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ENDOCRINE AND NUTRITIONAL FACTORS AFFECTING THE FIRST FOOD UPTAKE BY COD LARVAE

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ABSTRACT

The gastroendocrinal development of cod larvae was investigated with emphasize on the role different hormones from the feed might play in promoting growth and development or triggering gastric secretion. Pepsin like and chymotrypsin like activity was found in unfed larvae, indicating that the digestive system may be sufficently developed. None of the hormones and other substances added to the feed promoted growth. A diet based on cod roe gave significant growth, but no survival to metamorphosis.

INTRODUCTION

The start feeding of most marine fish is totally dependant on live feed. Even though many larvae readily eat dead food organisms and other inert diets the larvae will die after some time. The reason for this lack of ability to utilize dead food is not fully understood despite the fact that many experiments have been carried out to test different hypotheses concerning this problem.

The present study deals with the role different hormones might play in this process. Two hypotheses were tested:

1. The fish larvae are not fully developed at first feeding, but need a signal substance from the feed to start diegeston and growth.

2. The fish larvae are not fully developed at first feeding, but need a growth-and development promoting substance from the feed.

Little is known about substances present in live feed which might influence the development of the digestive apparatus of the fish larvae. Several hormones are known to influence development and differentiation. We picked as possible candidates ecdysone and thyroxine, and a synthetic cortisol analog, dexamethasone (D.E. Metzler, 1977).

Production and secretion from fully developed digestive enzyme glands is regulated by a number of peptides. The peptide caerulein which is isolated from amphibian skin, has an endocrine activity in teleosts which is similar to the activity of gastrin and cholecystokinin in mammals (L.I. Larsson and J.F. Rehfeld, 1977, G.J. Dockray, 1979). J.Flüchter (1982) demonstrated improved survival and growth in "whitefish" larvae after addition of an acetone extract of quickly frozen Artemia naupulii to the feed.

MATERIALS AND METHODS

Demonstration of digestive enzymes in larval extracts.

Pepsin like activity:

The fish species studied was cod (Gadus Morhua). Freshly collected larvae were filtered through double layers of cheese cloth to remove salt water. The larval mass was frozen and stored without further treatment. Extracts were prepared from 6 g of larvae in 5 volumes of 100 mM acetate buffer pH 5. The mixture was homogenized

and centrifuged at 12 000 x g for 15 min. An aliquot of the supernatant was consentrated by ultrafiltration to a small volume, diluted with 10 mM acetate buffer pH 5 and again concentrated. This procedure was repeated twice, and the whole treatment required 4 days in the cold room (2 - 4°). The extract was centrifuged and applied to a column of DEAE cellulose (50 x 8 mm) equilibrated with 10 mM acetate buffer pH 5. The colum was eluted stepwise with 10, 100 and 200 mM acetate buffer pH 5. The eluate was analyzed for pepsin like activity at pH 2 by the method of Rick and Fritsch (1974) with hemoglobin as a substrate. The method was modified to include a maximal volume of enzyme solution in the incubation mixture, and the incubation was carried out for 60 min. Pepsin like activity was present in all three fractions of eluate. Enzyme from a control extract of the stomach of a young cod behaved in a similar way: Some activity was eluted from the DEAE cellulose column when the colum was washed with 10 mM acetate buffer at pH 5. The bulk of the activity appeared in the fractions eluted with 50, 100 and 200 mM acetate buffer (pH 5).

Chymotrypsin like activity: An extract of 6 g of cod larvae was prepared in 5 volumes of 80 mM buffer, pH 7.8. The mixture was homogenized and centrifuged at 12 000 x g for 15 min. An aliquot of the extract was treated with 9 parts of methanol, and the precipiate removed by centrifugation. The supernatant was tested for chymotrypsin like activity by the method of Rick (1974) with N-benzoyl-L-tyrosine ethyl ester as substrate.
Significant chymotrypsin like activity was observed in the methanol treated larval extract.

Preparation of zooplancton extract: Freshly collected zooplancton were frozen in small portions in liquid butane which was again cooled in liquid nitrogen. The lumps of material were weighed and submerged in 4 volumes of acetone. The mixture was shaken until all frozen material was thawed and dispersed. The acetone was removed in a stream of nitrogen, and the residue was dissolved in a similar volume og 95% ethanol, which was added as such to the feed.

Start feeding experiment

Fertilized eggs were collected from a spawning pen and incubated and hatched in polyethylene cylinders (Huse and Jensen 1980,1981). Two to four days old larvae were transferred to the 100 l conical experimental tanks with an initial density of 20 larvae per l.

