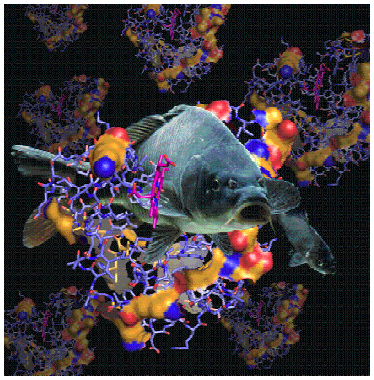

Project no. **022665**

Project acronym:

EUROCARP

Project title:

Disease and Stress Resistant Common Carp: Combining Quantitative, Genomic and Proteomic and Immunological marker technologies to identify high performance strains, families and individuals.

Instrument: STREP

Thematic Priority: Sustainable management of Europe's natural resources

Publishable final activity report

Period covered:
from January 1, 2006 to December 31, 2008

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Start date of project: January 1, 2006

Duration: 36 month

Project coordinator name:
Project coordinator organisation name:

Dr. Zsigmond Jeney
Research Institute for Fisheries, Aquaculture and Irrigation

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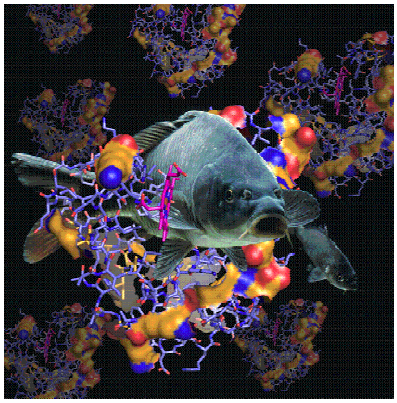
Publishable Executive Summary



Project title: Disease and Stress Resistant Common Carp: Combining Quantitative, Genomic and Proteomic and Immunological marker technologies to identify high performance strains, families and individuals.

Project no. 022665

Project acronym: EUROCARP

Public website of the project: http://eurocarp.haki.hu/

The common carp is the third most important farmed freshwater fish species in the world. Eastern European carp genebanks have been responsible for the selective improvement of carp for intensive and semi-intensive pond culture in Europe and their dissemination worldwide. Several serious disease problems such as Koi herpesvirus (KHV), and erythrodermatitis (*Aeromonas salmonicida* and *A. hydrophila*) threaten carp farming in many countries. Selection in carp has tended to develop high performing but inbred strains for crossbreeding. The inclusion of disease and stress resistance as traits within breeding programmes will require the use of modern quantitative and molecular genetic tools. Disease resistance has proved to be a difficult trait to assess and improve in fish, direct challenges on potential broodstock run the risk of turning such fish into carriers. Functional genomics, proteomics and gene mapping has the potential to identify candidate genes and Quantitative Trait Loci (QTL) for resistant fish without a challenge.

Using the HAKI carp genebank a series of crosses between two selected (TATA and Szarvas15) and two wild strains (Duna and Amur) produced 96 test families which were split into a number of replicates as a resource for the project. Controlled challenges for *Aeromonas* and KHV on two separate replicate groups identified families with high and low resistance for both diseases. The estimated heritability (on the underlying liability scale) was low (0.04 ± 0.03) for *A. hydrophila* resistance and very high (0.76 ± 0.15) for KHV resistance. Pure strain survival for KHV was Szarvas 15 (0%) < Amur (5%) < Tata (7%) < Duna (8%) and for *A. hydrophila*, Duna (35%) < Amur (36%) < Szarvas 15 (44%) < Tata (45%). The two challenge-tested diseases (KHV and *A. hydrophila*) had relatively high genetic correlation with each other (0.64 ± 0.28). These challenges enabled the families to be ranked in respect to their disease resistance. Tissue and serum samples were collected from the 10 highest and 10 lowest responding families for each disease as a resource for functional genomic analysis, proteomic and immunological assay.

The other sib replicates were used to assess culture performance under commercial pond and artificial tank conditions. Genetic parameters and heterosis effects were estimated for weight, length and survival traits at different time periods. Records of the fish weights at tagging (W0), first autumn (W1), second spring (W2), and second autumn (W3) were analysed. Total length (L1, L2, L3) of the fish was measured at the same time as the weight records except for time of tagging, and were also included in the analyses. Pond survival was defined as survival from second spring (W2) to second autumn (W3). Heritabilities for weight and length traits were estimated using univariate models, whereas genetic correlations and heritabilities for pond survival were estimated using multivariate models.

There were significant differences in survival between the pure strains. Duna (81%)> Amur (69%)> Tata (53%) > Szarvas 15 (51%). The level of heterosis (cross versus pure bred) varied from 0 - 29% for weight traits, from 1.7 - 8.3% for length traits, and from 8-37% for pond survival. However, significant differences between the strain combinations were only found for W3 and pond survival.

Heritabilities were low for W0, W1, W2, L1, and L2 (0.11-0.16) and high for W3 and L3 (0.49, 0.50 respectively). For pond survival the heritability on the underlying scale was 0.34 ± 0.09 corresponding to a heritability of ~0.2 on the observable scale. High correlations (0.81-0.98) were estimated between weight and length measured at the same time (W2/L2, and W3/ L3) and between weight measured in the first autumn (W1) and second spring (W2). Genetic correlations with pond survival during second growth season were low and non-significant for W2 (second autumn 0.06 ± 0.35), but high and significant for W3 (0.65 ± 0.15). The high levels of heritabilities for length and weight at the second autumn and the moderate heritability for pond survival indicates that selective breeding for these traits can be successful in common carp and give a considerable genetic gain in productivity. However, the estimated genetic correlations between pond survival and survival in disease challenge tests were not significantly different from zero for any disease (0.00 ± 0.31 and -0.24 ± 0.21 for *A. hydrophila* and KHV, respectively

The molecular analysis of the *A. hydrophila* and KHV disease ranked families was divided into three main themes, immunology, proteomics and functional genomics. The results from the immunology showed that there were significant differences between three immune parameters of common carp families with different genetic background and resistance against *A. hydrophila* infection. Characteristically, higher phagocytic and lysozyme activity could be measured in the resistant families than in the sensitive ones following challenge. In addition, the level of specific antibodies as measured by enzyme-linked immunosorbent assay (ELISA) was higher in resistant families after the challenge. Thus, it is concluded that these immunological parameters correlate with resistance against *A. hydrophila* and could be used as markers to identify resistant strains of carp.

The KHV immunology work was limited by the small number of fish surviving the cohabitation challenge. The plasma collected from control fish and from surviving fish at day 7 after the cohabitation challenge, and the sera collected from surviving fish at day 22 post challenge were both tested for antibody titre against KHV by titration in an ELISA. The ELISA antibody titres of the fish sampled at day 7 post cohabitation challenge ranged from 1:400 - 1: 1600 Of the 7 fish with titres ≥ 1:400, 6 were from resistant families and three were from the same family (#50).

The plasmas were also tested for virus neutralising activity. An optimised format for the neutralisation test was developed. Cytopathic effects were seen in the control virus at 14 days post inoculation. The virus grew through all dilutions of test plasma, but it was noticeable that the rate of development of CPE with many plasmas was much slower than the controls. The limited sample size makes it is unwise to draw firm conclusions from a correlation between ELISA titre and virus neutralisation. Although 6 of 7 fish with antibody detectable by the ELISA were from KHV-resistant families, it is not known to what extent that antibody influences the resistance. The antibody did not have strong neutralising activity, but it may have other effects such as opsonisation of the virus to enhance uptake by phagocytic cells.

Proteomic analysis was performed on the plasma collected from fish exhibiting high and low *A. hydrophila* resistance, before and after challenge. Two-dimensional differential gel electrophoresis using labelled dyes was used (ie 2D-DIGE) to compare the protein profiles of plasma samples. Numerous experiments were performed to optimise the method and then to assess resistant (A2 x D9) and susceptible (A3 x T9) families. The results indicated differences in the profiles obtained between resistant and sensitive families and between challenged and unchallenged fish, and there appears to be both up and down-regulation of different proteins following challenge. Several spots were identified as potential markers for resistance, however, mass spectrometric analysis of these spots was not possible in the timescale of the project. This work is being continued and will be taken to completion. It is interesting to note that for the susceptible family the major differences were observed in the down-regulation of specific proteins also observed in the functional genomic analysis.

The KHV proteomic samples were analysed using surface-enhanced laser desorption/ionization (SELDI) with an immobilized metal affinity capture (IMAC) chip. These comparisons showed that there was little difference between control resistant and control sensitive fish, and little difference

between control fish and sensitive fish sampled 1 day post challenge. However, the effects of disease (day 7 post challenge) were significant, especially in sensitive fish. The differences between controls *versus* fish sampled 7 days post challenge were significantly more different than sensitive family to resistant family differences. The peak heights were then analysed by partial least squares plots; broad groupings of the plasmas were apparent, with distinct clusters of plasma from sensitive and resistant fish although with some degree of overlap in the distributions. There were differences between sensitive and resistant fish, and between control and infected fish. The big differences are apparent in a small number of proteins, but this method of analysis does not identify those proteins. That was done by isobaric tag for relative and absolute quantification (iTRAQ) analysis.

Samples were selected based on the SELDI/IMAC results, the most significant results were seen in comparisons of control fish and fish sampled 7 days post KHV cohabitation challenge. To maximise information plasmas were selected where possible from this comparison to encompass families that were also shown to be resistant to *A. hydrophila*, and also to include families from crosses that included all the four parent strains of carp. The analysis of the proteins has been limited to those with a ratio > 2.0 or < 0.5 (each a factor of 2 away from parity, 1.0) in order to simplify a complex picture. In a comparison of KHV-challenged sensitive and resistant fish the differences were predominantly in comparisons between family #6 (sensitive) and the resistant families, #20 and #50. The proteins of relevance were α-globin, β-globin, haemoglobin β chains, haptoglobin, creatine kinase M2-CK and parvalbumin-α. Differences were also seen in vitellogenin and vitellogenin precursor, again when family #74 was being compared. When infected fish were compared with control fish from the same families, the major protein differences were generally seen in sensitive families. The proteins of relevance were α-globin, β-globin, haemoglobin β chains, creatine kinase M2-CK, parvalbumin-α and trypsin.

It is interesting to note that α-globin, β-globin and haemoglobin β chains are structural components of haemoglobin. As clinical signs of KHV disease include haemorrhage and anaemia, it is possible that the elevated levels of those proteins in the plasma from infected KHV-sensitive carp arose from degradation of carp erythrocytes as a result of the infection. That could also be a reason for the increased level of haptoglobin, as the function of that protein is to bind and remove free haemoglobin from the serum. Creatine kinase is found in muscle, and usually only found in plasma in cases of muscle damage; its presence in the carp plasma may also indicate muscle breakdown as a result of the KHV infection. There was no marked difference between ratios of immune-relevant proteins such as complement and immunoglobulin light and heavy chains.

There are clearcut differences in the sensitivity or resistance of carp families to KHV, and there are differences in the proteins detected in the fish plasmas. However, from these results is not possible at present to determine whether all those protein differences are responsible for the sensitivity/resistance of the fish and do not just represent an outcome of the disease pathogenesis i.e. the proteomic picture is of the disease process, rather than suggesting that, for instance innate immunity is a factor in the resistance. However, antibody was detected by the ELISA in 8% (7 of 89) of the fish sampled at day 7 post cohabitation challenge, and it is noteworthy that 6 of the 7 fish were from resistant families. Hence rapid development of acquired immunity may be an important attribute of the resistant families.

Functional Genomics

The EUROCARP project made possible the recent development of the carpBASE series of databases and the corresponding carpARRAY microarray chips. A significant advance that benefited the project substantial was the improvements in the annotation of carp ESTs based on two runs of the new 454 FLX high-throughput DNA sequencing system. This has generated a very large increase in the number of annotated genes. As a result, we have now generated a new carpBASE (version 7.1) containing the entire dataset, with a new and much more complete annotation.

