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ENZYME POLYMORPHISM EXPRESSED IN NEWLY HATCHED COD LARVAE AND GENETIC ANALYSIS OF LARVAE EXPOSED TO HYDROCARBONS

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ABSTRACT

Cod eggs and larvae, at different stage of development, have been analyzed by starch gel electrophoresis. Four common enzymes (phosphoglucose isomerase, phosphoglucose mutase, isocitrate dehydrogenase and lactate dehydrogenase) were easily demonstrated both in eggs and larvae. Some of the isozymes present in adult fish, were expressed in newly fertilized eggs. The others, including polymorphic forms of particular interest in population studies, seemed to be activated during the process of hatching, offering the possibility to perform detailed genetic investigations at an early stage of cod larval development.

Comparison between the phenotypes of parent fish and the offspring, was in complete concordance with the hypothesis of co-dominant inheritance of the alleles present. The parent fish material, however, was scarce.

Several groups of eggs and cod larvae were exposed to different

concentrations of the sea water soluble fraction of hydrocarbons for North Sea crude oil. Unfortunately, only a few phenotypes were present in the populations. No significant changes in the genetic compositions of cod larvae were observed in the exposed populations compared to the controls. The experiments described, however, suggest a rational approach for investigating possible effects of pollutants on gene pools and genetic variability.

INTRODUCTION

Protein polymorphism have been demonstrated to exist in natural populations of nearly all species (Avise, 1974, Powell, 1975). Mainly due to development of a number of electrophoretic techniques (Gordon, 1975, Brewer, 1970) combined with staining procedures for specific enzymes (Harris and Hopkinson, 1976, Siciliano and Shaw, 1976), protein variation are known to be very common on the molecular level. Recently, application of electrophoretic methods have been discussed in a number of books, (Lewontin, 1974, Ferguson, 1980), and the methods have been successfully used in different biologically disciplines (Nei, 1975, Masters and Holmes, 1975).

The early electrophoretic studies of different fish species, reviewed by de Ligny (1969), were mainly concentrated on descriptions of polymorphic systems. The electrophoretic data obtained, were further used to distinguish between different stocks of comercially important fish species (ICES, 1971, Jamieson, 1974). However, in the early investigations mostly serological methods and analysis of blood proteins were applied; these methods are known to have several limitations both for theoretical and technical reasons (Utter, Hodgins and Allendorf, 1974, Allendorf and Utter, 1979).

Electrophoretic analysis of different tissue enzymes have been used in more recent investigations (Ward and Beardmore, 1976, Allendorf et al., 1976). Multi loci studies in several fish species have provided valuable information about genetic variation and intraspecific groups within comercially important fish

species (Cross and Payne, 1978, Ryman et al.,1979). During the last years, identification and conservation of such genetic resources have received increasing attention (Smith et al., 1976). Basic knowledge about the population structure of fish resources, are necessary for proper management and monitoring of the available resources for the future. Such data can be obtained through detailed electrophoretic studies of a large number of loci (Lewontin, 1974).

In the 1960's several fish species occuring in Norwegian sea water were subject for electrophoretic studies (Møller, 1968, Naevdal, 1970). This work, however, was not continued until recently when the methods of enzyme electrophoresis are applied in stock identification of herring and cod in Norwegian coastal water.

Application of new methods to reveal enzyme variation in species, require careful interpretation of the results. As several workers have pointed out (Allendorf and Utter, 1979, Harris and Hopkinson, 1976), protein variation need not necessarily be genetically controlled. Usually, the variation observed is concluded to be of genetic nature if the distribution of the different protein variants in a population are in agreement with Hardy-Weinberg's expectations. More direct proof of the genetic control of protein variation can be obtained in inheritance studies. Breeding experiments have been reported for a number of enzyme loci in fish (Purdom et al.,1976, Allendorf et al.,1975), but in a limited number of fish species. Crossing experiments with fish are restricted to those species in which artificial reproduction and larval development can be controlled in the laboratory.

During the last years, methods of handling cod eggs and larvae in the laboratory have been improved, and thus given detailed information of early cod larval stages (Ellertsen et al., 1980). The methods permit breeding studies on this species.

This year, a number of experiments were designed to investigate sub lethal effects of hydrocarbon pollution on the early stage of cod eggs and larvae. The experiments also offered material for investigation on isozymes at different stage of larvae

development and for genetic analysis of hydrocarbon exposed cod larvae.

