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PRELIMINARY EVIDENCE OF *PGM-2** ALLELE FREQUENCY CHANGES IN MASS REARED WHITEFISH (*Coregonus lavaretus* L.) LARVAE

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A B S T R A C T. Enzyme gene variability was studied at 6 and 12 week in whitefish (*Coregonus lavaretus*) juveniles fed with *Artemia* nauplii and with three dry diets. Six of the 12 loci screened were monomorphic: *GPI-B1**, *LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2** and *sMDH-A1**, and the remaining 6 loci: *GPI-B2**, *sMDH-A2**, *sMDH-B1,2**, *PGDH**, and *PGM-2**, were variable. Most of the variability was at *PGM-2**, and the variation was reduced from 6 to 12 weeks on all diets. Pooled 12-week sample showed significantly ($P<0.05$) lower variability at this locus than pooled 6-week sample. After six weeks of rearing the heterozygosity values ranged from 0.037 to 0.048, and after 12 weeks from 0.025 to 0.037. The loss of genetic variation from 6 to 12 weeks may indicate selection operating during the rearing process. There was no correlation between genetic variation and growth of whitefish juveniles reared in a hatchery.

Key words: ALLOZYMES, BIOCHEMICAL GENETICS, Coregonus lavaretus, ELECTROPHORESIS, LARVAL REARING

INTRODUCTION

European coregonids (*Coregonus spp.*) have lost much of their natural habitat due to water pollution and eutrophication. One of the ways to compensate for this damage is to catch spawners and to stock newly hatched or prefed larvae (Luczynski, 1986; Salojärvi, 1986; Rösch, 1988).

Producing and maintaining a fish stock that will live and reproduce in the wild, must take into account the preservation of its genetic variation (usually expressed as heterozygosity). For instance it is known that loss of genetic variation in brook trout spawners has harmful effects on development, survival and growth rate of their offspring (Liskauskas & Ferguson, 1991). Also, the alterations in the gene pool of hatchery reared fish may cause deterioration of native stocks (Allendorf & Ryman, 1987). Such unintended, detrimental impacts of hatchery technology to native salmonid populations are well documented (for example Hindar et al. (1991) and references therein).

In contrast with the detailed studies on biochemical, physiological and anatomical aspects of rearing coregonid larvae on live versus dry diets (Dabrowski, 1984; Hofer, 1985; Segner & Rösch, 1990; Segner et al., 1989), there have been no analyses of genetic properties of reared fish. To trace the possible effects of the rearing technique on the genetic characteristics of the larvae, their genetic variation was determined during the rearing process.

MATERIAL AND METHODS

Larvae of the nearshore spawning whitefish (*Coregonus lavaretus* L.) (local name: Gangfisch) from Lake Constance (Bodensee-Obersee) were obtained from Langenargen hatchery and were reared for 12 weeks on three different dry diets (Kyowa, Az45 and Az30 Tetra-diets) and on *Artemia* nauplii. Rearing was performed in 50 l tanks at a temperature of 12 ^{o} C and at a water flow rate of about 1 l min^{-1}. Details of the rearing facilities are given in Rösch & Dabrowski (1986). Dry diets were provided from 8 a.m. to 6 p.m. hourly using automatic feeders. *Artemia* nauplii were fed twice a day. Dry diets were given in excess. Due to technical reasons *Artemia* nauplii were not permanently offered to the larvae. Rearing trials Az45 and Az30 were performed in duplicate tanks.

Two successive samples (48 to 177 fish per sample) were taken after 6 and 12 weeks of rearing. The fish were frozen immediately and kept at -20 ^{o}C until electrophoretic analysis.