Two major experiments, have been completed, designed to define the genes responding to bacterial or viral exposure, and to identify those responding genes that differentiate infected from control animals, and tolerant from susceptible phenotypes. The work focused on head kidney tissues due to its central

role in mediating immune defence and erythropoiesis. Each basic experiment was undertaken using a complex interwoven loop design in which we compared 6 disease tolerant families with 6 disease susceptible families. This maximises the statistical power for a given level of effort and cost. We used 6-fold biological replicates (i.e. tissue from 6 different specimens) on 72 arrays. This experiment was run separately for both bacterial and viral exposures requiring a total of 144 arrays. The hybridisations were performed at 42°C in Liverpool, followed by washing, imaging on a Genepix scanning fluorescence microscope and image extraction on Bluefuse software (BlueGnome, Cambridge). The data was spatially normalised, LOWESS corrected and statistically analysed all using scripts developed in Matlab following the ideas on ANOVA developed by Wit and McClure. Multiple sampling (FDR) corrections were employed throughout, and we then extracted genes that displayed a significant response (<0.05) in at least one ANOVA contrast. Overall responses and quality of the data were assessed using volcano plots, and P-value distributions. We clustered responding genes across all ANOVA comparisons and explored which clusters were linked to phenotypic responses of interest.

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Microarray data analysis

For the bacterial exposure experiment, we identified different gene clusters. Of these, bacterial cluster 3 displayed a substantial (3-4 fold) difference between control susceptible (SC) and tolerant (T) families. The tolerant group exposed to disease displayed an increase in gene expression, whilst the susceptible group showed a small decrease in expression. This resulted in little difference in the gene expression after long bacterial exposure. Cluster 1 displayed down-regulated responses on bacterial exposure but no difference between susceptible and tolerant families. Cluster 2 displayed up-regulated responses to bacterial exposure but again no differences between susceptible and tolerant families.

In the KHV samples a very large number (4268) of differentially expressed probes which formed 4 gene clusters were identified. Cluster 1 showed a small increase in expression at the early exposure time but a strong reduction in expression (4-8 fold) at long times (L). Susceptible families (SL/SC) displayed a greater decrease than tolerant animals (TL/TC), such that the comparison between them was significant (SL/TL, 2-3 fold response). By contrast, clusters 1 and 2 displayed a 2-fold increase in activity at long exposure period and cluster 4 displayed a ~2-fold decrease.

We have explored the extent to which the probes responding to bacterial infection are the same probes as responding to viral exposure, results suggest that this is largely true. By combining heatmaps we saw that clusters 1 and 2 together comprise the differentiated responses between TL/SL in the viral exposure and the TC/SC for the bacterial exposure. We also demonstrate that cluster 5 of this combined heatmap comprises genes which are up-regulated in late viral samples. Finally, it also demonstrates that the magnitude of the responses in the virus infection were some what larger than for bacterial exposure, in that the colours on the combined heatmap are more intense. Thus for the main cluster for each experiment we have identified a separate group of microarray probes that offer a means of predicting the tolerance status of families. We have analysed these lists using enrichment analysis of Gene Ontology categories in order to understand the types of processes involved. These data are being extended using mapping of gene lists onto networks of biological function using commercial packages (GeneGo and Ingenuity).

Finally, we have explored the genome-wide character of the transcriptomic profiles to identify gene probes that unambiguously identify the disease resistant phenotypes in individual specimens. For this it was necessary to screen all 26,000 carp gene probes to identify those which offered perfect discrimination between a training set of 12 specimens from 6 resistant families, and another 12 specimens from 6 susceptible families. For the bacterial exposure we identified ~250 such genes, and of these we selected 20 offering the greatest amplitude differences for inclusion in the diagnostic probe set. We then developed a test statistic that could be compared against a null distribution in order to provide a P-value that relates to the assignment to either a resistant or susceptible phenotype. We then used this to assess another set of 24 new specimens drawn from the same 12 families. We found that we could predict the disease-resistance phenotype of 19 out of 22 specimens, this being an 84% success rate. This provides proof-of-principle that a genomically-led strategy for the delineation of diagnostic probes is well able to generate meaningful characterisation of agriculturally-relevant phenotypes.

Genetic Mapping of Common Carp.

The genetic mapping of the common carp genome ran parallel to the other activities using some of the F1 resource families as reference material. A database was constructed of all published microsatellites for this species and other closely related cyprinids and EST data bases were mined for new repeat sequences. The database contains existing and new primer sequences based on using an end-labelling approach to visualisation. All primers were BLAST search against the *Danio rerio* genome assembly to assess their distribution across the genome. This information can be accessed through the EUROCARP website.

Analysis of the microsatellite markers in the reference families showed that approximately 50% of the published markers did not produce an amplification product and another 25% were homozygous in the reference families and therefore uninformative. A total of 210 microsatellite loci were analyzed by redesigning and synthesizing new primers. Finally after initial screening, we were able to score total 63 microsatellite loci for gene mapping in the reference families. In order to increase the marker density in the map AFLP analysis was performed with various selective primers to assess informative variation in the parents of family 94.
Seventy five primer combinations were assessed on parental fish and full sets of its progeny. A total 625 AFLP markers were assessed for all progeny of linkage map family #94 and recorded into the database. Deviation from HW equilibrium for each locus was assessed by JoinMap4 software, and small portion of loci with significant deviation from HW equilibrium was discarded from further analysis.

A major effort was put into the development of type I genetic markers based on SNP polymorphism of functionally important genes. This approach relied on the existing large collection of carp EST sequences organized in CarpBase and fairly well annotated genome of *Danio rerio.*
A total of 360 carp EST clusters from CarpBase 5.0 were picked for development of SNP-specific primers, based on following criteria:

-when mapped against zebrafish genome these genes were evenly distributed among zebrafish chromosomes, covering all chromosomes with several genes;
- The EST cluster represents genes of potential importance in stress or disease resistance, or other important biological function (decision was made based on GO category the gene belongs to). (GO = Gene Ontology see: http://www.geneontology.org/).

Primers are designed to reside on two neighboring exons and to amplify a region of carp genomic DNA including parts of two exons and intron (200-500bp) between it. Routinely we sequence four specimens – two parents (A3, D5) and two F1s. Heterozygotes are revealed by analysis of sequencing chromatograms and SNP-based primers are designed to screen a complete set of family#94 sexed progeny. The quality and robustness of the data improved after the 454 resequencing and better annotation in *Carpbase 7* , particularly the identification of paralogous genes.

All data obtained on segregation of genetic markers among progeny of family#94 (microcatellite loci, SNP-loci and AFLP markers) have been combined. Segregating markers were judged for deviations from the expected 1:1 (of AFLP marker and microsatellite and SNP DNA marker polymorphic in one parent) or 1:1:1:1 (of microsatellite DNA marker polymorphic in both parents) phenotypic ratios with $\chi 2$ testing. Those markers segregating at the expected ratios were used to construct linkage maps.

MapMaker v3.0 was used in determination of linkage groups and position of markers on within the each linkage group was obtained with assistance of JointMap v4.0 program.
Forty five linkage groups containing from two to ten markers have been identified by GROUP procedure and preliminary linkage map have been constructed. The longest LG has total length of 79cM. Ninety four markers (83 AFLP and 11 microsatellite DNA) did not show linkage to any other marker and were not assigned to any of the linkage groups. Sex was used as an additional marker; however it was not assigned to any linkage group produced during the analysis. Because sex determination mechanisms in polyploidy fish is not known, it is desired to continue with more fine mapping of sex determination locus in carp.
Despite first draft of carp linkage map have been prepared, additional analysis is required to produce final version of linkage map. Consortium will continue to work on preparation of the manuscript describing carp linkage map, which will be submitted for publication in 2009.

The three year EUROCARP project begun in January 2006, is being coordinated by Dr Zsigmond Jeney, head of the Fish Biology Department at the Research Institute of Fisheries, Aquaculture and Irrigation (HAKI), based at Szarvas in Hungary. Scientific coordination is shared with the University of Stirling's Institute of Aquaculture in Scotland. Also part of the consortium are the Centre for Environment, Fisheries and Aquaculture Science (Cefas) at Weymouth and the Centre for Environment, the University of Liverpool Laboratory for Environmental Gene Regulation, both in the UK and the Institute of Aquaculture Research (AKVAFORSK), in Norway. Eastern European, non-EU, R&D institutions taking part are the Division of Molecular Genetics at the Russian Federal Research Institute of Fisheries and Oceanography (VNIRO) and the Federal Centre of Fish Genetics and Selection, both based in Moscow.

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1. Project execution

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Project objectives and major achievements

1. **The identification of strains, families and individual carp with enhanced disease resistance response and growth performance by the end of year 2.**
2. **Develop tools that will help in the future management and improvement of common carp strains world-wide: disease and stress microarray, immunological and proteomic markers. Linkage map incorporating type1 and 2 markers by end of project.**
3. **Design of optimised breeding programmes informed by quantitative, functional genomic, proteomic and immunological data on disease response and how this correlates with other production traits by end of the project.**
4. **Dissemination of information on the tools and disease resistance and multi-trait selection programmes will be released continuously through existing networks to increase the sustainable production of carp worldwide.**

The project was aimed to assess the levels of diversity in four representative Hungarian common carp strains for reduced susceptibility to bacterial and viral infection presently causing major disease losses. The project adopted a multidisciplinary approach and combined data gathered through classical quantitative genetics, functional genomics, immunology and proteomics to identify strains, families and individual carp with different susceptibility to viral and bacterial infections. Ten carp families were found the most resistant against KHV (Koi Herpes Virus). The heritability estimate for KHV was sensitive to trait definition, but generally moderate to high. The heritabilities for the weight measures were also moderate to high. Both genetic and residual correlations between KHV and weights were close to zero. Ten other carp families were identified the most resistant against bacteria, *Aeromonas hydrophila*. The heritability estimated for the bacteria *A. hydrophila* was low wheras the genetic correlation between *A. hydrophila* and KHV were higher. Genetic and residual correlations between *A. hydrophila* and weights were low.
The microarrays and carpBASE developed in the first half of the project have been used to screen responses of large number of genes to both viral and bacterial infection. Data processing and pattern analysis has demonstrated an ability to discriminate genes responding to infection in each and both situations, but also those that discriminate between tolerance/susceptibility status.
High heritabilities were estimated for harvest weight (ca 1 kg) and pond survival and suggest that considerable genetic gain can be obtained for production performance by selective breeding. Gene sequences for all known carp transcripts have been collated into a searchable database, and the description of this collation has been published in the Journal of Fish Biology. Latter phases of EUROCARP incorporated the results from an additional 'next-generation' sequence collation which has dramatically extended our classification of carp genes. This data has been co-assembled with the pre-existing EUROCARP collation and the resulting database is presented at http://www.agf.liv.ac.uk/cb7pathways. These data sets was modelled to identify new optimised approaches to selective improvement of common carp strains worldwide. This information will be disseminated through the established international breeding and carp producer networks.

List of Consortium Partners

Partic. Role*	Partic. no.	Participant name	Participant short name	Country	Date enter project**	Date exit project**
CO	1	Research Institute of Fisheries, Aquaculture and Irrigation	HAKI	Hungary	Month 1	Month 36
CR	2	University of Stirling, Institute of Aquaculture	UoS	UK	Month 1	Month 36
CR	3	Centre for Environment, Fisheries and Aquaculture Science	CEFAS	UK	Month 1	Month 36
CR	4	University of Liverpool Laboratory for Environmental Gene Regulation	UoL	UK	Month 1	Month 36
CR	5	Akvaforsk The Institute of Aquaculture Research From January 1, 2008: Nofima Marin As.	AKVA Nofima	Norway	Month 1	Month 36
CR	6	Russian Federal Research Institute of Fisheries and Oceanography Division of Molecular Genetics	VNIRO	Russia	Month 1	Month 36
CR	7	Federal Center of Fish Genetics and Selection	FCFGS	Russia	Month 1	Month 36

*CO = Coordinator
CR = Contractor

In the following part the major achievements of the project are presented. The relations between Work packages are presented in Figure 1.

WORK PACKAGE 1

Carp Genetic Resources

Aims and achievements

The aims of WP 1 were to establish 4 resource strains for producing family resource material (96 families by diallel crossing), for WP 1, 2, 3 and 4; to identify families of carp with important production traits (survival, growth) at three different ages (advanced fingerling, autumn move and table-size fish); to evaluate the genotype-environment effect by rearing families in two different farming environments, and measuring important production traits; to provide experimental stocks for bacterial and viral challenges, as well as for genomics-, proteomics- and immunological studies; to generate reference family (Duna x Amur) for producing a gene linkage map (WP5) and to generate the F2 progeny as a future QTL mapping resource (WP5) and to catalogue of Eastern European carp genetic resources. Strains for producing family resource material were chosen and 96 families of carp were established. Important production traits and genotype-environment effect were identified. Reference family for producing gene linkage map was established. Catalogue of Eastern European carps was prepared for publishing.