The experimental tanks were supplied with filtered and UV-treated sea water from 55 m depth. The temperature varied between 6.0°C and 13.0°C during the experimental period. After each feeding the water circulation was shut off to enchance feeding success. This led to increased temperature in the tank water which explains the wide range of temperatures during the experiment. Salinities were relatively stable at 31.2 ± 0.50/00.

The different hormones tested were mixed into a standard diet based on hen's egg, proteose pepton, cod liver oil, and fish protein autolysate with vitamins and minerals added. Particles between 90 and 120µ were made by screening with plankton gauze. Hormones were added after this screening. The larvae were fed 2 - 3 times a day. The feed particles stayed in suspension for several hours before sedimenting. Feed level was kept at more than 2000 particles per l. Each experiment included one group of starving larvae and one group getting only standard diet.

Larval samples were collected by tube sampling every second or third day. Feeding incidence, length, myotome height, and dry weight was measured.

Five sets of experiments were carried out. The different diets tested are given in table 1.

TABLE 1. Diets and substances tested.

	EXPERIMENTAL SET NO.				
Diet	1	2	3	4	5
No food (starvation group)	x	x	x	x	x
Standard diet (S)	x	X	X	X	X
S+ Caerulein	X				
S+ Dexamethasone	X				
S+ Histamine		X			
S+ Thyroxine		X			
S+ Ecdysone+ Dexamethasone+ Thyroxine+ Caerulein				X	
Squid meal			X		
S+ zooplankton extract			X		
Cod roe				X	
Living collected zooplankton					X
Rotifers		X			

The substances tested in the standard feed were added in the following concentrations, mg per 500 g of feed:

Ecdysone	100
Dexamethasone	1000 and 10
Thyroxine	100
Caerulein	1
Histamine	1000
Zooplankton extract	5×10^5*

*Ethanol was evaporated at room temperature after mixing with standard diet.

RESULTS AND DISCUSSION

The development of dry weight over time for larvae fed different diets is given in FIGS. 1-5. Dry weight is chosen as a growth monitoring parameter as it is more sensitive than length and myotome height. The end point of the curves represents the last sample before the larval group died out. Two groups, However, survived beyond the experimental period and through metamorphosis. These were the groups fed live feed. All other groups died out during the experimental period.

The typical development in dry weight was a decrease during the first ten days and then either death, stabilization, or increase of dry weight. The stabilization and also often the increase was partly due to the dying out of the smallest larvae. This might mask the real results. There is, however, always the starvation group and the group fed the standard diet to compare with. In all experiments the group given the standard diet lived longer than the starvation group and had higher dry weight. This indicates that the larvae fed the standard diet were able to utilize energy from it while the starving larvae consumed themselves (Huse 1981). In no instance, however, did the larvae fed the standard diet grow significantly compared to the start sample.

No larvae fed the standard diet with hormones added lived longer than larvae fed the standard diet without hormones. On the contrary, where there was a difference in survival it was always in favour of the pure standard diet. This also was the case with

growth, althought not so pronounced. The only inert substance to perform better than the standard diet was a diet made from cod roe prepared by professor Jan Raa, The University of Tromsø. The group fed this diet grew significantly compared to the start sample, but nevertheless it died out after sixteen days simultanously with the standard diet group.

Both rotifers and living wild plankton gave survival to metamorphosis, indicating that both larvae and systems were of adequate quality.

While we regard the experiments with larval digestive enzymes as strictly qualitative, the results nevertheless give an indication that pepsin- and chymotrypsin- like activities are present in 5 day old unfed larvae. These result therefore support the first of the two hypotheses we wanted to test, that the fish larvae are fully developed at first feeding. However, the glands may be developed to a stage where enzymatically active proteins may be produced, while other functions of the digestive system could be far from maturity.

Preliminary experiments with young, fully developed cod, indicate that the levels of digestive enzymes are reduced to very low levels after 4 to 6 weeks of fasting. Intraperitoneal administration of dexamethasone resulted in significant increases of enzymes in the alimentary canal after three days. The development seems, therefore to be to some extent reversible.

The lack of succes in the feeding experiment with added hormones may have many causes. Even if we have guessed the right substances, the number of concentrations we have been able to test are few. We belive that further work along the same line may aid in answering the question: Are external factors needed to continue or to complete the development in young cod larvae?

