers are a solution to estimate relatedness and to build pedigrees and hence provide a tool to assist the design of breeding plans and to minimize inbreeding and maximize genetic diversity.

However, the current set of markers is not powerful enough to be used in parentage analysis (see also De Gelas et al. 2008). The main cause of this lack of power is the low variability of the markers. More, and more variable markers should be tested in order to increase the

power of genetic tests. These can be developed *de novo*, for example by using next-generation-sequencing technologies to detect new microsatellite markers. Alternatively, published markers for pike or related species could be optimized for further use.

Appendix:Genotypes for the analysed pike samples for 12 microsatellite loci

Sample Name	code	Elu002	Elu019	Elu025	Elu038	Elu051	Elu064	Elu076	Elu078	Elu087	Elu108	Elu252	Elu276
MATEN-01	0671 3127	215219	125125	153153	179179	112112	116116	141159	135135	142154	131135	115115	119187
MATEN-02	066D CC6C	215219	125127	153155	179179	112112	116116	159159	135135	142142	131135	113113	187187
MATEN-03	066D ECD0	219219	125125	153153	179179	112112	116116	159159	135135	142142	133135	113113	187187
MATEN-04	066E 1F60	219219	125125	153153	179179	112114	116116	159159	135135	142154	131135	113113	187203
MATEN-05	066D DFB3	219219	125125	153153	179179	112112	116116	159188	135135	154154	135135	113113	158187
MATEN-06	066E 17A6	219219	127127	153153	179179	112112	116116	159159	135135	154154	135137	113113	187191
MATEN-07	066E 1954	219219	125125	153153	179179	112112	116116	159159	135135	142154	131131	113113	187187
MATEN-08	066E 0867	219219	125125	153153	179179	112112	116116	159188	135135	142154	135137	113113	158187
MATEN-09	0671 269C	219219	125125	153153	179179	112112	116116	159159	135135	154154	135135	113113	158187
MATEN-10	0671 3D3B	191215	125125	151153	179179	112112	116116	159161	135135	142142	131139	113113	187187
MATEN-11	0671 442A	219219	125127	153155	179179	112112	116116	141182	135135	142154	131135	113115	119158
MATEN-12	066D FEBA	219219	125127	153153	179179	112112	116116	159188	135135	154154	131135	113113	158158
MATEN-13	066E 1F59	219219	127129	153153	179179	112112	116116	159188	135135	142154	133135	113113	158187
MATEN-14	066E 0F46	219219	125127	153153	179179	112112	116116	141182	135135	142160	131135	113113	119158

MATEN-15	066DD945	219219	125127	153155	179179	112112	116116	141182	135135	142154	131131	113113	119158
MATEN-16	066E2047	219219	125125	153153	179179	112112	116116	188188	135135	154154	135135	113113	158158
MATEN-17	06713D7C	219219	125125	153153	179179	112112	116116	159188	135135	154154	135135	113113	158187
MATEN-18	066E2DC5	219219	125125	153153	179179	112112	116116	159188	135135	142142	135137	113113	158187
MATEN-19	06711ECC	219219	125127	153153	179179	112112	116116	159188	135135	142154	133133	113113	158187
MATEN-20	066DF865	195219	125129	153157	179179	112112	116116	159159	135135	154154	131135	115115	187187
MATEN-21	066E01A9	219219	125127	153153	179179	112112	116116	188188	135135	154154	133135	113113	158158
MATEN-22	066DCBA6	219219	125127	153153	179179	112112	116116	159188	135135	154154	133135	113113	158158
MATEN-23	006DC3FB	219219	125125	153153	179179	112112	116116	188188	135135	142154	135137	113113	158158
MATEN-24	06713157	195219	125125	153153	179179	112112	116116	159159	135135	142154	131131	113113	187187
MATEN-25	066E05BF	219219	127129	148153	179179	112112	116116	159188	135135	142154	135135	113113	158187
MATEN-26	06700E45	219219	125125	148153	179179	112112	116116	141159	135135	154158	131135	113113	119187
MATEN-27	066DD5E0	219219	125125	153153	179179	112112	116116	178188	135135	142154	135137	113113	158158
MATEN-28	066DE22B	219219	125127	153153	179179	112112	116116	159188	135135	154154	135135	113113	158187

Appendix (continued)

Sample Name	code	Elu002	Elu019	Elu025	Elu038	Elu051	Elu064	Elu076	Elu078	Elu087	Elu108	Elu252	Elu276
Wik-01	066D FA3A	219219	125125	151153	179179	112112	116116	159159	135135	154154	131137	113113	187187
Wik-02	0671 28AA	219219	125125	153153	179179	112112	116116	159178	135135	154154	137137	113113	158187
Wik-03	066E 3FC6	219219	125125	151153	179179	112112	116116	141159	135135	142154	137137	113113	119187
Wik-04	0671 157A	219219	125125	151151	179179	112112	116116	159178	135135	154154	135137	113113	158187
Wik-05	0670 07DF	219219	125125	151155	179179	112112	116116	141159	135135	154154	135135	113113	119187
Wik-06	066D F9F0	219219	125125	151153	179179	112112	116116	141159	135135	142154	135137	113113	119187
Wik-07	066D D89E	219219	125125	151153	179179	112112	116116	141178	135135	142154	135137	113113	119158
Wik-08	066F FF83	219219	125125	153153	179179	112112	116116	141178	135135	154154	137137	113113	119158
Wik-09	066E 04CE	219219	125125	151151	179179	112112	116116	159178	135135	154154	131135	113113	158187
Wik-10	0671 2869	219219	125125	153153	179179	112112	116116	141159	135135	142154	131137	113113	119187
Wik-11	066E 1B96	199219	125125	151153	179179	112112	116116	159159	135135	154154	135137	113113	187187
Wik-12	066D CDEB	219219	125125	153153	179179	112112	116116	141159	135135	154154	137137	113113	119187
Wik-13	066E 2F85	219219	125125	153155	179179	112112	116116	141141	135135	154154	135137	113113	119119
Wik-14	066E 2507	219219	125125	153155	179179	112112	116116	141159	135135	154154	135137	113113	119187
Wik-15	066D FC77	219219	125125	151153	179179	112112	116116	159178	135135	154154	131137	113113	158187

Wik-16	066E0806	219219	125125	153153	179179	112112	116116	159159	135135	154154	131137	113113	187187
Wik-17	066DDCB2	219219	125125	153153	179179	112112	116116	159178	135135	154154	137137	113113	158187
Munster bos-18	066DED19	191219	125125	155159	179179	112112	116116	184184	135135	138142	131139	113113	158158
Munster bos-19	066DC930	191191	125125	153159	179179	112112	116116	159172	135135	142154	127139	115115	145187
Munster bos-20	066DCDF7	191219	125125	153159	179179	112112	116116	184184	135135	142142	127127	113113	158158
Munster bos-21	066E4175	191219	125125	155155	179179	112112	116116	163184	135135	142142	131137	113113	158187
Munster bos-22	066E186D	191219	125127	159159	179179	112112	116116	184184	135135	138142	127137	113113	158158
Munster bos-23	06713240	191219	125125	159159	179179	112112	112116	172184	135135	142154	139139	115115	145158
Munster bos-24	066DEB9C	191191	125125	155159	179179	112112	116116	172184	135135	142154	127131	115115	145158
Munster bos-25	066DE65D	191191	125125	151153	179179	112112	116116	159172	135135	142142	139139	115115	145187
Munster bos-26	066DC28F	191191	125125	153153	179179	112112	112116	159184	135141	138142	127131	115115	158187

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