Allele frequency data were derived from the electrophoretic separation of the enzyme products of 12 genetic loci. Whole larvae or muscle tissues (usually a mixture of red and white muscle) were homogenized in Tris-EDTA buffer, pH 7.0 (Ferguson, 1980) and the crude homogenate was used for horizontal starch gel electrophoresis. Two buffer systems were used: (A) a discontinuous lithium hydroxide-boric acid buffer, pH 8.1 (Ridgway et al., 1970) and (B) an N-(3-aminopropyl)-morpholine-citrate buffer pH 6.1 (Clayton & Tretiak, 1972). Electrophoresis and staining procedures followed Vuorinen (1984). The enzymes studied and the buffers used are given in Table 1. Locus and allele nomenclature follows that of Shaklee et al. (1990) and relative mobilities of the alleles and loci designations are according to Vuorinen (1984), Vuorinen & Piironen (1984) and Bodaly et al. (1991).

TABLE 1

Studied enzymes with their Enzyme Commission numbers, designation of loci, and buffers used.

Enzyme name	E.C. No.	Loci designation	Buffer
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-B1,2**	A
Lactate dehydrogenase	1.1.1.27	*LDH-A1,2**	A
		*LDH-B1,2**	A
Malate dehydrogenase	1.1.1.37	*sMDH-A1,2**	B
		*sMDH-B1,2**	B
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	B
Phosphoglucomutase	5.4.2.2	*PGM-2**	B

Allele frequencies were based on direct allele counts except for the duplicate *sMDH-B1,2**, which was treated as the two loci with equal allele frequencies. Chi-square tests were performed to evaluate deviations from Hardy-Weinberg equilibrium. Mean heterozygosity was calculated for each sample. All calculations were made by using the program BIOSYS-1 (release 1.7) of Swofford & Selander (1989).

RESULTS

GROWTH AND SURVIVAL OF THE LARVAE

During the first 6 weeks of rearing mortality ranged from 12 to 41% among diets, but during the next 6 weeks mortality was less than 15% (Table 2). Fish reared on Kyowa diet had the highest final weight and length; the remaining groups were similar in their body length but differed in their weight (Table 2).

GENETIC VARIABILITY

Insufficient activity of enzymes in newly hatched larvae prevented assessing of their genetic variability.

The following 6 loci showed no variability: *GPI-B1**, *LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2** and *sMDH-A1**. Locus *PGDH** was also monomorphic but one *PGDH*100/90* heterozygote was found among 1047 fish. The remaining 5 loci, *GPI-B2**, *sMDH-A2**, *sMDH-B1,2** and *PGM-2**, were polymorphic.

TABLE 2

Cumulative mortality (% of the initial number of fish), total body length (TL, mm; standard deviation in parentheses) and dry body weight (mg) in whitefish larvae fed with different diets (n.d.= no data).

Diet	Initial No. of fish	Mortality (%)		TL (mm)	Dry weight (mg)	
		0-6th week	6-12th week	12 weeks	6 weeks	12 weeks
Kyowa	2000	12.0	10.1	46.2 (4.4)	8.7	104.9
Artemia	2000	22.0	2.0	38.6 (3.6)	7.7	41.8
Az45 (I)	3000	18.8	10.0	38.0 (6.8)	n.d.	55.6
Az45 (II)	3000	29.7	8.4	39.5 (7.8)	n.d.	91.2
Az30 (I)	3000	41.0	5.5	40.6 (7.1)	7.4	62.6
Az30 (II)	3000	41.0	15.0	42.7 (6.7)	7.4	78.4

TABLE 3

Number of fish examined (N), allele frequencies, heterozygosity at *PGM-2** locus (HL, direct count estimate) and mean heterozygosity (H) in whitefish fingerlings fed with different diets. The minor alleles at loci segregating only for two alleles are: *sMDH-A1,2*50*, *sMDH-B1,2*100*. In addition, one *PGDH *100/90* heterozygote was observed in the sample Az30 (II) after 12 weeks of rearing. H-values are based on 12 loci.