Methodology and Approaches

Establishing 4 resource strains for producing family resource material

Resource families were selected from the live gene bank of common carp established and maintained by Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) (Bakos and Gorda, 2001). In order to "obtain" the possible widest genetic differences between the aimed families, resource strains were selected based on the following criteria:

- known origin and/or documented breeding history;
- sufficiently different genetic background („gene pool") and/or immunological potential (preliminary data available from previous studies);
- use of wild and cultured lines.

Results on genetic structure of the representatives of the gene bank suggested the following 4 strains: two wild strains "Duna" and "Amur" - native in the Danube and Amur rivers respectively and kept for at least 3 generations in the HAKI's live carp gene bank – and two noble carps: "Tata" and "15" – kept for at least 5 generations in the gene bank.

Establishing and growing 96 carp families

In order to implement activities related to the establishment of resource families, the available infrastructure had to be upgraded. The largest, to date, diallele cross of common carp needed major upgrade in facilities and husbandry approaches to produce tagged fish from 96 different carp families. This was successfully achieved and resulted in fish being available for bacterial and viral disease challenge and commercial production trials. The high and low responding families against viral (Koi Herpes Virus) and bacterial (*Aeromonas hydrophila*) diseases were identified (Table 1).

Table 1: High and Low responding families against bacterial and viral diseases

Female / Male	N°	Duna					Amur					Tata					15				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Duna	1	X							X									X			
	2	X							X									X			
	3		X									X							X		
	4		X									X							X		
	5			X									X								
	6			X									X								
	7						X							X							
	8						X							X							
	9							X									X				
	10							X									X				
Amur	1				X		X														X
	2				X		X														X
	3					X									X		X				
	4					X									X		X				
	5	X														X					
	6	X														X					
	7									X		X									
	8									X		X									
	9										X									X	
	10										X									X	
Tata	1		X							X									X		
	2		X							X									X		
	3			X									X							X	
	4			X									X							X	
	5				X									X							
	6				X									X							
	7							X							X						
	8							X							X						
	9								X									X			
	10								X									X			
15	1					X		X									X				
	2					X		X									X				
	3	X														X		X			
	4	X														X		X			
	5		X																		
	6		X																		
	7										X	X									
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	10						X						X								X

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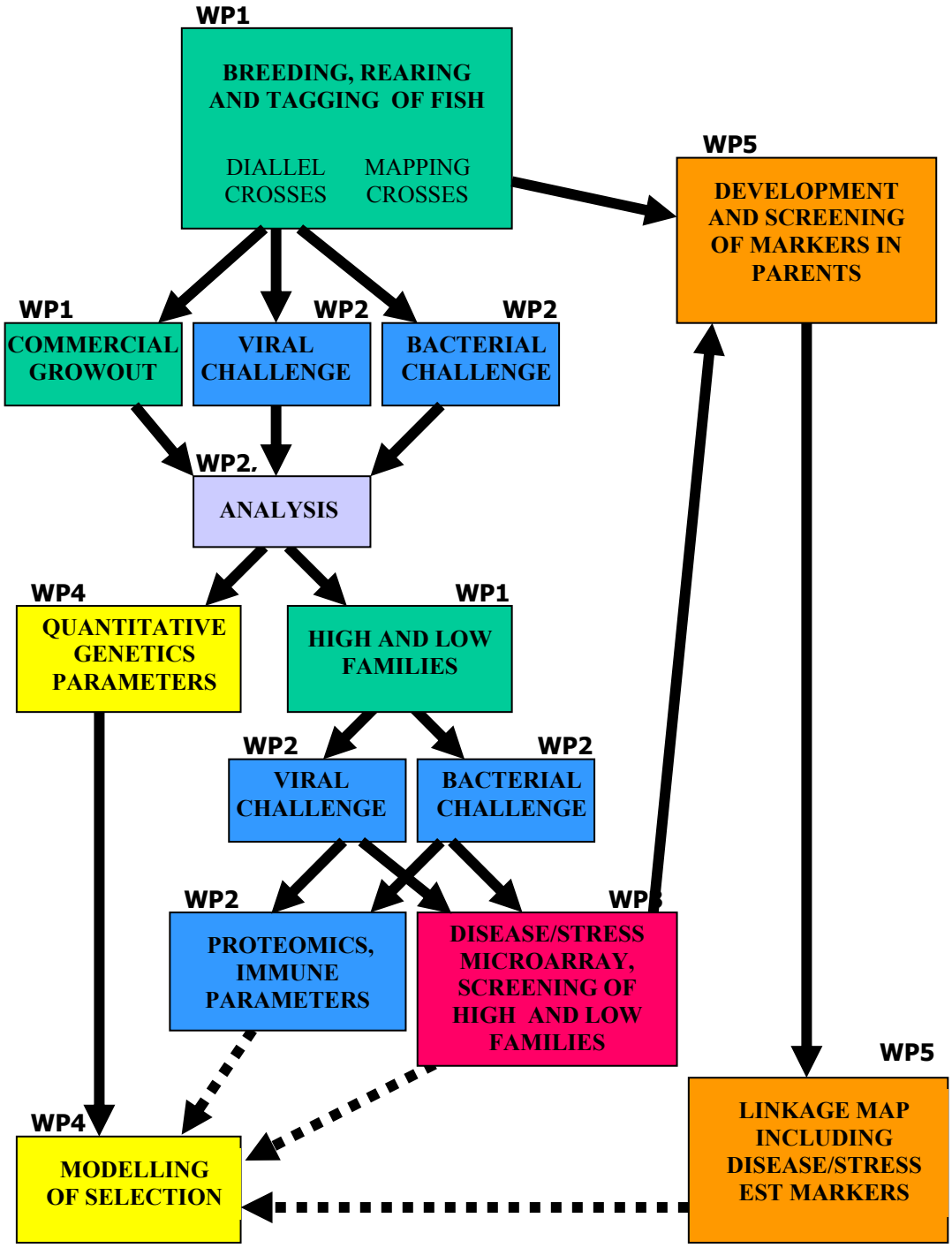


Figure 1: Outline structure of the project showing all main activities and interrelationship between the Work Packages

Figure **2:** Experimental facilities of the largest, to date, diallele cross of common carp

Identification of families of carp with important production traits and evaluation of the genotype-environment effect by rearing families in two different farming environments, and measuring important production traits

Estimating the magnitude of the genotype by test environment interaction for growth could unfortunately not be carried out as originally planned. The reason was that it was too costly to test the strains, crosses and families in two commercial farms. Hence, data recorded on reserve groups kept in the closed system in the HAKI experimental facilities was used as data representing a second farm environment. However, it is clear that these data were far from ideal for such analysis. First, the fish were not a random sample of the strains and families, second, the weighing was done at different times (26-138 days in difference and very different means (0.2 - 1 kg lower body weight)) from the commercial farm environments, and finally, the environment seemed to be rather limiting and creating a lot of extreme outliers. Hence, only very low but not significant genetic correlations (-0.27±0.27 – 0.49±0.33) were estimated. Very low genetic correlation between the two test environments indicate that growth in ponds and closed system tanks as in HAKI can be considered as completely different traits, which was very surprising. However, the very high S.E. also reflects the lack of power of the data to reveal significant genotype by environment interactions.

By use of production data (body weights and survival) from the commercial farm and disease challenge tests, breeding values for individual fish were predicted. These can be combined into a total merit selection index, including both disease resistance and production traits, that can be used for further selection and breeding in 2009 or 2010 when the tested fish are sexually mature and ready for breeding. See also results and breeding plan presented in WP4 description.

Generation of reference families for producing a gene linkage map and the F2 progeny as a future QTL mapping resource

In order to generate reference families for genotyping we chose the family 94. For genotyping we needed parents with a lot of heterozygosity, therefore we selected two most "wild" (and by assumption, most heterozygous) strains – Amur and Danube. Out of two Amur x Danube crosses only family 94 had enough F1 progeny to be sexed (14 males and 15 females), in addition to 65 unsexed ones.

According to the original plan, we have to generate the F2 progeny as a future QTL mapping resource. Since common carp reaches the sexual maturity earliest at an age of 3-4 years we were not able to produce them during the project period. In order to overcome this scheduling conflict, we chose the strategy with Amur and Danube strains, described above

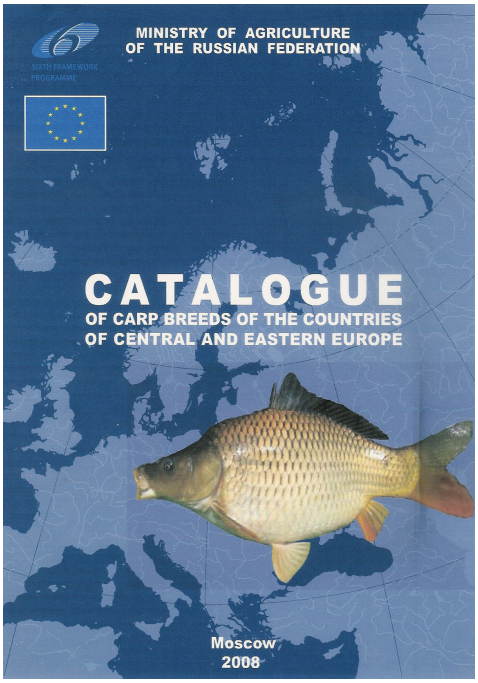
Catalogue Eastern European carp genetic resources

Pedigree fish-breeding farms of Hungary, Czech Republic, Poland, Russia, Ukraine, Belorussia and Moldova were monitored for collecting information on carp breeds origin, heritability, genetic variability and their resistance to diseases

Methodical requirements on compiling Fish Breeds Catalogue were prepared and the initial materials on carp breeds officially registered in seven countries of Central and Eastern Europe (Hungary, Czechia, Poland, Russia, Ukraine, Belorussia and Moldova) were collected and analyzed.

On the basis of this data the Catalogue of Carp Breeds *Cyprinus Carpio L.* of the countries of Central and Eastern Europe was prepared for publishing. This Catalogue describes 72 carp breeds rearing in different nature-climatic zones of Central and Eastern Europe. For each breed there are morphological, exterior, genetic, reproductive and fish-breeding characteristics by which breeds differ from each other.

The Catalogue content is exclusive and includes photos of cultivated carp breeds. For the first time in the world practice the Catalogue of such level is publishing both in English and Russian.



WORK PACKAGE 2

Disease challenge and immunological studies

Aims and achievements

The aims of WP 2 were to identify strains and families of carp exhibiting high and low disease resistance to a bacterial pathogen (*A. hydrophila*) and a viral pathogen (Koi herpesvirus KHV) and then to measure a variety of immune functions in these families to identify markers for disease resistance. In addition plasma samples were interrogated to identify plasma proteins from these fish to identify markers associated with disease resistance. All of the aims and objectives were achieved with the exception of spot analysis following proteomics of the plasma samples from *A. hydrophila* infected fish. Three immunological markers of disease resistance to *A. hydrophila* were identified: phagocytosis, lysozyme and pathogen-specific antibody. The results with KHV were not so clear cut, although pathogen-specific antibody responses may be worth following up in future studies. The proteomic work was time consuming and complicated and the results are inconclusive to date. This approach did however lead the participants to the application of new methodologies that may prove useful in the future. The immunological markers identified are easy to measure and such methods can now be applied to successfully identify resistant families.

Methodology and Approaches

Samples were collected from genetically different common carp (*Cyprinus carpio*) families that had been experimentally challenged with either *A. hydrophila* or KHV. Ten resistant and ten sensitive families that had been identified in WP1 were used for the experiment. Blood samples were collected 12 hours, one week and 21 days after the challenge and phagocytic and respiratory burst activity of phagocytic cells and lysozyme activity of the blood plasma were determined for the *A. hydrophila* challenged fish. The level of specific antibodies against *A. hydrophila* and level of natural antibodies were measured in the samples taken on the 28th day from the survivors. Non-infected fish were used as controls. The presence of KHV-specific antibodies and their neutralising activity were investigated in the KHV challenged fish. Plasma samples were also analysed by proteomics to identify any marker protein associated with disease resistance to either *A. hydrophila* or KHV. Two-dimensional differential gel electrophoresis using labelled dyes (ie 2D-DIGE) was performed with the *A. hydrophila* challenged fish samples. An alternative method was used for the KHV samples ie analysis by surface-enhanced laser desorption/ionization (SELDI) with an immobilized metal affinity capture (IMAC) chip. These methods can produce as much as or more information than 2-D DIGE, and so were considered to be a suitable alternative way of obtaining the information. Identification of the proteins was achieved by isobaric tag for relative and absolute quantification (iTRAQ) analysis. In the iTRAQ system, trypsin digests of the plasma are labelled, fractionated then analysed by tandem mass spectrometry. The labels enable relative quantification of the peptides, and databases are searched in order to identify the peptide fragments.