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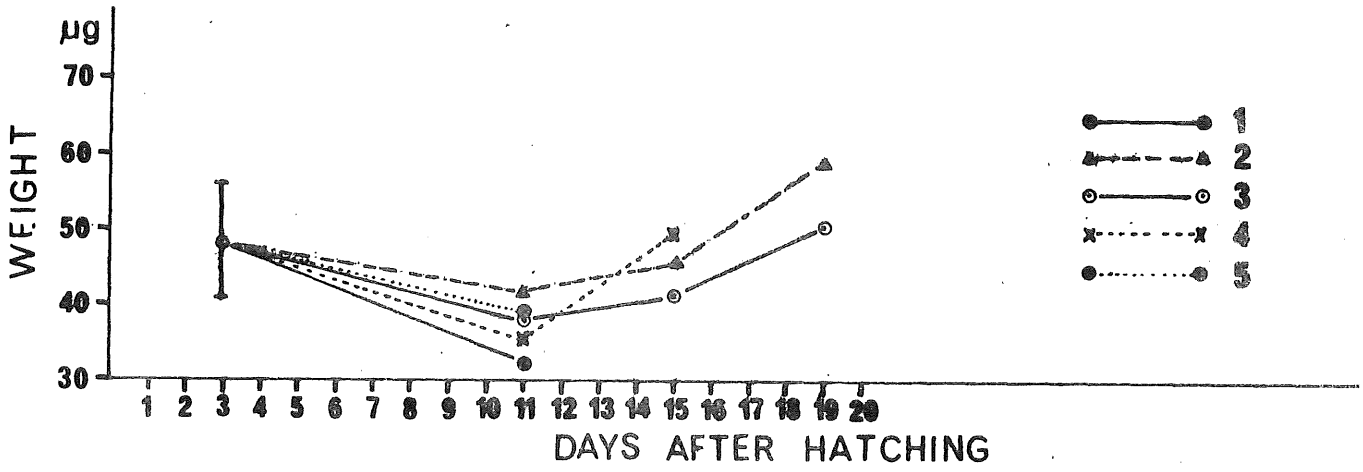

Fig.1. Growth (µg dry weight means) of cod larvae start fed with: 1.No food. 2.Standard diet (S). 3.S+Caerulein. 4.S + Dexamethasone (10mg/500g). 5.S+Dexamethasone (1000mg/500g).