MATERIALS AND METHODS

Brood stocks of cod were received in the spawning season from commercial fishermen and the fishes were kept alive. Eggs and sperm were obtained by stripping the fishes, and the eggs were artificially fertilized and incubated at a seawater temperature of 5C.

Except for one experiment (Results, part c), only eggs from one male and female were used, and samples of white muscle and liver from these were frozen at -85°C until analysis of tissue enzymes. In the experiment using cod larvae at feeding conditions, however, larvae from unknown parents were used. These were obtained by collecting fertilized eggs from fish which have spawned in captivity at Statens Biologiske Stasjon Fløde-vigen, Arendal, and sent by air to Institute of Marine Research.

The fertilized eggs and later cod larvae were incubated and treated as described by Ellertsen et al. (1980). Samples of individual eggs and larvae were carefully transferred from incubation containers to small pieces of filterpapers (1x8 mm) on which they were destroyed and soaked into the papers. These filterpapers were then stored at -85°C and analyzed by starch gel electrophoresis as soon as possible.

Four different enzymes (phosphoglucose isomerase, phosphoglucomutase, isocitrate dehydrogenase and lactate dehydrogenase) were analyzed using histidine gel pH 7.0. Details concerning electrophoretic equipment; conditions during electrophoresis etc. will be described elsewhere (Jørstad, in preparation). The staining of the different enzymes followed the procedures described by Ward and Beardmore (1977), and Harris and Hopkinson (1976).

The technical arrangement used for exposing the eggs and cod larvae to different concentrations of sea water soluble hydrocarbons, are reported by Johannessen (1978). In this system cod eggs and larvae were continously exposed to two different

levels (150 ppb and 50 ppb) of sea water dissolved hydrocarbons. Details about keeping the larval system under proper control will be discussed elsewhere by Tilseth et al. (in preparation).

The nomenclature used for designing the different isozymes and multiple alleles at corresponding loci, followed the principles proposed by Allendorf and Utter (1979).

RESULTS

a. Enzyme activities at different stage of cod larvae development.

Activity for all four enzymes could be demonstrated in newly fertilized eggs by using an extended time for staining of the gels combined with increased amounts of substrate. However, of the LDH isozymes reported (Odense et al., 1966) only LDH-2 (normally expressed in white muscle tissue in adult fish) was detected in newly fertilized cod eggs. Similarly, only PGI-2 activity was seen on the gels stained for phosphoglucose isomerase. In adult fish this enzyme is most active in liver and heart tissue (Dando, 1974, Cross and Payne, 1978). Enzyme activity was also demonstrated in gels stained for phosphoglucomutase, but the bands were rather weak and need high amounts of substrate to be observed. Isocitrate dehydrogenase isozymes, on the other hand, seemed to be expressed with high activity in the eggs. IDH-1 and IDH-2, which are most active in liver tissue and white muscle in adult fishes, were both present in newly fertilized cod eggs. Possible due to the buffer system used, the separation of the two isozymes, were sometimes not satisfactory for routine typing of the individual eggs and cod larvae.

The expression of the enzymes as described above, were not changed until hatching of the eggs started to occur. Hatching of cod eggs normally takes place about 3 weeks after fertilization at $5^{o}C$ (Ellertsen et al., 1980). Analysis of the newly hatched cod larvae demonstrated that additional isozymes have been activated. LDH-3, in adult fish most active in heart tissue, was clearly expressed at this stage of larval development.

The PGI-1 isozyme, which are active in white muscle in adult

fish, was also observed. These two enzymes are both known to be polymorphic and informative in population studies of cod (Jamieson,1975, Cross and Payne, 1978). Photographs of starch gels stained for the different enzymes, are shown in fig. 1-4.

Unfortunately, the analysis of the parent fish showed that a limited number of enzyme phenotypes could be expected in the offspring. The male and female used in this particular crossing, were both heterocygous for the LDH-3 locus, and the male was heterocygous for IDH-1 locus. For the other enzyme loci analysed, both parental fish were homocygotes for the same alleles.

However, a limited number of the offspring from the crossing were analyzed and compared to its parents. The results are summerized in Table 1.

No significant deviation for Mendelian expectations were seen, which are in agreement with investigations on other fish species (Purdom et al., 1976, Allendorf, Utter and May, 1975 and Ward and Beardmore, 1977).

