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The Propagation of God *Gadus morhua* L.

IMPORTANCE OF GENETIC VARIATION IN THE PROPAGATION OF COD

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ABSTRACT

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During the last decade the existence of genetic diversity within species and populations has been generally accepted. The complex structure of populations as well as the variations within populations is believed to be of fundamental importance for adapting to a changing environment and for further evolution.

Several genetic studies have suggested a complex population structure for the cod in Norwegian waters. Cod stocks in enhancement programmes may suffer the unwanted loss of genetic variation which has been observed in stocking and enhancement programmes for anadromous species. In preliminary experiments with cod in the laboratory, differential selection against some genotypes was observed and is in fact likely to take place when producing cod fry. When producing large quantities of fry from a few parent fish, genetic drift will also greatly effect the gene pool.

At present, however, methods for genotyping parental fish and genetic analysis of cod eggs and larvae offer the opportunity of genetic control at different stages of development. The importance of genetic surveillance of local populations, application of genetic principles and methods to prevent loss of genetic variation, and the possibility of using "genetically tagged" cod fry are discussed in relation to artificial propagation programmes for cod.

INTRODUCTION

Under natural conditions the genetic variation of population units is characterized by their particular gene pool,

geographic distribution, preferred environment and general life history. This variation is believed to be of fundamental significance for adaptation - already in 1930 Fisher recognized that the amount of genetic variation is positively correlated with the rate of evolutionary change by natural selection (Fisher, 1930). Genetic variability is in some respects related to overall fitness (survival and reproduction) both for populations in their natural environment and for stocked fish whose surroundings abruptly change when they are transferred from a protected to a natural environment.

This discussion of the significance of genetic variation in the artificial propagation of cod is based, in large part, on the investigations carried out on species which have been in culture or semiculture for some time. Although few studies have been conducted on the genetics of artificially reared marine fish, we present some of the methods available for studies of artificially propagated cod as well as some preliminary results.

TYPES AND LEVELS OF GENETIC VARIATION

Genetic variation is usually divided into the two categories of quantitative variation and polymorphism. Quantitative variations are continuous and usually influenced both by genetic and environmental factors. Usually also several genes are involved. To find the degree of influence of genetic factors on variation in quantitative traits, we have to carry out breeding experiments or in other ways have information about correlations between relatives.

Polymorphic variations are usually independent of environmental factors except that they may be influenced by natural selection at least during adaption to a specific environment. The individuals may be easily separated into well-defined groups (morphs), the genetic control is simple with only one or a few loci involved and may be studied by population data.

Genetic variation in natural populations also exist on two levels of organization: within and between population units. The amount of genetic variation may be estimated and subdivided into the two categories, by for instance, examination of allele frequencies in polymorphic proteins or enzymes. The amount of variation within and between populations varies with the species. For some species the greater part of the variation observed seem to exist within populations, but significant variations between populations are also frequently found.

The cod is a widespread species with a number of subspecies described by morphological variation. Even within subspecies much variation can be found in traits such as growth rate, colour pattern, migration and behaviour patterns as well as morphometric and meristic characteristics. To our knowledge no studies on the influence of genetic factors on these characteristics have been undertaken, and we can make no more than a guess about the degree of genetic influence. In contrast however, much genetic variation is found in polymorphic characteristics. Studies on blood types, blood protein and tissue enzyme were started in the early sixties (see review by de Ligny, 1969), and during recent years new techniques of population genetic (Harris and Hopkinson, 1976; Allendorf and Utter, 1979; Ferguson, 1980) have been more applied.

A number of investigations were instigated to find genetic markers for studying the population structure of the species. This is still a major problem since neither quantitative or polymorphic characteristics, nor tagging experiments have led to clear-cut conclusions about the numbers and delineations of the population units of cod, their relationships and their isolating mechanisms.