Population	N	Allele frequencies								HL	H (%)
		*GPI-B1,2**			*sMDH-A1,2**	*sMDH-B1,2**	*PGM-2**				
		**100*	**135*	**50*	**100*	**120*	**-100*	**-125*	**-155*		
6 WEEKS											
Kyowa	48	1.000			0.990	0.948	0.781	0.219		0.313	4.5
Artemia	50	0.990	0.010		0.990	0.975	0.810	0.190		0.340	4.0
Az45 (I)	58	0.991	0.009		0.991	0.948	0.793	0.207		0.276	4.3
Az45 (II)	65	0.992	0.008		0.992	0.961	0.805	0.195		0.391	4.8
Az30 (I)	57	1.000			0.982	0.961	0.833	0.167		0.263	3.8
Az30 (II)	52	1.000			0.990	0.971	0.827	0.173		0.308	3.7
Total after 6 weeks	330	0.995	0.005		0.989	0.960	0.809	0.191		0.316	4.1
12 WEEKS											
Kyowa	116	1.000			0.991	0.968	0.849	0.147	0.004	0.241	3.2
Artemia	75	1.000			0.980	0.963	0.833	0.167		0.253	3.7
Az45 (I)	125	1.000			0.996	0.970	0.848	0.148	0.004	0.272	3.3
Az45 (II)	103	0.995	0.005		0.990	0.968	0.830	0.165	0.005	0.282	3.6
Az30 (I)	121	1.000			0.996	0.957	0.909	0.091		0.149	2.6
Az30 (II)	177	0.992	0.006	0.003	0.983	0.983	0.886	0.114		0.170	2.5
Total after 12 weeks	717	0.997	0.002	0.001	0.990	0.969	0.864	0.134	0.002	0.221	3.2

Table 3 summarizes the amount of genetic variation in different trials after 6 and 12 weeks of rearing. Greater number of fish examined in 12-week samples enabled detection of the rare *PGM-2*-155* allele. On all diets the larvae showed less genetic variation after 12 weeks compared to six weeks of rearing. The fish fed with the diet Az30 were least variable.

Most of the variability was at *PGM-2**, where the frequency of the variant allele was greater than 0.050 in all samples. The variability at *PGM-2** was reduced from 6 to 12 weeks on all diets (Table 3). The phenotypic proportions did not differ significantly from Hardy-Weinberg expectations except for the sample Az30 (II) after 12 weeks of rearing. Contingency table analysis (Table 4) revealed that the genetic variation after 12 weeks of rearing did not differ significantly from respective values after 6 weeks, except for the diet Az30 (I). However, pooled 12-week sample had significantly lower variability than pooled 6-week sample (Table 4).

DISCUSSION

Growth rate and mortality of reared whitefish larvae remained within the ranges usually reported for experimental rearing of coregonid larvae (Champigneulle, 1988; Champigneulle & Rojas-Beltran, 1990); good growth and low mortality for the Kyowa group confirmed the results reported by Harris and Hulsman (1991).

Genetic variation among Gangfisch was low and confirmed the results previously reported for Lake Constance whitefish; the heterozygosity values (2.5-4.8 %) were similar to those reported earlier by Vuorinen et al. (1.5 %) and Hecht et al. (5.6 %).

Four of the eight variant alleles (*GPI-B2*50*, *GPI-B2*135*, *sMDH-A2*50* and *PGDH*90*) were not reported by Vuorinen et al. (1.5; 1986) for Lake Constance population. *PGDH*90* allele has been found in two North American coregonids, *Coregonus sardinella* and *Stenodus leucichthys* (Bodaly et al., 1991), also in one population of European whitefish (J. Vuorinen, University of Joensuu, Finland, personal communication).

The loss of genetic variation from 6 to 12-week old fish may indicate an erosion of the gene pool of whitefish larvae fed with dry diets. On the other hand, it could be expected that subjecting whitefish fingerlings to such environmental factors as crowding, dry diet, etc. should rather increase their heterozygosity enhancing the adaptability of the population to the rearing conditions. For instance, Liskauskas & Ferguson (1991) studying allele frequencies and the number of heterozygous loci per individual

TABLE 4

Contingency table analysis of the homogeneity of allele frequencies at *PGM-2** locus (5% criterion) between the respective samples of whitefish fingerlings reared for 6 and 12 weeks and fed on different diets. For the tests the rare **-155* allele was pooled with the common **-100*.