The hypothesis of co-dominant inheritance of the present enzymes as indicated from population data, seems to be confirmed by the breeding experiments.

b. Exposion to hydrocarbons during larval development.

In this experiment the technical arrangement described by Johannessen (1978) was used. Groups of fertilized eggs were incubated under identical conditions except for exposion to hydrocarbons. Samples of cod larvae from the different groups were taken short time after hatching and before mass mortality starts to occur (Ellertsen et al., 1980). The samples were analyzed for IDH and LDH phenotypes. The results, shown in table 2, indicate no significant differences with regards to the LDH-3 phenotypes present in exposed and control population of cod larvae.

c. Short time exposion to hydrocarbons followed by incubation of cod larvae at feeding conditions.

In this experiment cod larvae from unknown parental fish were used. Cod eggs were obtained from Statens Biologiske Stasjon, Flødevigen, (see MATERIAL AND METHODS). Enzyme analysis performed four days after hatching, showed that only part of the normal genetic variation of these enzymes was present in this population. One larvae group, however, was exposed to hydrocarbons (350 ppb) for twenty-four hours, transferred to feeding container and incubated in parallel with control larvae. Sixteen days after hatching, all larvae in the containers were removed for analysis. At this time the number of larvae in the two groups was reduced to about 25 percent, suggesting that the mass mortality period had not ended (Ellertsen et al., 1980). Table 3 summerize the results of the genetic analysis, showing no significant differences between the two groups of larvae.

DISCUSSION

The data reported in this work seems to confirm that the analyzed enzyme systems are under Mendelian control. Several polymorphic loci are expressed in cod larvae already at hatching of the eggs. This fact permits a number of interesting investigations to be done.

a. Genetic analysis of larval populations in nature can be performed. Use of the larval stage to identify fish stocks have earlier been suggested (Jamieson, 1974), but this approach have been rarely applied because of technical problems.

   Adolescent and mature fish are usually used in genetic investigation of marine fish. This, however, cause problems concerning mixture of age groups, migration and possible selection before the fish are sampled in the spawning population. It is assumed that detailed genetic analysis of larval populations present in spawning area will support better information about the spawning population.

b. Controlled breeding studies of cod parental fish of known genotypes can be done in the laboratory, and thus establish the basic genetic relationship between different loci and alleles.

c. Cod larvae populations of known genetic composition can be used in laboratory experiments concerned with a number of different ecological factors; for instance possible effects of different pollutants on the genetic composition of larvae can be investigated.

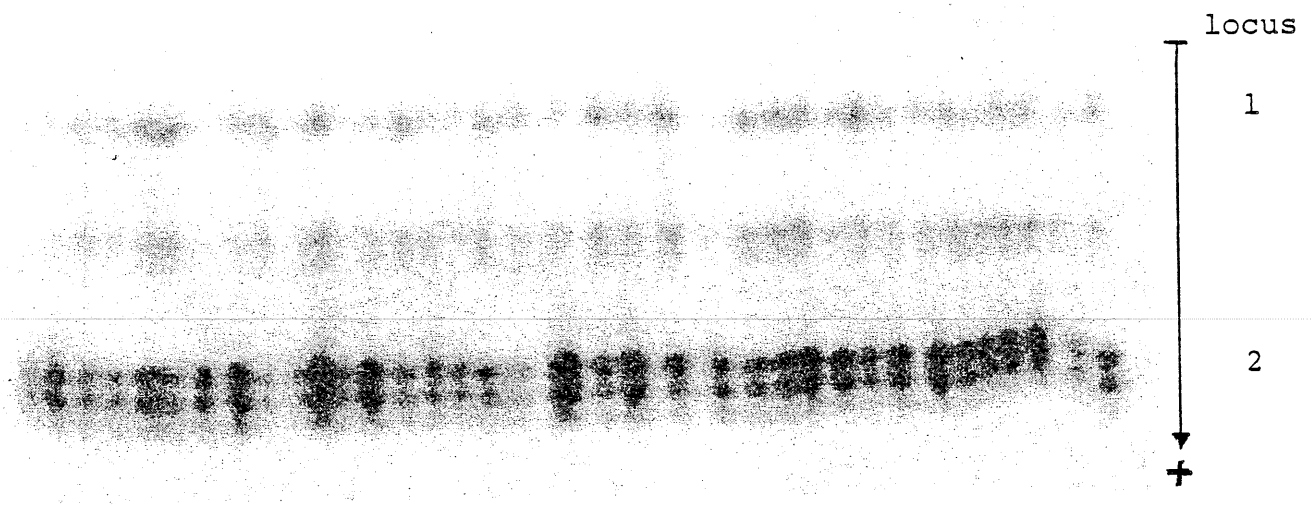

Fig.1. Phosphoglucose isomerase activity present in newly hatched cod larvae.
Observed phenotypes: PGI-1(100/100)
PGI-2(100/100)

