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POPULATION GENETIC STRUCTURE OF WRASSES USED AS CLEANERFISH IN ATLANTIC SALMON FARMING IN NORWAY

by

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ABSTRACT

The increasing use of wrasses as cleaner fish in the salmon farming industry has created local fishery in many regions in Norway. In several cases, live fish have been transported between regions without knowledge of the population structure of the three species in question (goldsinny, *Ctenolabrus rupestris*, rock cook, *Crenolabrus exoletus*, and corkwing, *Symphodus melops*). Genetic studies based on variation in proteins were carried out, and polymorphic enzymes of potential use in popoulation studies were identified for all three species. For the most common wrasse the goldsinny, four loci (*SDH-1*; PGM-1*; GPI-1* and GPI-2**) were used to study genetic variation between geographic regions. Five samples collected in the southern part of Norway were compared and significant variation in allele frequencies were detected for *SDH-1** and *PGM-1**. The variation seemed to be associated with specific fjord - coastal area distribution.

INTRODUCTION

The use of chemicals in salmon-lice treatment is causing health hazard to the farmers and pollution in the marine environment. In addition, traditional treatment reduce growth of fish and thus influence the economics of the industry.

During the last years, there has been an increasing interest in application of wrasses in the Norwegian fish farming industry (Bjordal and Kårdal, 1989). In 1992 about 280 000 individual fish were used by the industry. The number is expected to increase in the future.

The need for live wrasses in the salmon farming industry, has developed a local fishery in many regions. The abundance of wrasses along the coast is unknown, and there are transportation of fish between different regions. At present, the population structure of the different species is unknown. The question of local stocks of wrasses is important and the future management of wrasse resources should be based on genetic information.

In this contribution, we describe the preliminary results from a study where polymorphic proteins in the different species have been identified. Further, comparison of allele frequencies between samples from five different geographic regions is presented.

MATERIALS AND METHODS

The initial screening of enzymes to identify polymorphic enzymes were carried out on a sample of wrasses collected in the Bergen area. At this time the fish were found at about 20m of depth and were caught with gillnets (12.5/16.5mm mesh size) and traps.

During May and June, samples of wrasses were collected in collaboration with local fishermen. The different sampling localities are given in Fig.1.

The fish were frozen immediately and transported to the laboratory in Bergen and stored at minus 70°C until analyses. Initially, 5 different tissues were sampled from each fish and 28 different proteins were analysed by horizontal starch gel electrophoresis and isoeletric focusing (Tab.1).

Starch gel (12%) and the following buffers were used: a) histidine, pH=7.0 b) TCB: tris-citrate-borate, pH=8.6 (Skaala and Jørstad, 1987) c) AM: citrate aminomorpholine, pH=6.1. The different enzymes were stained as described by Brewer (1970) and Harris and Hopkinson (1976).

The BIOSYS-1 program package (Swofford and Selander, 1981) was used in the statistical comparison of the different samples.