Diet	*PGM-2**	
	χ^2	P
Kyowa	2.536	0.11127
Artemia	0.225	0.63508
Az45 (I)	1.979	0.15950
Az45 (II)	0.497	0.48075
Az30 (I)	4.364	0.03670
Az30 (II)	2.555	0.10994
Pooled samples reared for 6 weeks versus pooled samples reared for 12 weeks	11.519	0.00069

in young of the year (YOY) cohort of *Salvelinus fontinalis* have found that YOY sampled in June (exogenous feeding of fish) had significantly more heterozygous loci than YOY sampled in May (yolk-sac stage). A similar example of the selective advantage of heterozygosity has been reported by Ferguson & Drahushchak (1990), who showed that more heterozygous rainbow trout had superior disease resistance over the less heterozygous fish.

The data in Table 3 show that the loss of genetic variation was mostly due to the reduction of genetic variability at *PGM-2** locus. It can be postulated that the selection may either directly affect *PGM-2** locus or some other locus (or loci) closely linked with it. Further studies on genetic, physiological and behavioural aspects of rearing of coregonid larvae and juveniles are still needed to validate these hypotheses.

There was no correlation between genetic variation and growth (Table 2 versus Table 3), what suggests that genetic changes at loci coding for electrophoretically screened enzymes may not contribute to the genes controlling the growth of whitefish larvae and fingerlings (Kimura, 1983).

Little is known on how the observed genetic changes could contribute to genetic diversity of the wild coregonid stocks. Several studies with natural populations of other fish species (Philipp et al., 1985; DiMichele & Powers, 1991) have shown a highly significant correlation between the allelic frequencies at several enzyme loci and different variables, such as latitude, length of growing season, developmental rate, thermal tolerance of developing fish, etc. In this regard the loss of genetic variation is generally expected to reduce the ability of populations to adapt to altered environmental conditions (Allendorf & Leary, 1986; Liskauskas & Ferguson, 1991).

Vuorinen et al. (1986) have shown that coregonids inhabiting subalpine lakes are rather uniform biochemically and maintain unusually high genetic variation at *PGM-2** locus in comparison with other European whitefish populations. It seems likely that variation at *PGM-2** locus alone or in association with other loci (Gauldie, 1984) constitutes an adaptive feature of coregonid populations to unique environmental conditions of subalpine lakes. As several millions of prefed coregonid larvae are stocked yearly into Lake Constance (IBK, 1991), it can be postulated that the planted fish may affect the natural population by decreasing its original genetic variation. As so far the stocking efficiency of coregonids in Lake Constance is still under discussion (Eckmann et al., 1988; Hartmann, 1990), this presumption waits for its validation.

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STRESZCZENIE

OBSERWACJE ZMIAN FREKWENCJI ALLELI LOCUS *PGM-2** W GRUPACH LARW SIEI (*Coregonus lavaretus* L.) PODCHOWYWANYCH W BASENACH

Badano zmienność genetyczną narybku siei (*Coregonus Lavaretus*) po 6 i 12 tygodniach podchowu na nauplisach *Artemii* oraz trzech paszach sztucznych. Sześć spośród 12 badanych loci było monomorficznych (*GPI-B1**, *LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**, *sMDH-A1**), a w pozostałych - sześciu loci: *GPI-B2**, *sMDH-A2**, *sMDH-B1,2**, *PGDH**, *PGM2**, zanotowano zmienność. Najbardziej polimorficznym locus był *PGM-2**. W locus tym zanotowano istotny ($P<0,05$) spadek zmienności genetycznej we wszystkich grupach eksperymentalnych między 6 a 12 tygodniem podchowu.

Heterozygotyczność po 6 tygodniach podchowu wahała się między 0,037 a 0,048, a po 12 tygodniach między 0,025 do 0,037. Utrata zróżnicowania genetycznego między 6 a 12 tygodniem może wskazywać na działanie procesu selekcji podczas podchowu larw siei w warunkach podchowu basenowego. Nie zanotowano korelacji między zmiennością genetyczną i wzrostem podchowywanego narybku siei.

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