Immunological markers

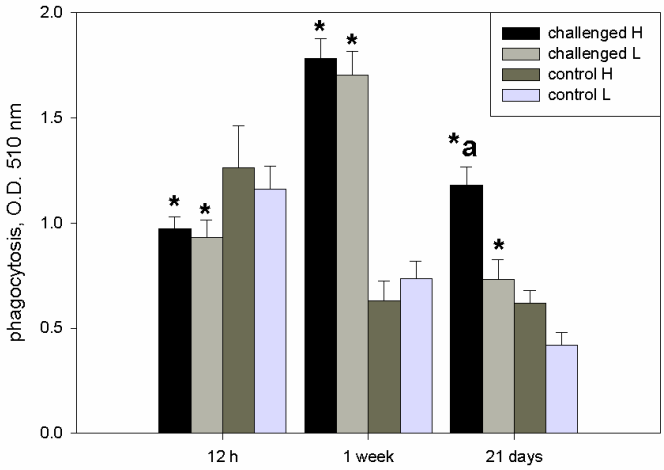


Figure 3. Phagocytic activity of isolated phagocytic cells 12 hours, one week and 21 days after infection of fish with *A. hydrophila*. Data are expressed as the mean of five fish ± SEM. Significant differences ($p<0,05$) between the infected groups and their relevant control groups are marked with an asterisk. Significant differences ($p<0,05$) between the two challenged groups or the two control groups are marked with "a".

Significant differences were observed between *A. hydrophila* challenged resistant families for phagocytic and lysozyme activies, as shown in Fig 3 and 4. The level of specific antibodies between the challenged resistant and challenged sensitive families was also found to be significantly different (Fig 5). There were no significant differences in the parameters between the control groups. It can be concluded that phagocytic activity of leukocytes, plasma lysozyme activity and specific antibody titre are significantly higher in the resistant families than in the sensitive ones following infection with *A. hydrophila*.

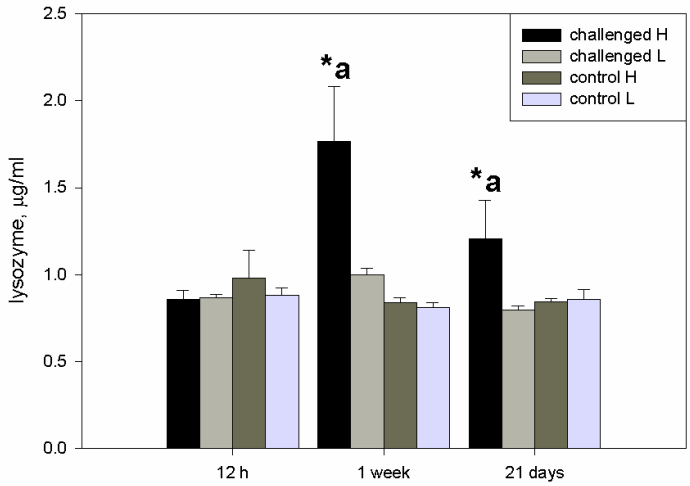


Figure 4. Plasma lysozyme activity 12 hours, one week and 21 days after the infection of fish with *A. hydrophila*. Data are expressed as the mean of five fish ± SEM. Significant differences ($p<0,05$) between the infected groups and their relevant control groups are marked with an asterisk. Significant differences ($p<0,05$) between the two challenged groups or the two control groups are marked with "a".

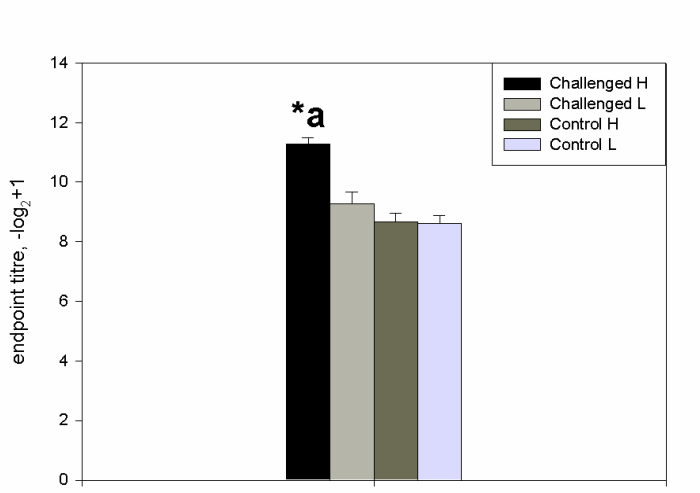


Figure 5. Endpoint titre of specific antibodies against *A. hydrophila* 21 days after infection of fish with *A. hydrophila*. Data are expressed as the mean of all survivors belonging to each experimental groups (± SEM). Data are expressed as the mean of five fish ± SEM. Significant differences ($p<0,05$) between the infected groups and their relevant control groups are marked with an asterisk. Significant differences ($p<0,05$) between the two challenged groups or the two control groups are marked with "a".

KHV-specific antibody was detected by the ELISA in 8% of the fish sampled at day 7 post challenge, and it is noteworthy that 6 of the 7 fish were from resistant families. Hence rapid development of acquired humoral immunity (ie antibodies) may also be an important attribute of the KHV resistant families. The plasma samples were also tested for virus neutralising activity, and although sample numbers were low there did not appear to be a correlation between ELISA titre and neutralisation.

Protein markers

Proteomic analysis was preformed on the plasma collected from fish exhibiting high and low disease resistance, before and after challenge with *Aeromonas hydrophila* or KHV. The 2D-DIGE was optimisation and standardised and results indicated that were differences in the profiles obtained between resistant and sensitive families, and between challenged and unchallenged fish. There appears to be both up and down-regulation of proteins following challenge. Representative gels are shown in Figure 6 Gels 6a and 6b show a comparison of proteins in the serum from resistant family A2 x D9 before and after challenge. Several spots were identified as potential markers for resistance, however, analysis of these by mass spectrometry was not possible in the timescale of the project. This work will nevertheless be continued and taken to completion. It is interesting to note that for the susceptible family (Figure 6c and 6d) major differences were observed in the down regulation of specific proteins.

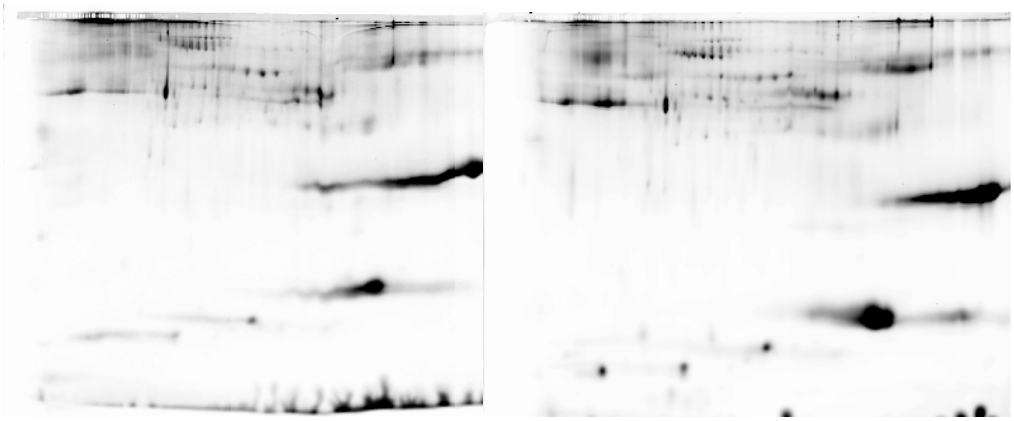


Figure 6 (a and b): Analysis of serum from resistant family A2 x D9 by DIGE; Gel 1
a) Cy 3 resistant control b)Cy 5 resistant 18hrs

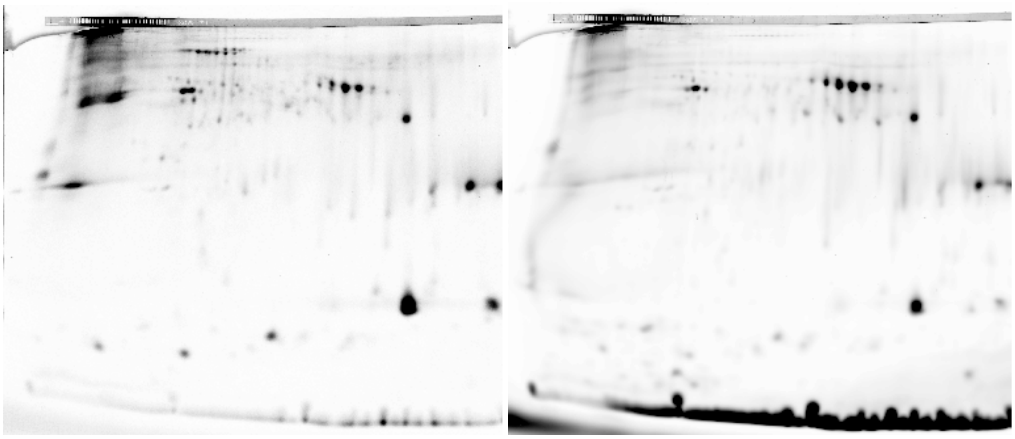
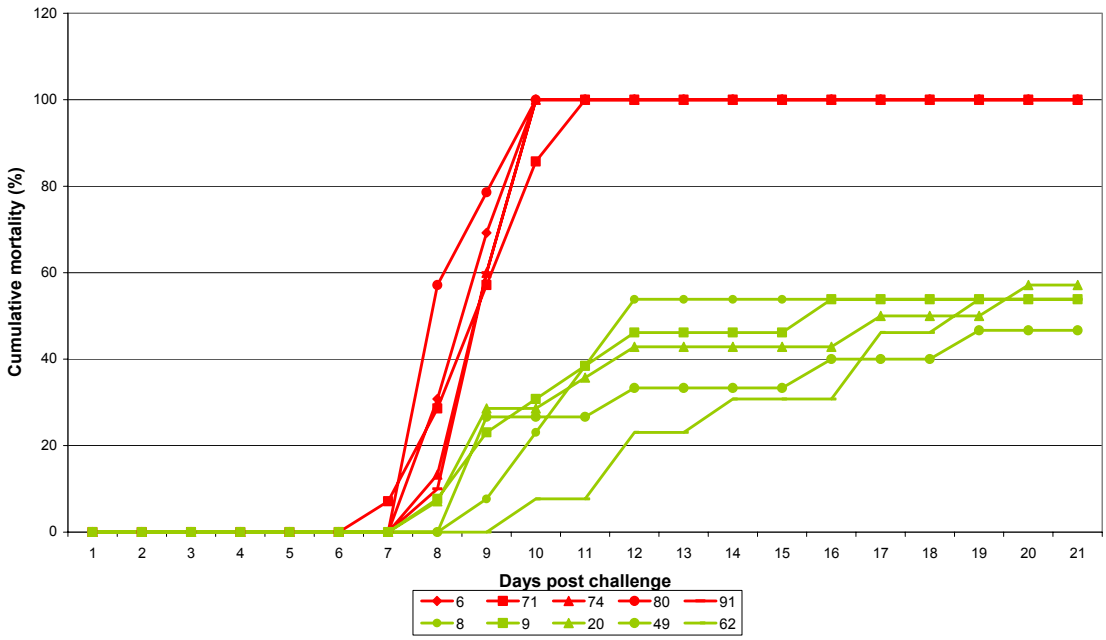


Figure 6 (c and d): Gel 6 Analysis of serum for susceptible family A3 x T9 by DIGE; Gel 6



c)Cy3 susceptible control d) Cy 5 susceptible 18hrs

Figure 7. KHV challenge study. Cumulative mortality of the five most sensitive families (red lines) and five most resistant families (green lines). Numbers are family numbers

The proteomics results from the KHV challenged fish indicated that there was little difference between control resistant and control sensitive fish, and little difference between control fish and sensitive fish sampled one day post challenge. However, the effects of disease (day 7 post challenge) are significant, especially in sensitive fish. The differences between controls *versus* fish sampled 7 days post challenge were significantly more different than sensitive family to resistant family differences. There were clearly differences in the sensitivity or resistance of carp families to KHV (Figure 7), and there were differences in the proteins detected in the fish plasmas. However, from these results is not possible at present to determine whether all those protein differences are responsible for the sensitivity/resistance of the fish and do not just represent an outcome of the disease pathogenesis i.e. the proteomic picture is of the disease process, rather than suggesting that, for instance innate immunity is a factor in the resistance

WORK PACKAGE 3

Gene regulatory basis of immune resistance in infection tolerant carp strains

Aims and achievements

This workpackage was directed at the discovery of DNA sequences of gene transcripts, and the use of cloned carp sequences to generate a DNA microarray. This was then to be used to screen across the entire set of gene probes for differences in expression between disease-susceptible and –tolerant individuals and families. Gene sequences for all known carp transcripts have been collated into a searchable database, and the description of this collation has been published in the Journal of Fish Biology. Latter phases of EUROCARP incorporated the results from an additional 'next-generation' sequence collation which has dramatically extended our classification of carp genes. This new data collation has been co-assembled with the pre-existing EUROCARP collation and the resulting database is presented at http://www.agf.liv.ac.uk/cb7pathways/.