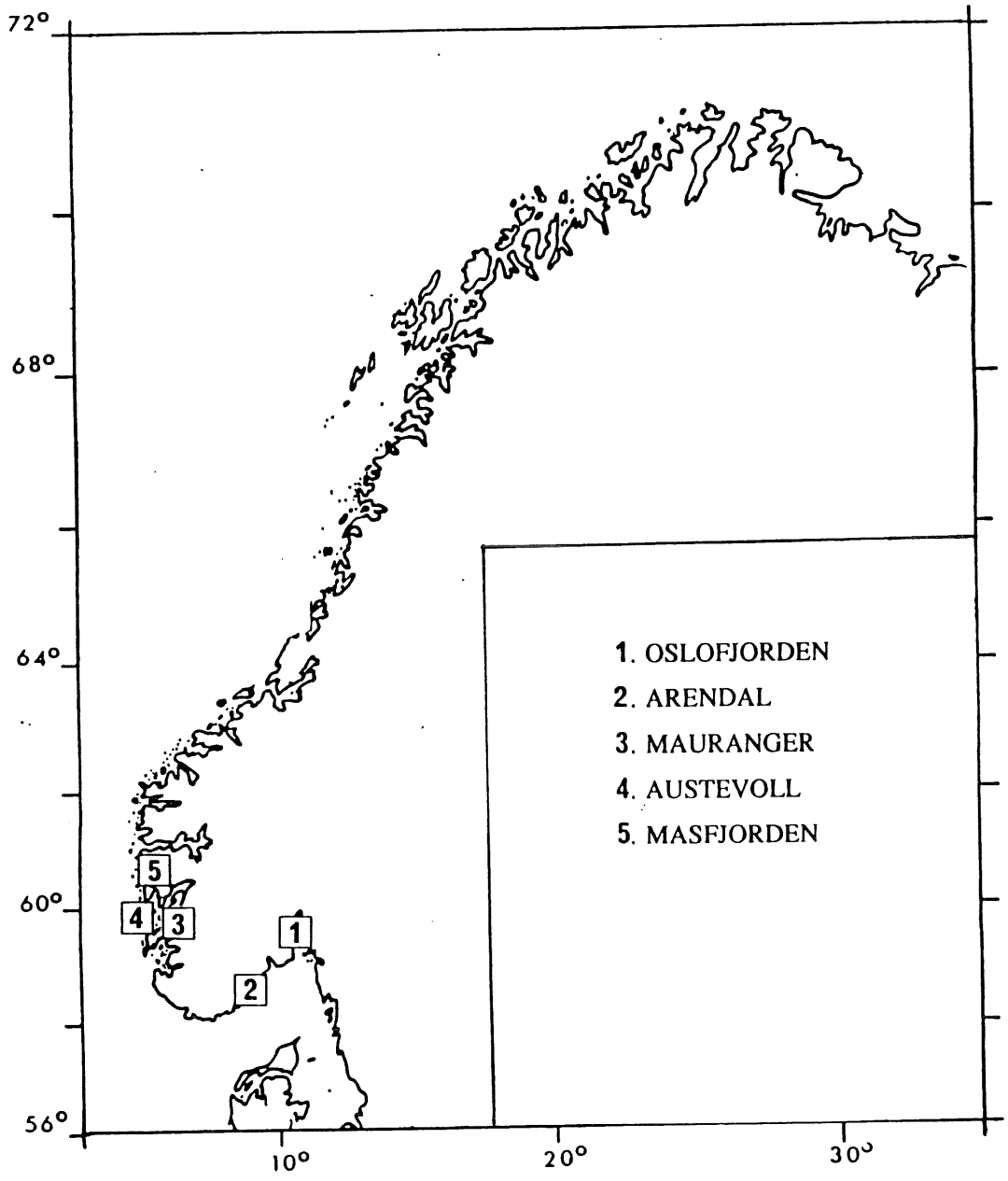

Figure 1. Sampling localities.

RESULTS

Description of polymorphic enzymes

Goldsinny

The 28 different proteins studied in samples of goldsinny are given in Table 1. Seven enzymes were stained weakly under the conditions used. Individual variation was detected in five different loci which accounts for 15% of the tentative loci investigated. The polymorphic loci found are discussed below.

*SDH-1**

This enzyme was only detected in liver, only isoelectric focusing gave the resolution needed for this study. Heterozygotes are identified by a five banded pattern suggesting a tetramer protein structure (Fig 2). Five different alleles were found in the samples analyses.

*PGM-1**

High enzyme activity was detected in white muscle, liver, eye and heart. Optimal separation of 6 different alleles was obtained in starch gel electrophoresis using histidine buffer (p=7.0). As in other species, the enzyme is a monomer giving two band for the heterozygote on the gels (Fig. 3).

*GPI-1**

White muscle and heart were the tissue with highest activity. Heterozygote were identified by a three banding pattern which is according to dimer structure of active enzyme. In the total material analyses, 5 different alleles were found, and the starch gel with histidine buffer gave sufficient separation.

*GPI-2**

This enzyme was strongly expressed in white muscle, liver, eye and heart and as for *GPI-1** the active enzyme has a dimer protein structure. Three different alleles were detected, as shown in Fig. 4.