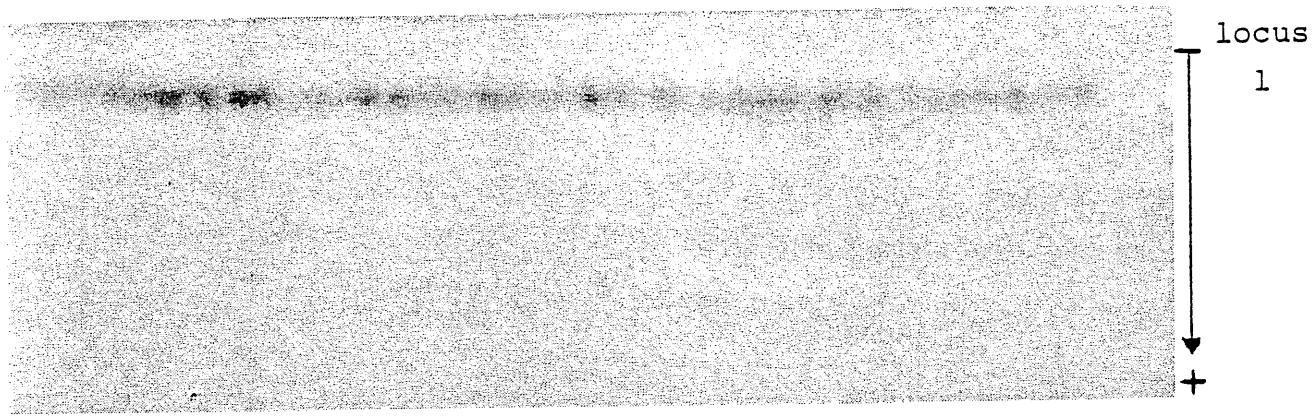

Fig.2. Phosphoglucomutase activity present in newly hatched cod larvae.
Observed phenotypes: PGM-1(100/100).

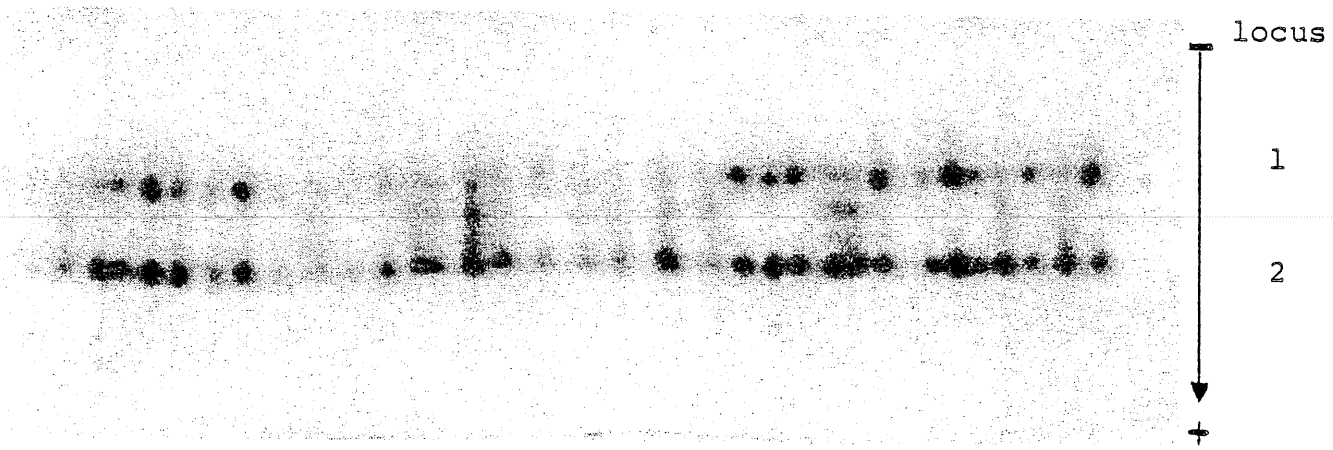

Fig.3. Isocitrate dehydrogenase activity present in newly hatched cod larvae.
Observed phenotypes: IDH-1(100/100)
IDH-2(100/100),IDH-2(100/120).