The new 26K carp array was used in an extensive experiment in which the expression profile of 288 specimens was analysed in a complex statistical design seeking to identify responses to viral and bacterial infection, and responses that differentiate families exhibiting a disease tolerance phenotype from those which are diseases susceptible. We now profile over 4000 gene probes that are responding to experimental treatment across the full range of 12 families, and by exploring their functional meaning can describe the properties that account for infection responses, and those accounting for the tolerant or susceptible status. This data set forms the basis for developing a screening test that is capable of predicting the tolerance/susceptible status of individual carp specimens in WP4.

Methodology and Approaches

Further development of sequence data and fabrication of cDNA microarrays

Within the EUROCARP programme we have extended the number of cDNA clones to include those generated from the tissues of diseased (bacterial and KHV) and control carp. Combining all cDNA clones generated in Liverpool with those generated by colleagues in Singapore, and those working on the closely related goldfish in Ottawa provided a list of over 40K clones. Given the limitations on gene probes placed onto glass slides, we reduced the

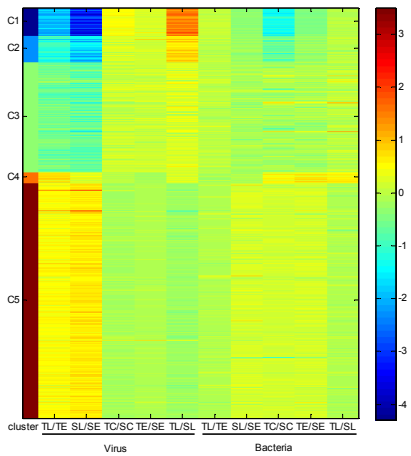
considerable redundancy in some genes by removing clones for the same assembled contigs. We thus reduced the entire clone-set down to 26K, and used this clone set to generate PCR amplicons for printing at high density onto coated glass slides using one of two robotic printers. We subsequently extended our annotation of gene sequences by incorporating very extensive sequence data generated from 2 runs of the Roche 454 GLX next-generation sequencer now installed at Liverpool. This created 559,000 new reads comprising 120Mb sequence. We co-assembled this new data with the existing Sanger reads, BLAST-identified the contigs and used this to generate a new and much more informative gene annotation with which to interrogate the outcomes of the array experiments. All of the results reported here used this new annotation.

Microarray experiments

These microarrays have been used in a major screening experiment to determine which genes are regulated during exposure to either of the two pathogens, and whether carp families judged to be particularly disease-tolerant or –susceptible displayed any differences at the level of gene transcription. We adopted an interwoven loop design for each pathogen exposure that compared 6 tolerant with 6 susceptible families, and for each family we compared control, uninfected individuals with those after a short and long time-points of pathogen exposure. The arrays were used with two channels allowing 2-way comparisons between pairs of tissue samples. The experiment used a complex plan to maximise statistical power and to minimise effort and cost. Thus final design incorporated 144 RNA preparations, run on 72 arrays, for each of the two pathogen exposures. We hybridised the arrays using Maui hyb chambers, imaged the arrays using an 5µm Agilent scanning fluorescence microscope, extracted the resulting intensity data using a Bluefuse algorithm, and normalised the data using conventional spatial and LOWESS intensity-dependent procedures. All informatic protocols were performed using MatLab scripts. The significance of the resulting data was assessed using ANOVA statistics and the quality of the resulting values assessed using volcano plots. P-value distributions were examined in order to define the multiple sampling corrections and the resulting confidence in the differences. This allowed us to extract a list of gene probes that displayed statistically significant responses for each of the comparisons, tolerant/susceptible (T/S) and control (C)/infected at early and late time periods (E or L).

Statistical analysis of the microarray data

For both viral and bacterial exposures we have identified ~4000 responding gene probes which were hierarchically clustered and functionally characterised. In each experiment we separately identified a cluster that unambiguously separated T from S specimens. Thus for the viral infection we found that the greatest discrimination between T and S families was obtained from the comparison of infected specimens at the long time interval. By contrast, for the bacterial infection the greatest T/S discrimination occurred for the comparison of uninfected controls. Combining the virus and bacteria profiles from both experiments into a single presentation (see adjacent figure) indicated that the cluster C1 (and to a lesser extent, cluster C2) of genes for both effects was identical. This indicates the central role of this cluster in discriminating T from S families.



We also identified large numbers of gene probes that were regulated in infected specimens (E or L) relative to uninfected controls (C), but with little or

no discrimination between S and T families. Thus. a second major cluster (C4) contained up-regulated genes in virus-infected carp. The same cluster was also up-regulated in bacterially-infected carp though to a lesser extent. A third cluster (C2) showed down-regulated responses in virus-infected carp but with no noticeable effect on bacterially-infected carp.

The different cluster were examined using enrichment analysis of Gene Ontology categories, using FDR protocols. We found that many of the genes mapped onto 'immunity' and 'infection' pathways and processes but we have identified a range of other pathways and processes that were consistently regulated in infected carp. This included 'cell cycle' and 'cell differentiation' processes, and a range of developmental processes particularly including for the nervous system.
Despite the fact that most of the carp gene probes showed BLAST homology to zebrafish sequences, many of them possessed no functional annotation, and thus we are unable to comment on their significance in terms of infection and disease. Continuing improvements in gene databases will certainly lead to the identification of some of these unknown transcripts over the next few years. Nevertheless, the gene list of responding probes suggests a rich mechanistic understanding of processes that are typically affected by infection or which are part of the infection response. This analysis could be repeated for other tissues.

Provision of gene expression dataset for further delineation of disease tolerance selection in WP4

We have generated tables and files containing all of the data obtained in WP3 to allow the interrogation of gene responses for those able to distinguish disease-tolerant from disease–susceptible individuals. These outcomes are described below in WP4 deliverable 4.4.

WORK PACKAGE 4

Integration of functional genomic approaches with selective breeding

Aims and achievements

The aims of WP 4 were to estimate the genetic variance within and between strains of common carp for disease and production traits; estimate the genetic correlations between different traits; estimate the magnitude of the strain heterosis effect for disease resistance and growth; estimate the magnitude of the genotype (strain and family) by test environment interaction for growth; begin to selectively breed common carp with the goal of producing a population which is highly resistant to disease and highly productive; develop experimental and statistical means to utilise whole genome expression profiles as a tool for selectively breeding animals with a generally better level of disease resistance; and finally design, model, evaluate and recommend more practical and efficient selective breeding programs incorporating these new technologies. All of the aims and objectives were achieved successfully with the exception of estimating the magnitude of the genotype (strain and family) by test environment interaction for growth. As mentioned under WP1, the reason was that it was too costly to test the strains, crosses and families in two commercial farms. Hence, data recorded on reserve family groups kept in the closed production system in the HAKI experimental facilities were used as a second test environment. However, the correlation between e.g. harvest weight in the farm pond (recorded October 2007 with mean=1.2 kg) and the final corresponding closed system weight (recorded June 2007 with mean=0.2 kg) was very low (-0.27±0.27) and not significantly different from zero. Also, correlations between earlier weights (in November and December 2006 with corresponding mean body weights of

120 and 103 g for ponds and closed system, respectively) were low (0.49±0.33). These results indicate high G×E and that growth in the two environments can be regarded as different traits. However, the very high S.E. reflects the lack of power of the data to reveal significant genotype by environment interactions. Problems with the data are probably due to the facts that the closed system fish were not a random sample of the strains and families (due to sampling of fish from certain families for other experiments, weighing of the fish was not done at the same dates as at the commercial farm (difference of 26-138 days), and that the tank environment at the research station seemed to be rather limiting resulting in generally much lower growth (0.2 kg (20%) - 1 kg (>500%) lower mean body weight). Furthermore, the data contained extreme outliers indicating problems associated with the environment and/or data recording.

Estimation of strain differences, heterosis and genetic parameters

The variance between the strains with respect to survival during the second growth season (4 April-26 October 2007) was clearly significant with the wild strains having the highest survival rate (81% and 69%) compared to the domesticated strains (53% and 51%). The level of heterosis (crosses versus pure bred) varied from 0 to 29% for weight traits and from 8 to 37% for pond survival. However, significant differences between the strain combinations were only found for harvest weight (2. autumn) and pond survival during the same period. Heritabilities were low for early weights (0.11-0.16) and high for harvest weight (0.49). For pond survival the heritability estimate was moderate (0.34 ± 0.09 on the underlying scale corresponding to a heritability of ~0.2 on the observable scale). Genetic correlation with pond survival during second growth season was high and significant for harvest weight (0.65 ± 0.15). The high heritabilities estimated for length and weight at the second autumn and the moderate heritability for pond survival indicates that selective breeding for these traits can be successful in common carp and give a considerable genetic gain in productivity (Table 2).

Table 2

Heritabilities (on diagonals), genetic correlations (above diagonal) and phenotypic correlations below diagonals) for pond survival, W2 (weight recorded in April 2007) and W3 (harvest weight recorded in October 2007).

	Trait		
	Pond survival	W2	W3
Pond survival	0.34 ± 0.09	0.06 ± 0.35	0.65 ± 0.15
W2	0.25	0.11 ± 0.08	0.38 ± 0.27
W3	0.42	0.64	0.50 ± 0.12

Based on the challenge tests for KHV, the domesticated strain Szarvas 15 obtained the lowest survival rate (0%) whereas the highest expected survival rate was estimated for the wild

strain, Duna (8%). Based on the challenge tests for resistance to *A. hydrophila*, the estimated strain differences in survival rates were of the same magnitude with the lowest survival rate for the domesticated strains, (44-45% compared to 35-36 for the two wild strains). The estimated heritability (on the underlying liability scale) was low (0.04 ± 0.03) for *A. hydrophila* resistance, but very high (0.76 ± 0.15) for KHV resistance. The two challenge-tested diseases (KHV and *A. hydrophila*) were highly genetic correlated with each other (0.64 ± 0.28). However, the estimated genetic correlations between pond survival and survival in disease challenge tests were low and not significantly different from zero for any of the diseases. The latter was not surprising as the KHV virus had not yet been observed in Hungary at the time of the experiment. For pond survival and resistance to the new pathogen in Hungary, KHV, the wild (and probably less inbred) strains (Duna and Amur) performed best. For body weight (W3) and *A. hydrophila* the domesticated Tata strain performed better. Both domesticated strains showed a higher survival after *A. hydrophila* challenge, probably due to an adaptation to the common pathogen in carp farm environments. Based on the high heritability there are good prospects for improving resistance to KHV infection and pond survival in common carps through selective breeding. Furthermore, selection for improved KHV resistance is also likely to improve resistance against *A. hydrophila* infection due to the favourable genetic correlation between the two traits. However, the estimated correlations between body weights and disease resistance were low and not significant.