Interest has been aroused in recent years because cod had proved itself well suited for studying the significance of genetic variation, the rate of selection and adaptation to particular environments and the existence of sympatric gene pools. Ultimately the destruction of genetic resources by

selective fishing on particular stocks or by artificial propagation may also be studied.

GENETIC DRIFT AND THE SIGNIFICANCE OF PARENT NUMBER

Genetic drift is the change in gene frequencies caused by sampling effect. When a generation is based on a few parent fish it is probable that the gene pool of the offspring will be significantly altered relative to the parent gene pool. With low effective numbers of parents the chance of losing genes is high. Although this may be expressed in mathematical terms it is more valuable here to find some theoretical limits which may state the minimum number of parent fish which must be maintained to avoid any serious reduction of the genetic variation of the next generation. Frankel and Soulé (1981) discuss the basic rules of conservation genetics, and are of the opinion that a 1% change in inbreeding rate is the maximum tolerable before loss of genetic variation brings a decline in fitness. Below this threshold natural selection is able to offset the tendencies of fixation of genes which, according to Wright's expression, corresponds to an effective contributing number of parent fish of about 50 for both sexes. This figure may be used as a rough estimate when planning the experiments, but it should also be emphasized that this is effective numbers. The actual numbers of parents must, in most practical experiments, be far higher.

GENE MARKERS FOR CONTROL OF SURVIVAL RATES OF RELEASED FISH

Gene markers may be a very helpful way of controlling the effect of releasing artificially reared cod. By using a polymorphic system, presumed to be selectively neutral, and choosing parent fish which change the frequency of one or a few alleles to a frequency as different as possible from the

natural populations in the release area, proportions of released fish in later samples may be estimated. This is in alternative or supplement to traditional tagging procedures, and is especially valuable when releasing young larvae. For more detailed discussion of the use of genetic markers in hatchery stocks and enhancement studies see Allendorf and Utter (1979). In large scale enhancement programmes, however, the problems and guidelines discussed by Hynes et al., (1981) should be considered.

INDICATIONS OF STRATEGIES

Genetic controls in release programmes have a twofold purpose: to use genetic principles to increase the success rate of the programme, and to prevent undesirable effects on the natural populations.

The first point requires a comprehensive study of the genetics of the natural populations in the release area by applying as many polymorphic systems as possible before the release. Similarly the artificially propagated populations have to be studied at various life stages starting with the spawners and continuing with the fertilized eggs, larvae, postlarvae and 0-group until their release. If possible, some of the fish should be kept in net pens and studied throughout the life cycle. In this way good estimates of selection rates for the different alleles may be obtained and the relationship between gene pools of natural and released fish may also be studied.

Undoubtedly we must consider the within-population variation when releasing fish, despite our insufficient knowledge of the significance of the variation. This may be provided by using as varied parent fish as possible and ensuring that the number of parent fish is far above 50. As we may assume that local populations are well-adapted to the environment, we would also prefer to use parent fish from the release area and to have the released fry adapted to the environment.

Only when gene markers are used for genetic tagging should the released fish differ genetically from the natural populations, and then only for one or a few alleles. In any case, this strategy would monitor the genetic variation of the natural and the artificial populations, and be particularly sensitive to changes.

Thus we will gain knowledge about the significance of the variations both in polymorphic traits and in quantitative traits like growth rate, and adjust further strategies according to the results obtained.

TENTATIVE RESULTS FROM STUDIES ON ARTIFICIALLY HATCHED COD LARVAE

Some analyses have been carried out in recent years on the genetic variation in artificially hatched larvae. Table 1 gives an account of polymorphic systems detectable in yolk sac larvae of cod with the observed range of variation of the frequencies of some of the alleles in Norwegian cod populations. These systems will be valuable tools in more extensive analyses of variations in artificially hatched larvae and genetic control of larvae groups chosen for mass propagation.