EST

Enzyme activity was detected in all the different tissue extracts tested ,and varied between individuals. In the present material it was, however, not possible to work out a model which explained the variation observed.

Figure 2. Picture of gel stained for sorbitol dehydrogenase (SDH)

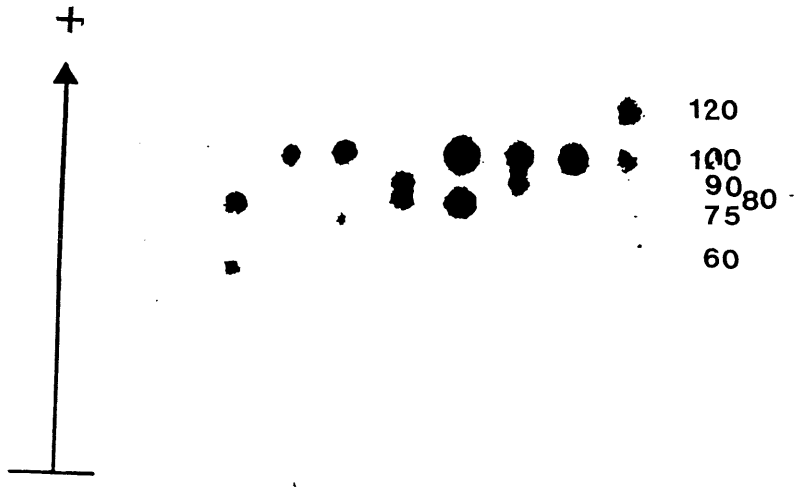

Figure 3. Picture of gel stained for Phosphoglucomutase (PGM)

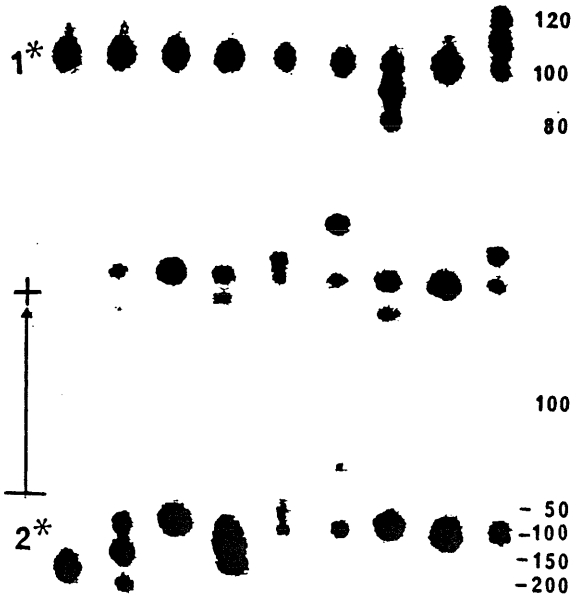

Figure 4. Picture of gel stained for Glucose phosphate isomerase (GPI)

Table 1. Account of proteins tested for Goldsinny *Ctenolabrus rupestris*, from western Norway, EC number, abbreviations used in the text, number of alleles, tissue (muscle, liver, eye, heart and blood) and optimal electrophoretic method. + = strong staining, - = no staining, w = week staining, var. = variation. ? = insufficient resolution. IE = isoelectric focusing A = Histidin p 7.0, B = TCB p 8.6