Model, evaluation and recommendation of incorporating new technologies in selective breeding programs

A selective breeding plan aiming at producing a population of common carp with a high growth rate, a high survival rate, and which is highly resistant to Koi herpes virus (KHV) and the bacteria *Aeromonas hydrophila* is proposed. The four traits growth and survival until market size, and resistance to KHV and *A. hydrophila* were included in the breeding goal. Almost the same response in *A. hydrophila* was obtained when selecting for resistance for KHV only compared to selection for resistance to both KHV and *A. hydrophila*. This was due to a low heritability for *A. hydrophila* and a high genetic correlation between resistance to KHV and resistance to *A. hydrophila* as reported above. It was concluded that the structure of a selective breeding program for common carp should follow the general structure for large-scale breeding programs for aquaculture species, which is divided into breeding nucleus, multipliers and grow out units. A family based selective breeding program is suggested where each of 100 males are mated with two different females to produce 200 full-sib families. For each trait, 20 individuals should be tested in each full-sib family. After tagging, the fingerlings should be communally stocked and reared under commercial grow-out conditions for performance testing. The selective breeding program for common carp should be based on index selection with combined individual and sib selection. Individual body weight and survival should be recorded after two growth seasons (mean body weight of ca 1 kg). In order to select for resistance to KHV, challenge tests with tagged individuals from the full-sib families in the breeding nucleus should be performed. Altogether, selection for improved pond survival, resistance to KHV and *A. hydrophila* in addition to growth will contribute to develop a more robust and productive common carp for farming. Assuming a realized selection intensity of 1 (implying 15-20% selected), a genetic gain of minimum 150 g higher body weights at harvest (2^{nd} autumn), 9 %-units higher pond survival and 2-4 %-units higher resistance to KHV can be expected per generation. This gain will be accumulated for each generation of selection.

A method using random regression (BLUP) with cross validation is proposed and evaluated for predicting phenotypes from gene expression data for animal breeding. The new approach resulted in a moderate but significant correlation between predicted and actual phenotype (0.32). Binary classification of the predicted phenotypes yielded similar classification error rates to those found by other authors (35%). Unlike prior methods, the new method gave a quantitative estimate of phenotype that could be used to rank animals or patients and choose those with extreme phenotypic performance.

The use of such gene expression profiling with selective breeding for improved disease resistance was evaluated using a computer simulation model for Atlantic salmon. Genetic response was evaluated under four different selection criteria: family breeding value from disease challenge tests (CRIT1), predictor of individual challenge test phenotype using gene expression profiling (CRIT2), a combination of criteria 1 and 2 (CRIT3), and, direct selection of disease challenge test survivors (CRIT4). The best genetic response was achieved by using disease challenge tests to select the best families in combination with gene expression tests to select the best individuals within families (CRIT3). Disease resistance was doubled after 6-7 generations of selection, and varying the phenotypic and genetic correlation had a relatively small effect on the overall genetic response after 10 generations. With 10 generations of selection under CRIT3 the model predicted a benefit-cost ratio of more than 17:1, total added value per kg of fish of 0.29 Euro/kg and a nominal economic effect on operating income of over 175 million Euros. CRIT3 was almost as profitable an option as CRIT1, providing the cost of gene expression testing was less than €280/individual and the genetic correlation was higher than 0.3, was more profitable than CRIT2 under all scenarios and resulted in greater total added value and higher nominal effect on operating income than all other selection criteria.

Methodology and approaches

Genetic parameters

Genetic parameters and heterosis effects were estimated for weight, length and survival traits for common carp reported at different time periods at a commercial farm. The diallel cross between the two land race strains (Duna and Amur) and the two farmed strains (Tata and Szarvas 15) was used. Five females and 10 males per strain were used to produce six families for each of the 16 cross combinations resulting in 96 families each with 20 fish. Records of the fish weights at tagging (W0), first autumn (W1), second spring (W2), and second autumn (W3) were analysed. Total length (L1, L2, L3) of the fish was measured at the same time as the weight records except for time of tagging, and were also included in the analyses. Pond survival was defined as survival from second spring (4 April 2007, W2 recording) to second autumn (26 October 2007, W3 recording). Hertitabilities for weight and length traits were estimated using univariate models, whereas genetic correlations and heritabilities for pond survival were estimated using multivariate models.

Estimates of strain effects and heritabilities for resistance to *A. hydrophila* and koi herpes virus (KHV) and pond survival, as well as genetic correlations among the traits were obtained from the same diallel cross of the total of 92 full-sib families of common carp produced. Pond survival was included as the same trait as defined above. Disease resistance was obtained from survival data from the challenge-tests using intraperitoneal injection for *A. hydrophila* and cohabitants for KHV. Two replicated challenge-tests were conducted for each disease. The overall survival rates were 44% and 34% for the two replicated tests of *A. hydrophila*, 7% and 5% for the two replicated tests of KHV and 78% for pond survival. Threshold models

including sire, dam, common environment before tagging and challenge test (first or second for the disease traits) and cross sectional data (dead or alive at end of test) were used to estimate heritabilities.

Incorporation of new technologies in selective breeding programs

A method using random regression (BLUP) with cross validation is proposed and evaluated for predicting phenotypes from gene expression data for animal breeding. The method accounts for the distribution of variation in the trait and utilizes different subsets of patients or animals to perform a complete validation of predictive ability Breast tumour data published by Van't Veer et al. (2002) was used to test the method. The new approach resulted in a moderate but significant correlation between predicted and actual phenotype (0.32).

The use of such gene expression profiling with selective breeding for improved disease resistance was evaluated using a computer simulation model for Atlantic salmon. Disease resistance was assumed to be improved by selection for survival after challenge test, or by selection for predicted survival from analysis of the gene expression response of cells challenged in-vitro to disease, or a combination of both traits. As the correlation between gene expression level and survival is unknown and will be dependant on the prediction equation used, different levels of genetic and phenotypic correlation (r_g and r_y) between the two traits were modelled. Genetic response was evaluated under four different selection criteria: family breeding value from disease challenge tests (CRIT1), predictor of individual challenge test phenotype using gene expression profiling (CRIT2), a combination of criteria 1 and 2 (CRIT3), and, direct selection of disease challenge test survivors (CRIT4). Economic evaluation was performed using estimates and records from the industry, and accounting for the costs of gene expression profiling, under an opportunity cost model.

We have sought to use the genome-wide expression data to identify gene probes capable of providing the basis for a diagnostic text differentiating disease-tolerant from susceptible carp. The outcome of the ANOVA analysis, which seeks an overview of the entire dataset of 14 individual animals, proved unsuitable for the discrimination of individual specimens. This was because we found considerable variation in the expression values both absolute and relative within families for the 'T' and the 'S' groups. Instead, we devised a screen of the data to identify any gene probes that correctly (100%) discriminated the S/T status of 24 individual specimens. This identified ~250 which exceeds by a factor of ~10-fold that expected by chance alone (~25). Of these we selected the 20 best performing gene probes based on the magnitude of the differences between T and S comparisons. We then used this training set to develop a protocol that would test the S/T status of another 24 specimens from the same range of 6 x T and 6 x S families. We tested the properties of each specimen against a null distribution and calculated a P-value from which significance was called. 2 of the specimens failed display a significant outcome, and of the remaining 22 specimens 18 were correctly called. Thus of those specimens demonstrating a significant distribution we find a success rate of ~80%. This test has the advantage of using control, uninfected animals. We would need to expand the test to incorporate specimens from crosses other than those tested, and to demonstrate the validity of outcomes from animals with a more variable history in respect of environ mental conditions. The EUROCARP protocol was strictly controlled resulting in specimens with a common recent history of environmental/rearing conditions. We cannot be certain that the results obtain are robust to different ages, different feeding regimes etc etc.

WORKPACKAGE 5

Gene linkage mapping

Aims and achievements

The aims of WP5 were to produce linkage mapping family by crossing two presumably most geterozygous carp strains, to generate large number (appr. 500) of genetic markers, geterozygous in at least one parent, and to construct carp linkage map by genotyping informative genetic markers in F1 progeny of this experimental linkage mapping carp family. Genetic markers were of type II - non-coding markers, such as AFLP and microsatellite loci, not associated with known genes, and type I (coding) markers, derived from SNP and intron length polymorphism of know and expressed genes, and microsatellite markers, derived from carp EST database.

All of the aims and objectives were achieved successfully, 625 AFLP, 52 micrtosatellite loci and 38 SNP loci have been found polymorphic in parents and informative for linkage mapping analysis, and preliminary type-I marker enriched carp linkage map have been produced.

Methodology and approaches

Production of carp linkage mapping family.

A linkage mapping family (family #94) was produced by crossing parents from Amur (specimen A3, male) and Danube (specimen D5б female) strains. 65 F1s have been fixed at age 2 month, and 29 specimens (14 males and 15 females) were grown up till the sex can be determined by dissection, sexed and fixed in ethanol.

Genotyping with AFLP markers

AFLP analysis.

AFLP analysis was performed with various selective primers to assess informative variation in the parents of family 94. AFLP analysis was carried out essentially as described by Vos et al. (1995). DNA was digested with EcoR I and Mse I before ligation to restriction sitespecific adaptors. Pre-amplification was carried out using adaptor-specific primers with one selective base overhang each primer (EcoR I adaptor primer with A and Mse I adaptor primer with C). MspI and EcoRI adapters were produced from the following primers:

```
Eco-F: 5'-CTC GTA GAC TGC GTA CC-3'
Eco-R: 5'-AAT TGG TAC GCA GTC TAC-3'
Msp-F: 5'-GAC GAT GAG TCC TGA G-3'
Msp-R: 5'-CGC TCA GGA CTC AT-3'
```

The pre-amplification product was diluted (10-fold) and used for selective amplification. Selective amplification was carried out with diluted pre-amplification product and primers with three selective bases overhang each primer. In total, 91 primer combinations were selected for AFLP analysis.

The second amplification was performed with Fam-labled EcoR primers to produce fluorescent-labled PCR products. The products of selective amplification were separated by capillary electrophoresis have been imported and analysed by GeneMarker software (SoftGenetics).

Mse I and EcoR I selective primers were named with capital letters and numbers, respectively (Table 1). AFLP marker was named with the name of Mse I selective primer and that of EcoR I selective primer followed by letter f (fragment) and the size of the fragment in base pairs (Zhang et al., 2006), e.g., A1f169 referred to the 169 bp fragment generated by Mse I primer A (AAA) and EcoR I primer 1 (ACT).

	E_1ACT	**E_2ACA**	**E_3ATT**	**E_4AAG**	**E_5AGC**	Total
M_AAAA	8	11		9	7	35
M_BAAC	13	17	9			39
M_CAAG	7	11	2	9	8	37
M_DAAT	2	10	10	8	4	34
M_EACA	9	16	4	10		39
M_FACC	17	15	9	9		50
M_GACT	11	23	12	20	13	79
M_HAGA	8	8	2	9	8	35
M_IAGC	7	15		12	6	40
M_JAGG	13	19	4	12		48
M_KAGT	8	12	1	13		34
M_LATC	13	8	4	6		31
M_MACG	17	0	5	21		43
M_NATG	8	15	3	6	5	37
M_OATT	14	11	7	11	1	44
Total	155	191	72	155	52	**625**

Table. The number of polymorphic loci detected with AFLP analysis

Seventy five primer combinations were assessed on parental fish and full set of its progeny. Number of informative loci for each primer combination is summarized in Table 1. Total 625 AFLP markers were assessed for all progeny of linkage map family #94 and recorded into database. Deviation from HW equilibrium for each locus was assessed by JoinMap4 software, and small portion of loci with significant deviation from HW equilibrium was discarded from further analysis..

Type-I genetic markers discovery and genotyping in carp mapping family.

Major emphasis was done on development of type I genetic markers based on SNP polymorphism of functionally important genes. This approach relay on existing large collection of carp EST sequences organized in CarpBase and fairly well annotated genome of Danio rerio.

All carp EST (appr. 32121) were first compared with manually curated database of zebrafish genes (RefSeq, NCBI). All Carp ESTs were blasted against zebrafish RefSeq database (http://www.ncbi.nlm.nih.gov/RefSeq/) by using standalone BLAST program incorporated into MS Access database. Over 6000 carp ESTs were found (with cut off blast value of e-100)

to have strong homology with over 2500 known or predicted zebrafish genes. Zebrafish RefSeq genes were located on the last Danio rerio genome assembly (July 2007) with assistance of web-based genome browser at UCSC, USA (http://genome.ucsc.edu/cgi-bin/hgBlat) . Because of close relationship between carp and Danio, we expect a large conordance between genomic maps of these two cyprinids. Allocation of Carp genes on danio genome allow us to use zebrafish genome map as a blueprint for carp linkage map construction

360 carp EST clusters from CarpBase 5.0 were picked for development of SNP-specific primers, based on following criteria: 1. when mapped against zebrafish genome this genes are evenly distributed among zebrafish chromosome, covering all chromosomes with several genes; and 2. EST cluster represents gene of potential importance in stress or disease resistance, or other important biological function (decision was made based on GO category the gene belongs to). (GO = Gene Ontology see: http://www.geneontology.org/).