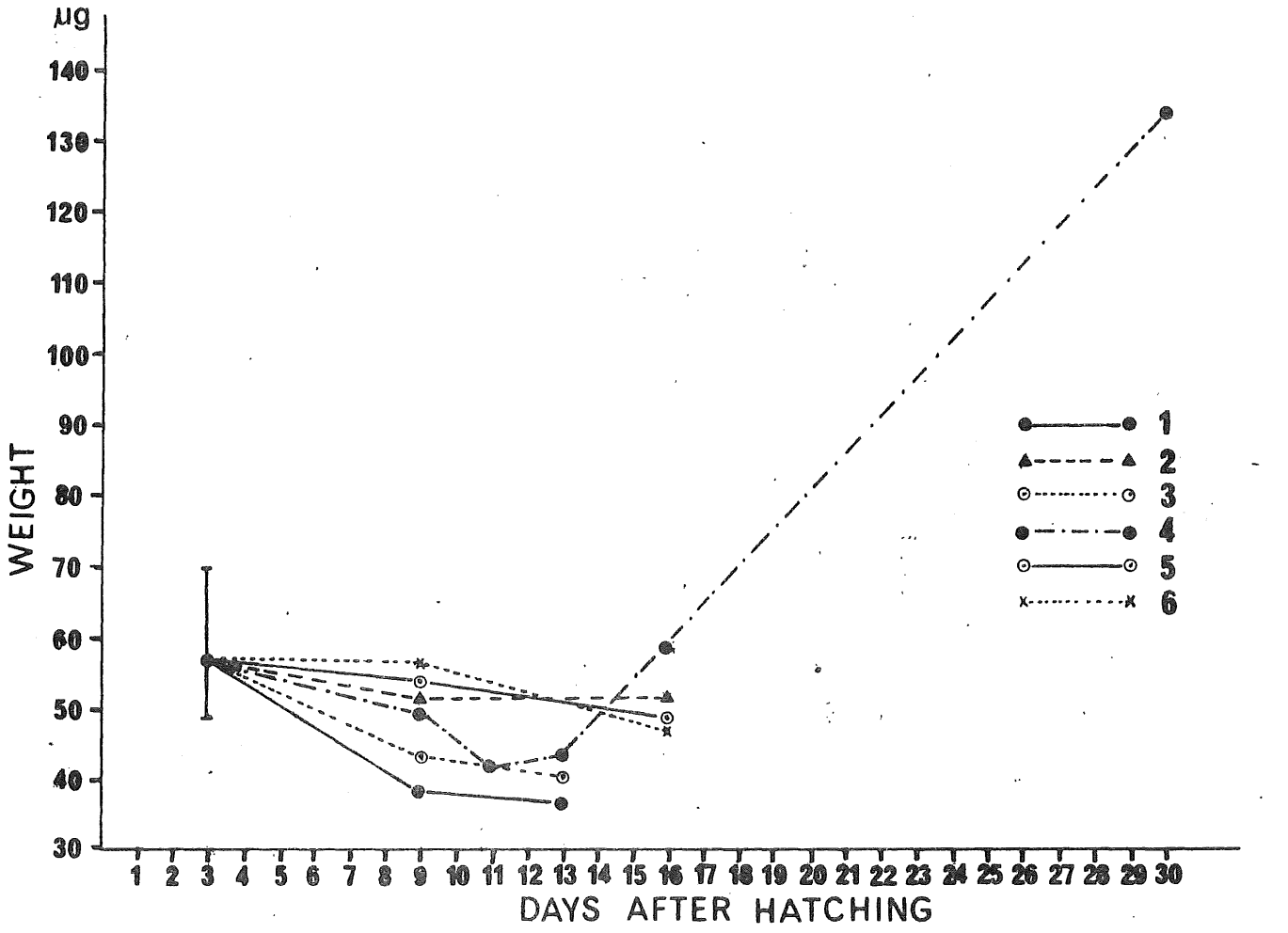

Fig.2. Growth (µg dry weight means) of cod larvae start fed with: 1.No food. 2.Standard diet (S). 3.S+Histamine. 4.Rotifers. 5.S+Thyroxine. 6.S+Ecdysone.

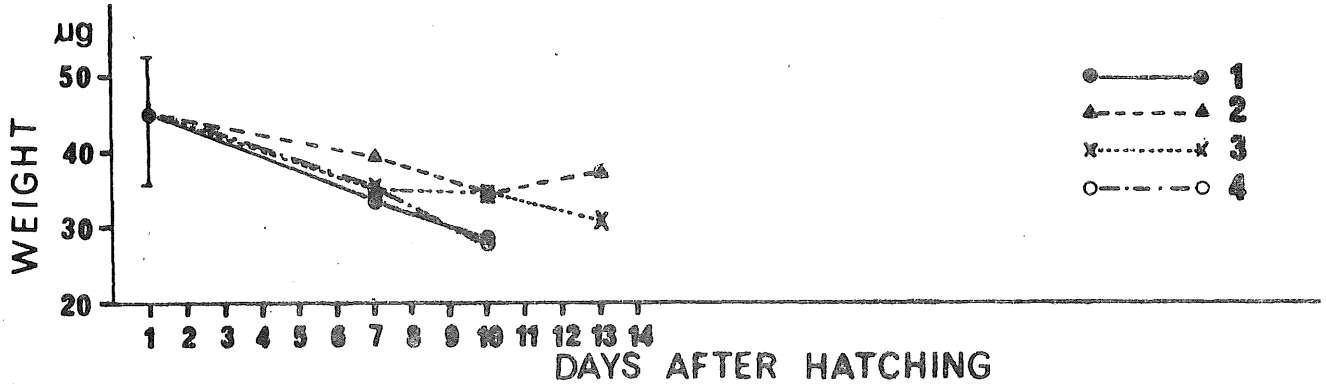

Fig.3. Growth (µg dry weight means) of cod larvae start fed with: 1.No food. 2.Standard diet (S). 3.Squid meal. 4.S+Zooplankton extract.