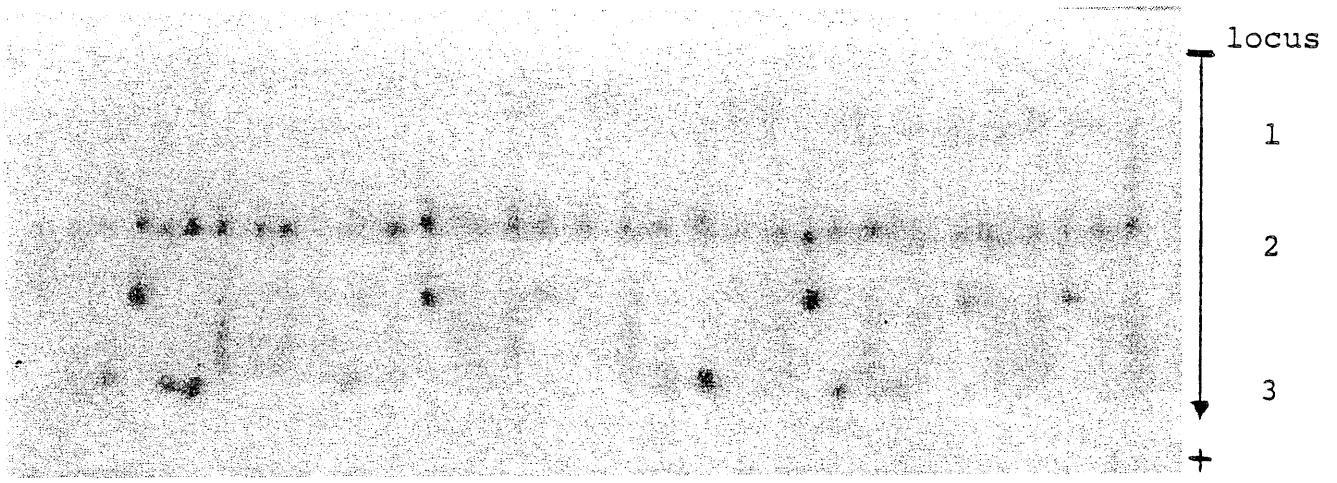

Fig.4. Lactate dehydrogenase activity present in newly hatched cod larvae.
Phenotypes observed: LDH-2(100/100)
LDH-3(100/100),LDH-3(100/70),
LDH-3(70/70).

Table 1.

Genetic analysis of cod larvae hatched twenty days after fertilization.

locus	phenotypes detected in natural populations (Jørstad, unpublished data)	parental phenotypes male	parental phenotypes female	offspring phenotypes
LDH-3	70/70	1	1	28
	70/100			50
	100/100			27
	100/120			0
IDH-1	100/100	1	1	48
	100/120			26
	120/120			0
IDH-2	100/80	1	1	0
	100/100			74
	100/120			0
PGI-1	100/30	1	1	0
	100/100			85
	100/150			0
	150/150			0
	30/150			0
	100/170			0
PGI-2	100/100	1	1	78
	100/90			0
PGM-1	30/100	1	1	0
	100/100			85
	100/70			0

Table 2.

Long term exposion to hydrocarbons during cod larval development

larval sample	LDH-3 phenotypes			IDH-1 phenotypes	
	70/70	70/100	100/100	100/100	100/120
control larvae day 1	8	19	8	21	14
control larvae day 11	10	19	12	-	
exposed larvae day 1	11	17	12	27	12
exposed larvae day 11	11	16	9	-	-

Table 3.

Short term exposion to hydrocarbons followed by incubation of cod larvae under feeding conditions.

larval sample	LDH-3 phenotypes		allel frequencies		PGI-1 phenotypes		allel frequencies	
	70/100	100/100	70	100	100/100	100/150	100	150
control larvae day 4	91	52	0.318	0.682	56	42	0.786	0.214
control larvae day 16	34	30	0.266	0.734	35	37	0.743	0.257
exposed larvae day 16	45	44	0.253	0.747	47	38	0.776	0.224

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