In 2008 the new version of Carpbase was produced by UoL partner. This version (Carpbase 7) differs from previous versions as it contains not only contigs from Sanger sequencing but also hybrid contigs (containing data sequenced using 454 sequencing technology and Sanger clones). Total 388514 reads from 454 and 19065 sanger sequences deposited in GenBank form 71051 clusters. Analysis of gene duplication in carpbase 7 resulted in designing additional 148 primers for paralogous type-I loci

Primers are designed to reside on two neighboring exons and to amplify a region of carp genomic DNA including parts of two exons and intron (200-500bp) between it. All designed carp specific exon based intron targeted primers was assessed on four specimens – two parents and two randomly chosen F1s. Heterozygotes and indels, revealed by analysis of sequencing chromatograms was used to design SNP or indel-specific primer, allowing to discriminate allele segregation during F1 genotyping procedure. Visualization of PCR reaction product of each SNP allele-specific PCR reaction was performed by agarose electrophoresis for each allele, and presence of allele-specific band was scored for each F1 individual of complete set of family#94 sexed progeny.

Microsatellite loci analysis.

All previously known carp microsatellite markers (Crooijmans *et al*., 1997; Aliah *et al*., 1999; Desvignes *et al*., 2001; David *et al*., 2001; Sun and Liang, 2004) have been reassessed .and primers were screened against DNA sequences deposited in GenBank to reveal typos. All primers were manually inspected and assessed on self-dimer formation and appropriate annealing temperature (algorithm implemented in ***FastPCR professional 5.0***, PrimerDigital Ltd).

After exhaustive search and assessment of robustness and polymorphism of published Carp microsatellite primers, a we included in our microsatellite database a large set of microsatellite primers published ore deposited in GenBank for closely related to Carp other cyprinid species. These species are : crucian carp, goldfish, bighead carp, and white amur. All primers were manually inspected on presence of typos, assessed on self-dimer formation and appropriate annealing temperature (algorithm implemented in ***FastPCR professional 5.0***, PrimerDigital Ltd).

All EST sequences from derived from Carp (appr. 32000 entries as to July 2008) have been downloaded as a fasta file and screened on presence of short (2-4) nucleotide repeats as by RepeatFinder program. All loci revealed were compared with previously published EST-bised microsatellite loci to avoid duplicates, and for newly discovered loci the sat of primers was designed and these loci were added to our Carp microsatellite database.

Presence of heterozygotic state of microsatellite markers for either or both parents of family#94 (A3, D5) was assessed for presence of heterozotes in parental specimens of gene linked family #94. On average, 50% of all published microsatellite markers fail to produce specific amplification product, and within remaining about half of the loci studied (25% of total microsatellite markers) do not reveal heterozygote state in at least one of the parental fish (i.e. both parental specimens are homozygous) and therefore 75% of all assessed lociwere excluded from further gene linkage analysis as an uninformative.

The genetic analysis was redesigned in order to improve reliability and performance, and most of the microsatellite markers were first tested on A3 and D5 parents, and, if parents were found heterozygous, all sexed progeny A3xD5 cross were screened with these primers, allele composition of parental specimens and offspring are screened and recorded in linkage mapping database for further analysis. Total 210 microsatellite loci were analyzed by designing and synthesis of primers, assessment of PCR performance and suitability for gene linkage analysis. Finally after initial screening, we were able to score total 53 microsatellite loci to include in gene mapping.

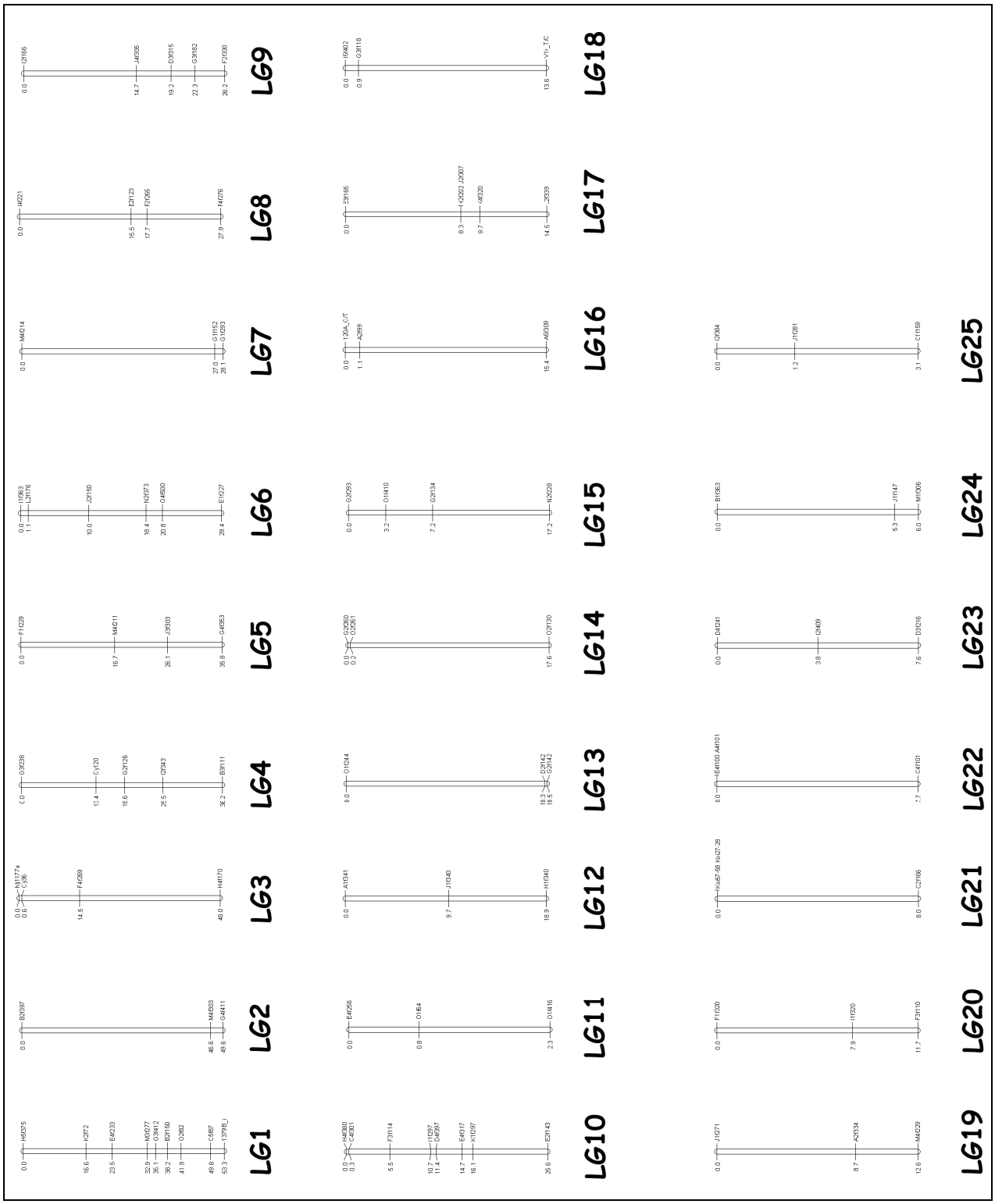
Construction of type-I marker enriched carp linkage map.

All data obtained on segregation of genetic markers among progeny of family#94 (microcatellite loci, SNP-loci and AFLP markers) have been combined. Segregating markers were judged for deviations from the expected 1:1 (of AFLP marker and microsatellite and SNP DNA marker polymorphic in one parent) or 1:1:1:1 (of microsatellite DNA marker polymorphic in both parents) phenotypic ratios with $\chi 2$ testing. Those markers segregating at the expected ratios were used to construct linkage maps.

MapMaker v3.0 was used in determination of linkage groups and position of markers on within the each linkage group was obtained with assistance of JointMap v4.0 program.

Population code CP was used (population resulting from a cross between two heterogeneously heterozygous and homozygous diploid parents, linkage phases originally unknown). Because all microsatellite loci analyzed revealed clear diploid inheritance (possibly as a result of allotetraploidy of carp), and all duplicated genes were treated separately, linkage mapping analysis was conducting by assuming of diploid nature of all genetic markers used.

Forty five linkage groups containing from two to ten markers have been identified by GROUP procedure and preliminary linkage map have been constructed. The longest LG has total length of 79cM. Ninety four markers (83 AFLP and 11 microsatellite DNA) did not show linkage to any other marker and were not assigned to any of the linkage groups.



Draft on Carp linkage map. Only groups, containing 3 and more markers are shown.
Additional 20 linkage groups, containing duplets, are not shown.

2. Dissemination and use

Appendix 1 – Plan for using and disseminating the knowledge

Section 1 – Exploitable knowledge and its use

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
New fish-breeds product with improved consumer qualities	*Special strains of carp*	*1. Fish-farming* *2. Science* *3. Selective breeding*	*2009-2010* *2009-2010* *2009-2010*	*Know-how*	***All consortium***
1 generation of Carp-specific genetic markers and map	*Marker-assisted selection*	*1. Aquaculture, carp strain selection*	*2008*	*2008*	***All consortium***
Breeding plan for common carp	*Report*	*1. Fish-farming* *2. Selective breeding*	*2009-2010*		***All consortium***
Genetic parameters for production and disease resistance	*2 Scientific papers*	*1. Fish-farming* *2. Science* *3. Selective breeding*	*2009-2010*		***All consortium***
Use of gene expression data for predicting continuous phenotypes	*Statistical method*	*1. Fish-farming* *2. Science* *3. Selective breeding*			***All consortium***

- What the exploitable result is (functionality, purpose, innovation etc.);
  Second generation genetic map that will enable more rapid genetic improvement of carp. Identification of possible candidate genes with important impacts on commercial traits.

  Development of new fish-breeds with improved customer qualities

  Breeding plan for common carp

  Genetic parameters for production and disease resistance

  Use of gene expression data for predicting continuous phenotypes
- Partner(s) involved in the exploitation, role and activities

  All consortium partners

NACEE (Network of Aquaculture Centers in Central and Eastern Europe),
Rosrybkhoz,
Russian Association of Agricultural Farmers,
Association of Fishing Cooperative Farms of Russia
Akvaforsk Genetics Center
Carp breeders

- How the result might be exploited (products, processes) - directly (spin offs etc) or indirectly (licensing) – on an individual basis or as a consortium/group of partners;
  It can be used both directly and indirectly on an individual basis or as a consortium/group of partners/Joint venture between private commercial actors and research institutes
  - any technical and economic market considerations – commercial and technical thresholds etc.
    In NACEE region production volumes of carp in the perspective will exceed 200000 tonnes including more than 100000 tonnes in Russia
  - any obstacles identified which might prove to be barriers to commercialization
    Limited number of carp fish farms, financial resources and lack of skilled staff
    - the existence or development of similar or competing technologies / solution elsewhere

    It is not known
    - third party rights (eg patents belonging to competitors), standards,…

    No
    - analysis of any (potential) non-technical obstacles

    No
  - any form of non-commercial use or impact, relating e.g. to the development of new standards or policies
    Yes
- Further additional research and development work, including need for further collaboration and who they may be

  For implementing disease resistance in the breeding program, there is a need for confirming the high heritability for KHV resistance and potential for selection response by breeding broodfish from high resistant families (or if possible survivors from a challenge test in these resistant families) to see if the offsprings really are more resistant to KHV.

- Intellectual Property Rights protection measures (patents, design rights, database rights, plant varieties, etc – include references and details)

  Database rights

- Any commercial contacts already taken, demonstrations given to potential licensees and/or investors and any comments received (market requirements, potential etc.)

  Database, promoting of planning results on the Project among potential customers

Where possible, also include any other potential impact from the exploitation of the result (socio-economic impact).