PROTEINS	EC no.	NUMB. LOCI	ALLELES	TISSUE M L E H B	BUFFER
Adenosine deaminase	3.5.4.4.	*ADA**	?	- - - - -	IEF
Adenylate kinase	2.7.4.3.	*AK-1**	?	+ w - w -	IEF
		*AK-2**	?	- + - - -	IEF
Alcaline phosphatase	3.1.3.1.	*ALP**	?	- w - - -	IEF
Alcohol dehydrogenase	1.1.1.1.	*ADH-1**	1	- + - - -	IEF
		*ADH-2**	1	w w + w -	IEF
Aldolase	4.1.2 13.	*ALD-1**	1	+ - - - -	A
		*ALD-2**	1	- w w + -	A
		*ALD-3**	1	- + - - -	A
Aspartate aminotransferase	2.6.1.1.	*AAT-1**	1	- + w - -	B
		*AAT-2**	1	- + w - -	B
		*AAT-3**	1	+ w + + -	B
Creatine phosphokinase	2.7.3.2.2	*CPK-1**	1	+ - w w -	IEF
		*CPK-2**	1	+ - - - -	IEF
Diaphorase	1.6.2.2.	*DIA**	?	- - - - -	IEF
Esterase	3.1.1.1.	*EST '*	var.	- + - + -	IEF
Fructose-1.6-diphosphatase	3.1.3.11.	*FDP**	1	- + - - -	IEF
Glucose phosphate isomerase	5.3.1.9.	*GPI-1**	5	+ w w + -	A
		*GPI-2**	3	w + + + -	A
Glutamate dehydrogenase	1.4.1.3.	*GDH**	2	- w w + -	A
Glyceraldehyd-3-PDH	1.2.1.12.	*GAP-1**	1	+ - - - -	A
		*GAP-2**	1	- + - w -	A
α-Glyserophosphate dehydrogenase	1.1.1.8.	*GPD-1 **	1	- + - - -	A
Hexokinase	2.7.1.1.	*HK-1**	1	- + - - -	A
		*HK-2**	?	- + - - -	A
Isocitric dehydrogenase	1.1.1.42.	*IDH-1**	1	w - w + -	B
		*IDH-2**	1	- + - - -	B
Lactate dehydrogenase	1.1.1.27.	*LDH-1**	1	+ - - - -	A
		*LDH-2**	1	+ + w + -	A
		*LDH-3**	1	- - + - -	A
Mannose phosphate isomerase	5.3.1.8.	*MPI**	?	- - - - -	IEF
Malate dehydrogenase	1.1.1.37.	*MDH-1**	1	- - + - -	IEF
		*MDH-1**		+ + + w -	IEF
		*MDH-3**	1	w + + + -	IEF
Malic enzyme	1.1.1.40.	*ME**	1	- + - - -	B
Phosphoglucomutase	2.7.5.1.	*PGM-1**	6	+ + + + w	A
		*PGM-2**	?	- w w - -	A
Phosphogluconate dehydrogenase	1.1.1.44.	*PGD**	1	- + - w +	A
Purine nucleosidphosphorylase	2.4.2.1.	*NP**	?	- - - - -	IEF
Sorbitol dehydrogenase	1.1.1.44.	*SDH-1**	5	- + - - -	IEF
		*SDH-2**	1	- + - - -	IEF
Superoxide dismutase	1.15.1.1.	*SOD-1**	1	- w - + w	IEF
		*SOD-2**	1	- + - - -	IEF
Xanthine dehydrogenase	1.2.1.37.	*XDH**	1	- + - - -	A
Hemoglobin	--	HEM		no var.	IEF
Serum protein	--	SP		no var.	IEF

Rock cook and Corkwing

A limited number of individuals (35; 25) for these two species were also screened for some of the same proteins and the results are summarized in Table 2.

For the Rock cook three loci were found polymorphic and variation was also observed for esterase. In all cases, starch gel with histidine gel buffer (p=7.0) gave sufficient separation. For *CPK-2** highest activity was found in eye tissue and heterozygote with 2 bands suggests a dimeric structure. Only 2 different alleles were detected. White muscle and heart have high activities for *IDH-1** and heterozygotes with 3 bands (dimeric structure) were detected. Two different alleles were observed.

With respect to corkwing, little variation was seen. Esterase staining suggested individual variation but it was unclear if the variation was genetically controlled. One enzyme locus, *GPI-1**, demonstrated variation between individuals as heterozygote with a tree banded pattern were detected. Two different alleles were found for this loci. As for the other species, good separation was obtained using starch gel and histidine buffer.