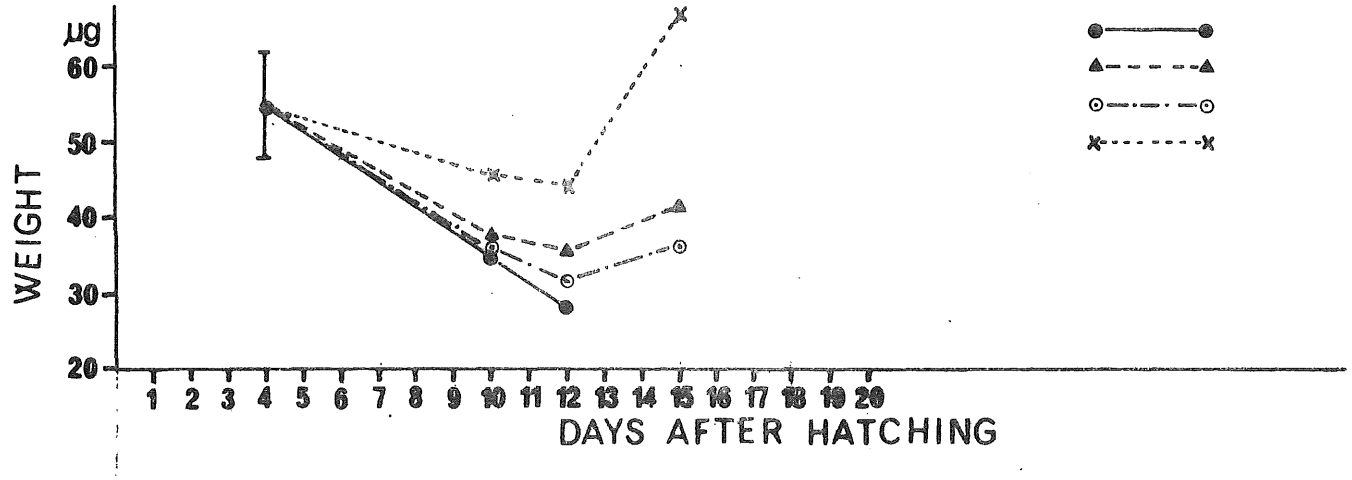

Fig.4. Growth (µg dry weight means) of cod larvae start fed with: 1.No food. 2.Standard diet (S). 3.S+Ecdysone+Caerulein+ Dexamethasone+Thyroxine. 4.Cod roe.

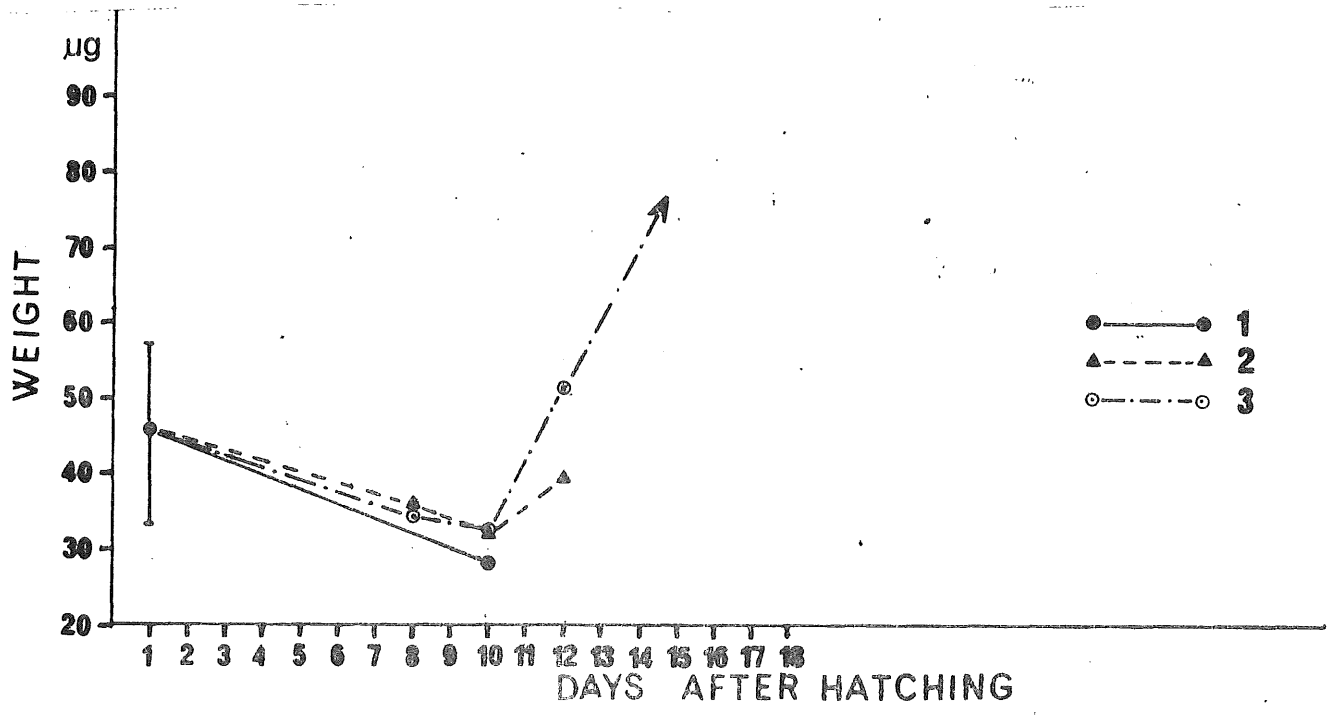

Fig. 5. Growth (µg dry weight means) of cod larvae start fed with: 1.No food. 2.Standard diet. 3.Living collected zooplankton.