Assuming a realized selection intensity of 1 (implying 15-20% selected), a genetic gain of minimum 150 g higher body weights at harvest (2^{nd} autumn), 9 %-units higher pond survival and 2-4 %-units higher resistance to KHV can be expected per generation. This gain will be accumulated for each generation of selection

Section 2 – Dissemination and use

Overview table

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	Seminars				
April, 2007, Moscow	*Seminars: "About the tasks of the administrative areas of the Russian Federation on realization the National project in the field of aquaculture";*	*Research*	NACEE	*60 persons*	*Ministry of Agriculture of Russia*
June, 2007, Moscow	*Pedigree fish-breeding is an important part of aquaculture direction in the priority national project "Development of the agroindustrial complex"*	*Research*	NACEE	*200 persons*	*Ministry of Agriculture of Russia*
May, 2007, Stavropol	*Aquaculture in the priority national project "Development of Agroindustrial Complex"*	*Heads and specialists of fish-breeding farms*	NACEE	*260 persons*	*Association Rosrybkhoz*
April, 2007, Moscow	*"The Role of Machinery of Government of Agriculture of the regions of the Russian Federation in Aquaculture Development"*	*Heads of machinery of government of agriculture of 60 administrative areas of Russia*	Worldwide	*95 persons*	*Ministry of Agriculture of Russia*
April, 2008, Moscow	*International seminar "Molecular-Genetic Methods in Aquaculture"*	*Scientists and specialists in the field of fish genetics and selection*	NACEE	*86*	*EAS, with participation of EC specialists*
August, 2008, Saint-Petersburg	*"Pedigree Fish-Breeding: status, problems and ways for Perfection"*	*Heads and specialists of pedigree fish-breeding farms, scientists-selectionists*	NACEE, countries of the Caucasus	*60 persons*	*Ministry of Agriculture of Russia, Rosrybkhoz Association*
September, 2009, Sochi	*Scientific-practical seminar on pedigree fish-breeding)*	*Heads and specialists of pedigree fish-breeding farms, Heads of Departments of pedigree cattle-breeding of administrative areas of Russia and other countries of Eastern Europe*	Norway	*160 persons*	*Ministry of Agriculture of Russia, Rosrybkhoz Association*
			USA		
	Animal Breeding seminar at Dep. Animal and		Canada	*15*	*Dep. Animal and Aquaculture Sci., UMB,*

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
May 2008, Ås, Norway	*Aquaculture Sci., Norwegian Univ. Life Sciences (UMB).*	*Animal breeders and researchers*			*Nofima Marin*
November 2008	*Research Seminar*	*Stanford University Martine Laboratory, Pacific Groves, CA*		*40 persons*	*Stanford University*
November 2008	*Research Seminar*	*School of Biological Sciences, University of British Columbia*		*60 persons*	*University of British Columbia*
	Conferences				
September 2005	*Koi herpesvirus workshop at the 12th international Conference of the EAFP, Copenhagen*	*Research and Policy makers/advisors*	Worldwide	*75*	*Cefas*
May, 2006	*The 30th Annual Meeting on Fisheries Research, Szarvas, Hungary*	*Researchers, farmers stake holders*	Hungary	*170*	*HAKI*
September, 2006, Astrakhan	*"Problems and perspectives of aquaculture development in Russia"*	*Research*	NACEE	*95*	*Government of the Russian Federation*
February, 2007	*"Fishery" Conference, Belgrade, Serbia*	*Researchers, farmers stake holders*	Serbia, Norway	*80*	*Akvaforsk, Norway Faculty of Agriculture, University of Belgrade, Serbia*
April, 2007	*Keynote conference presentation (Experimental Biology 2007)*	*Academic research*	Worldewide	*100*	*UoL*
May, 2007	*The 31st Annual Meeting on Fisheries Research, Szarvas, Hungary*	*Researchers, farmers stake holders*	Hungary	*180*	*HAKI*
May, 2007, Stavropol krai	*"Aquaculture in the priority National project "Development of agroindustrial complex"*	Research	NACEE	*100*	*Ministry of Agriculture of Russia*
June, 2007	*Oral presentation at Fish Breeders' Roundtable, Ålesund, Norway*	*Researchers, farmers, breeders, stake holders*	Worldwide	*50*	*Akvaforsk (Nofima)*
August, 2007	*Conference of World Aquaculture Science*	*Research*	Worldwide	*2000*	*HAKI*
September 2007	*Koi herpesvirus workshop held at the 13th International Conference of the EAFP, Grado, Italy,*	*Research and Policy makers/advisors*	Worldwide	*100*	*Cefas*

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
October, 2007	*Plenary conference presentation*	*Academic reaserch*	China	*500*	*UoL*
April 2008	*International Mini-Symposium, Molecular Genetic Methods in Aquaculture. VNIRO, Moscow, Russia*	*Research and Policy makers/advisors*	*Worldwide*	*86*	*Cefas*
May, 2008	*The 32nd Annual Meeting on Fisheries Research, Szarvas, Hungary ()*	*Researchers, farmers stake holders*	Hungary	*150*	*HAKI*
July, 2008	*Poster at XX congeress of Genetics, Berlin*	*Researchers*	Worldwide	*3000*	*VNIRO*
October, 2008, Pushchino - Moscow)	*Tthe 5th International Symposium "EC-RUSSIA; COOPERATION IN THE FIELD OF BIODIVERSITY, AGRICULTURE, FORESTRY, FISH INDUSTRY AND FOOD IN EC 7TH FRAMEWORK PROGRAM", Section "Fish Industry and Aquaculture"*	*Heads, specialists and scientists in the fiels of fish industry and aquaculture*	NACEE, EC	*65 persons*	*Together with EC*
May 14-15, 2008	*The 32nd Annual Meeting on Fisheries Research, Szarvas, Hungary ()*	*Researchers, farmers stake holders*	Hungary	*170 person*	*HAKI*
August 2008	*Oral presentation at Skretting Australasian Aquaculture, Brisbane, Australia*	*Researchers, farmers, stake holders*	Australia, New Zealand, Asia, Pacific	*100*	*Nofima Marin*
September 2008	*Oral presentation at Fish Breeders' Roundtable, Ålesund, Norway*	*Researchers, farmers stake holders*	Worldwide	*50*	*AKVAFORSK*
	Oral presentation at Aquaculture Europe 2008, Krakow, Poland	*Researchers, farmers, breeders, stake holders*	Worldwide		*EAS, Polish Fisheries Association,. AKVAFORSK*
September 2009	*14th International Conference of the EAFP, Prague*	*Research and Policy makers/advisors*	Worldwide	*300*	*Cefas, HAKI*
	Exhibitions				
October, 2006, Moscow	*All-Russian Agroindustrial Exhibition "Gold Autumn-2006"*	*Heads and specialists of fish-breeding and farmers` farms, scientists, teachers of colleges and universities, students*	NACEE, Europe	*500-600 Persons*	*Ministry of Agriculture of Russia*
October 2007, Moscow	*All-Russian Agroindustrial Exhibition "Gold Autumn-2007"*	*Heads and specialists of fish-breeding and farmers` farms, scientists, teachers of colleges and universities, students*	NACEE, Europe Eastern Europe	*500-600 Persons*	*Ministry of Agriculture of Russia*
April, 2008,	*Agrofarm-2008*		NACEE, Europe		

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
Moscow *October 2008, Moscow* *October 2009, Moscow*	*All-Russian Agroindustrial Exhibition "Gold Autumn-2008"* *All-Russian Agroindustrial Exhibition "Gold Autumn-2009"*	*Heads and specialists of fish-breeding farms* *Heads and specialists of fish-breeding and farmers` farms, scientists, teachers of colleges and universities, students*  *Heads and specialists of fish-breeding and farmers` farms, scientists, teachers of colleges and universities, students*	NACEE, Europe	*150-200 persons* *500-600 Persons* *More than 600 persons*	*Ministry of Agriculture of Russia* *Ministry of Agriculture of Russia* *Ministry of Agriculture of Russia*
	Publications				
2006	*Haenen, O. and Hedrick, R. (2006). Koi herpesvirus workshop. Bulletin of the European Association of Fish Pathologists, **26**: 26-37.*	*Research and Policy makers/advisors*	Worldwide	*1000 persons*	*Cefas*
	Reference Book «Pedigree Fish-Breeding Farms of the Russian Federation»	*Industry, General public*	NACEE	*900-1000 persons*	
2007	*Stress and Disease Resistant Common Carp. In: The way to success. Hungarian coordinators in the EU 6th Framework Programme, p. 13-14.*	*General Public*	Hungary	*200*	*HAKI*
2007	*Annual report of OIE Reference Laboratories and Collaborating centres: Koi Herpesvirus*	*Research and Policy makers/advisors*	Worldwide	*1000 persons*	*Cefas*
2008	*Annual report of OIE Reference Laboratories and Collaborating centres: Koi Herpesvirus*	*Research and Policy makers/advisors*	Worldwide	*1000 persons*	*Cefas*
2008	*Catalogue of Carp Breeds of the countries of Central and Eastern Europe*	*Industry, research, general public*	Worldwide	*1000 persons*	*EC, Ministry of Agriculture of Russia*
September-October, 2008	*Virus-resistant carp the objective of European project.* Hatchery International	*Industry, research, general public*	Worldwide		*Cefas*
March-April, 2008	*Common Carp: Selection and Development of Different European Breeds.* Hatchery International	*Industry, research, general public*	Worldwide	*1000 persons*	*HAKI, FCFGS*
2008:	*Common Carp: Selection Genomic resources and microarrays for the*	*Research*	Worldwide	*1000 persons*	*UoL, UoS, HAKI, Cefas*

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	common carp (Cyprinus carpio L.). J. of Fish Biology, *72:1-23*				
2008	*Modelling the use of gene expression profiles with selective breeding for improved disease resistance in Atlantic salmon (Salmo salar).* Aquaculture: 285: 38-46	*Research*	Worldwide	*1000 person*	*Nofima Marin*
2008	*Use of gene expression data for predicting continuous phenotypes for animal production and breeding.* Animal Breeding, : 2:, 1413-1420	*Research*	Worldwide	*1000 person*	*Nofima Marin*
2009	*Genetic parameters and heterosis estimates for growth traits and pond survival in four Hungarian carp strains.* Aquaculture	*Research*	Worldwide	*1000 person*	*Nofima Marin, HAKI*
2009	*Genetic parameters for pond survival and resistance to Koi herpesvirus and Aeromonas hydrophila in common carp (Cyprinus carpio).* Aquaculture	*Research*	Worldwide	*1000 person*	*Nofima Marin, HAKI, CEFAS*
2009	*Breeding plan for common carp in Europe. Nofima report*	*Industry, research*	Worldwide	*50 persons*	*Nofima Marin*
2009	*Comparison of the resistance of selected families of common carp, Cyprinus carpio (L.), to koi herpesvirus: preliminary study. Submitted to Journal of Fish Diseases*	*Research*	Worldwide	*1000 person*	*Cefas, HAKI*
	Web site				
2006	*Lab informatics website (http://legr.li.ac.uk)*	*Academic research*	Worldwide	*Not known*	*UoL*
2008	*CarpBASE 7.1 website http://www.agf.liv.ac.uk/cb7pathways/*	*Academic research*	Worldwide	*Not known*	*UoL*
2006	*Project website (http://haki.hu/eurocarp)*	*Industry, research, general public*	Worldwide	*Not known*	*HAKI*

Section 3 - Publishable results

1. Ten carp families were found the most resistant against KHV (Koi Herpes Virus). The heritability estimate for KHV was sensitive to trait definition, but generally moderate to high. The heritabilities for the weight measures were also moderate to high. Both genetic and residual correlations between KHV and weights were close to zero.

2. Ten other carp families were identified the most resistant agains bacteria, *Aeromonas hydrophila.* The heritability estimated for the bacteria *A. hydrophila* was low whereas the genetic correlation between *A. hydrophila* and KHV were higher. Genetic and residual correlations between *A. hydrophila* and weights were low.

3. High heritabilities were estimated for harvest weight (ca 1 kg) and pond survival and suggest that considerable genetic gain can be obtained for production performance by selective breeding.

4. A method developed to use gene expression profiles for phenotype prediction can be applied and integrated in animal breeding programs for increasing genetic gain of disease resistance.

5.Gene sequences for all known carp transcripts have been collated into a searchable database, and the description of this collation has been published in the Journal of Fish Biology.

6. Latter phases of EUROCARP incorporated the results from an additional 'next-generation' sequence collation which has dramatically extended our classification of carp genes. This data has been co-assembled with the pre-existing EUROCARP collation and the resulting database is presented at http://www.agf.liv.ac.uk/cb7pathways/.