Table 2. Account of proteins analyses in Rock cook *Crenolabrus exoletus*, and Corkwing, *Symphodus melops*, their E.C. numbers, abbreviations used in the text, number of alleles, staining intensity in respectively muscle (M), liver (L), eye (E), heart (H) and blood (B). (+ = strong staining, w = weak staining, - = no staining) and gel type used , var. = variation not understood (N = 35 and 25)

			ROCK COOK		CORKWING		
PROTEINS	E nr.	ANT. LOCi	ALLELE	TISSUE M L E H	NO. ALLELES	TISSUE M L E H	BUFFER
Adenylate kinase	2.7.4.3.	*AK-1**	1	+ - - -	1	+ - - -	IE
		*AK-2**	1	+ + + +	1	+ + + +	IE
		*AK-3**	1	- + - w	1	- + - w	IEF
Alcaline phosphatase	3.1.3.1.	*ALP**	?	- - - -	1?	- + - -	IEF
Aspartate aminotransferase	2.6.1.1.	*AAT-1**	1	w + - w	1	- w - -	B
		*AAT-2**	1	- + - -	1	- w w -	B
Creatine phosphokinase	2.7.3.2.2	*CPK-1**	1	+ - w w	1	+ - - -	IEF
		*CPK-2**	2	+ w + +	1	+ + + +	IEF
		*CPK-3**	1	- - + -	1	- - + -	IEF
Fructose-1.6-diphosphatase	3.1.3.11.	*FDP-1**	1?	- + - -	1?	+ - - -	IEF
		*FDP-2**	1?	- + - -	1?	- + - -	IEF
Glucose phosphate isomerase	5.3.1.9.	*GPI-1**	1	+ w w +	2	+ w w +	A
		*GPI-2**	1	w + + +	1	w + + +	A
Glutamate dehydrogenase	1.4.1.3.	*GDH**	?	- - - -	?	- - - -	A
Glyceraldehyd-3-PDH	1.2.1.12.	*GAP-1**	?	- - - -	?	- - - -	A
		*GAP-2**	?	- - - -	?	- - - -	A
α-Glyserophosphate dehydrogenase	1.1.1.8.	*GPD-1**	1	w + - w	1	w + + +	A
		*GPD-2**	1	- + - -	1	+ w w w	A
		*GPD-3**	1	+ - - w			A
Isocitric dehydrogenase	1.1.1.42.	*IDH-1**	2	+ - w +	1	+ - w +	B
		*IDH-2**	1	- + w +	1	- + w -	B
Lactate dehydrogenase	1.1.1.27.	*LDH-1**	1	+ - w w	1	+ - w w	A
		*LDH-2**	1	w w + +	1	+ w + +	A
		*LDH-3**	1	- - + -	1	- - + -	A
Mannose phosphate isomerase	5.3.1.8.	*MPI**	1	w + w +	1	w + w +	IEF
Malate dehydrogenase	1.1.1.37.	*MDH-1**	1	w + + +	1	w + + +	IEF
		*MDH-2**	1	+ w w w	1	+ - - w	IEF
Malic enzyme	1.1.1.40.	*ME**	1	w + w w	1?	w + - w	B
Phosphoglucomutase	2.7.5.1	*PGM-1**	2	+ + + +	1	+ + + +	A
		*PGM-2**	?	- + - w	?	- + - -	A
Sorbitol dehydrogenase	1.1.1.44.	*SDH-1**	1	- + - -	1	- + - -	IEF
Superoxide dismutase	1.15.1.1.	*SOD-2**	1	- + - -	1	- + - -	IEF
Xanthine dehydrogenase	1.2.1.37.	*XDH**	?	- - - -	?	- - - -	A

Geographic variation - Goldsinny

The sampling localities are distributed from Oslofjord in the south-eastern part of Norway to coastal and fjord areas in mid-western Norway (Fig. 1.). These samples were analyses for the polymorphic loci identified (see above).