Table 2 gives some data from repetitive sampling of two batches of yolksac larvae under laboratory conditions. Evidently, great changes in distribution of genotypes has taken place in about two weeks, suggesting differential selection. However, in this case it is not possible to decide whether this is a real selection against some of the genes or genotypes or whether it is familial selection. The material is composed of a limited number of unidentifiable sub groups, and different survival rates for the different families could have resulted in the present observation. Genetic changes in hatchery stocks have been reported for other fish species (Allendorf and Pelps, 1980; Ryman and

TABLE 1

Polymorphic enzymes detectable in yolksac larvae by use of starch gel electrophoresis. (Data from Jørstad et al., 1980; Reisegg, 1983). For comparisons of nomenclature see Moth-Poulsen (1980).

Enzyme	Loci	Alleles	Frequency (range)
Lactate dehydrogenase	*LDH-3*	*70* *100* *150*	.51 - .80
Isocitrate dehydrogenase	*IDH-1*	*100* *120*	.91 - .80
	IDH-2	*100* *120*	.97 - 1.00
Phosphoglucomutase	*PGM*	*30* *70* *100* *120*	.93 - .99
Phosphoglucose isomerase	*PGI-1*	*30* *70* *100* *150* *200*	.60 - .75
	PGI-2	*90* *100* *120*	.97 - 1.00

Ståhl, 1980; Tanigusha et al., 1983) and the data summarized above suggest that changes are likely to take place also in artificial propagation of cod.

Table 3 shows some data on the genotype composition of yolksac larvae in a basin whose parents had genotypes *LDH-*

TABLE 2

Changes in genotype frequencies in two groups of yolksac larvae under laboratory conditions, sampled at two and fifteen days of age (data from Jørstad et al., 1981). Homogeneity test is described by Sokal and Rohlf (1969).

		Genotype frequencies						
		LDH-3			*PGI-1*			
Gr.	Age	*70/70*	*70/100*	*100/100*	*30/100*	*100/100*	*100/150*	*100/150*
1	2	.07	.64	.30	-	.30	.51	.29
1	15	.05	.86	.08	-	-	.52	.48
2	2	.15	.48	.37	.05	-	.47	.47
2	15	.34	.49	.17	.22	.04	.54	.21
Homogeneity test, group 1:		P=0.037				P=0.02		
Homogeneity test, group 2:		P=0.001				P=0.01		

3(70/70) and *LDH-3(100/100)*. On two arbitrarily chosen days the distributions of genotypes are significantly different. This suggests that at any given time the effective number of spawners actually contributing to the daily spawning is probably quite low compared with the total numbers. This is actually the case in the release programme where only a fraction of the fertilized eggs are used for hatching and thus represent only a portion of the gene pool of the spawners.

TABLE 3

Genotype frequencies in two groups of yolksac larvae of cod spawned in a basin one week apart by the same spawning population (data from Jørstad et al., 1983). The two groups of larvae were tested for homogeneity by using the G-test described by Sokal and Rohlf (1969).

		LDH-3 Genotype frequencies		
Group	Day	*70/70*	*70/100*	*100/100*
1	15	.88	.08	.04
2	22	.53	.28	.19
Homogeneity test, P=0				

CONCLUDING REMARKS

During the last years much attention have focused on the problems of identification and conservation of the genetic resources in fish. Several international meetings have evaluated the problems and proposed recommendations (Ryman, 1981; FAO, 1981) relevant to artificial propagation and enhancement programmes of cod. The essential problem, considering fish in their natural environment or under artificial conditions, is how to maintain the genetic variability present.

In this paper, the problem is discussed in relation to theory, in comparison with non marine species and a few preliminary studies on cod larvae under controlled conditions. As discussed, incorporation of genetic studies in propagation of cod will possibly increase the chance of a successful programme. On the other hand, an enhancement programme should be designed in such a way that no harmful

genetic changes occur in the native stock. This requires, however, that genetic studies of native populations, of the parental fish used for propagation and of the fry released, are incorporated in enhancement programmes (Hynes et al., 1981). To measure potential long term effects on the native populations a genetic monitoring programme should be established.

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