The allele frequencies for the 4 polymorphic loci are summarized in Table 3. The distribution of geneotypes for all loci were in agreement with the expected Hardy-Weinberg's proportions. For two of the loci analysed, (*GPI-1*; GPI-2**), only rare variant alleles were found and there were no variation between localities. With respect to *PGM-1**, six different alleles were found and two of these were rare. The frequency of the most common allele (*PGM-1**) varied from 0.88 to 0.94 and a G-test for homogeneity based on the allele frequencies demonstrated significant variation (P=0.003; Tab. 3) in the material analyses. Pairwise statistical comparisons revealed significant differences between several of the sample collections. The sample from the inner part of Masfjord was significant different from all the other samples including the sample from Austevoll (G=15.2; P=0.002) and Mauranger (G=19.9; P=0.0003). Further, no differences were detected among the samples from the coastal area.

The results obtained for the *SDH-1** locus were also informative. The allele frequencies are given in Tab. 3 and a homogeneity test did not reveal significant variation. As seen in the table, however, the frequency of the most common allele (*SDH-1*100*) varied from 0.408 (Mauranger) and 0.53 (Austevoll) and a pairwise G-test demonstrated a significant difference (G=10.2; P=0.018).

Table 3. Allele frequencies for 4 polymorphic loci in goldsinny samples from the coast of Norway (Fig. 1). Results from G-test (for heterogenity), pooled material is also given for each enzyme.

	LOCALITY					
LOCUS	1	2	3	4	5	G-TEST
*SDH-1**						
(N)	129	132	76	83	130	
A	.496	.477	.408	.530	.504	
B	.074	.091	.138	.048	.088	
C	.345	.322	.362	.349	.319	G = 13.43
D	.081	.106	.092	.072	.088	df = 12
E	.004	.004	.000	.000	.000	P = 0.347
*PGM-1**						
(N)	144	144	114	144	144	
A	.003	.000	.000	.000	.000	
B	.042	.031	.044	.031	.003	
C	.042	.066	.018	.049	.056	
D	.024	.017	.022	.031	.003	G = 30.133
E	.882	.882	.912	.885	.938	df = 12
F	.007	.003	.004	.000	.000	P = 0.003
*PGI-1**						
(N)	144	144	114	144	144	
A	.010	.007	.013	.024	.010	
B	.000	.000	.000	.000	.003	
C	.990	.993	.987	.976	.979	G = 3.598
D	.000	.000	.000	.000	.003	df = 4
E	.000	.000	.000	.000	.003	P = 0.492
*PGI-2**						
(N)	144	144	114	144	144	
A	.010	.017	.004	.003	.007	G = 3.929
B	.990	.976	.996	.997	.990	df = 4
C	.000	.007	.000	.000	.003	P = 0.495

DISCUSSION

This pilot study on population structure of wrasses in Norwegian waters has identified several polymorphic enzymes which can be used for more detailed investigations. At present, the sample sizes of Norwegian rock cook and corkwing are small and the main emphasis has been payed on goldsinny.

For this species, significant genetic variation of allele frequencies was detected between different localities. The variation was found in the *SDH-1** and *PGI-1** loci and was associated to fjord areas. The genetic differentiation found in the preliminary sample collection was small, but suggests that local populations of wrasses (goldsinny) does exist, at least in some fjords. The sample sizes of the other species and the limited number of localities, does not so far permit final conclusions about population structures.

The results obtained for goldsinny, are, however, not surprising. Wrasses in Scandinavian waters are known to be stationary and found in the same area from year to year (Hillden, 1981). Large migration have not been found and this behaviour correspond to similar species in other areas. For instance, experimental studies of *Crenolabrus ocellatus* have demonstrated a return migration after experimental transfer to other areas (Fiedler, 1964).

The different species in question have also different strategies with respect to reproduction (Darwall *et al.* 1992). The goldsinny has small, pelagic eggs which are transported according to the local current conditions. These eggs are commonly found in most of the fjords in Norway in the summer (Lie *et al*, 1978; Camo Lopes, 1979). On the other side, rock cook and corkwing spawn their eggs in nests. For this reason, these species have a more restricted distribution area at early life stages which could be reflected in the genetic population structure.

The increased use of wrasses in the salmon lice treatment in the industry must be based on the naturally occurring fish resources, at least in the near future. The question of local stocks for the different species must be clarified and management principles must be developed. This must include fishing options specific for the various species and regions and an evaluation of transplantation of stocks between different geographic regions.

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