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The Propagation of Cod *Gadus morhua* L.

THE PECULIARITY OF THE DEVELOPMENT OF WHITE SEA COD

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ABSTRACT

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The paper discusses the main results of many years of research into spawning conditions and embryonic and larval development of the White Sea cod, i.e., peculiarities in morphogenesis, in the formation, structure and functioning of the digestive system, increase of protein and utilization of yolk substances. It presents results of experimental work on the effects of temperature on the character of morphogenetic and metabolic processes in developing cod eggs.

INTRODUCTION

In our research we proceed from the assumption that fish development can be looked upon as a historical compromise between the environment and the adaptative power of a species. This process may also be regarded as the dynamic equilibrium between a living organism and the everchanging factors of the environment. The very nature of the organism-environment relationship undergoes considerable changes throughout ontogenesis as the organism becomes complex in the process of its

development and the degree of its adaptive capacities increases.

When discussing the early stages of individual development, it should be remembered that, as S.G. Kryzhanovsky (1948, 1950) one of the founders of ecological embryology of fish noted, "adaptation of fish to reproduction and developmental conditions reflects not only essential ecological factors of the embryonic period, but also essential phases of all the other life periods. They produce an impact on the biology of adult fish, determine the nature of migration, the possibilities of movements and boundaries of distribution".

MATERIALS AND METHODS

The time and character of cod spawning have been determined using catches of mature males and females and sampling of pelagic eggs in the plankton. Eggs obtained by natural spawning in laboratory conditions or by artificial fertilization were used in experimental work. The effects of temperature conditions on the character of embryonic and larval development were studied using eggs obtained from one pair of parents which were incubated at different temperatures within the natural range for the particular species. Buoyancy was determined using techniques developed by Zaitsev (1954). Morphogenetic peculiarities were examined with living material by means of side microscopy and conventional microscopy (Chernyaev, 1962, 1981). Methods of experimental embryology (Kostomarova, 1974) were used to study the properties of the cells forming the embryonic disc. The histological structure of the digestive system was examined using conventional methods (Verigina et al., 1981). Acid proteinase activity was determined by Anson's technique (Anson, 1938), and α-amylase in accordance with the method developed by A.M. Ugoleva (1968). Separation methods of embryo and yolk in the eggs to study regularities of protein increase and resorption of reserve yolk substances were also described earlier (Kuftina and Novikov, 1980).

RESULTS AND DISCUSSION

The condition of reproduction and development for White Sea cod (*Gadus morhua marisalbi Derjugin*) inhabiting the Subarctic Sea, are characterized by marked variations in temperature and salinity. Its spawning period is prolonged with one female producing 5 to 6 batches of eggs; it begins early in March under the ice at ~1.5°C with salinity of about 29°/oo and it is completed in June, when the sea is free of ice. At the end of the spawning period the surface waters have increased to 4°C and salinity is reduced to 24-25°/oo. The developmental conditions of the initial and the subsequent batches of eggs vary and are identical with the variation in reproduction conditions. The temperatures which allow normal development vary from -1.5° to 10°C. The buoyancy of eggs varies greatly even within one batch. On the average, zero buoyancy is achieved at 24.2°/oo of salinity. The embryonic period in the initial batches is 45-50 days, for the final 12-14 days. The size of the fertilized egg varies in different females from 1.40 mm to 1.75 mm (Makhotin and Soin, 1974).

Proceeding from our present knowledge of bony fish (Ballard, 1973, 1981; Trinkaus, 1973, 1976) and relying upon qualitative characteristics of morphological movements in their early ontogenesis (Makhotin, 1978) we have established ten stages in embryonic and larval development of the cod before the time of complete external feeding of larvae, as shown in Fig. 1 and 2.

Stage I. Zygote formation. This is characterized by the rearrangement of the nuclear apparatus and the cortex of the egg, as well as the initiation of ooplasmic aggregation at the animal pole of the egg. The development of the cod is initiated only by penetration of the egg by sperm. The stage continues until the first cleavage appears. It lasts for about 12 h at -1.5°C, 8 h at 2°C and 6 h at 6°C.

Stage II. Cleavage. This lasts for nine mitotic cycles which are practically synchronous. Cleavage divisions (from II to IX) occur at nearly equal intervals of 6 h (at -1-5°), 4 h (at 2°) and 3 h (at 6°).

The ratio of the time for the initial cleavage to the time

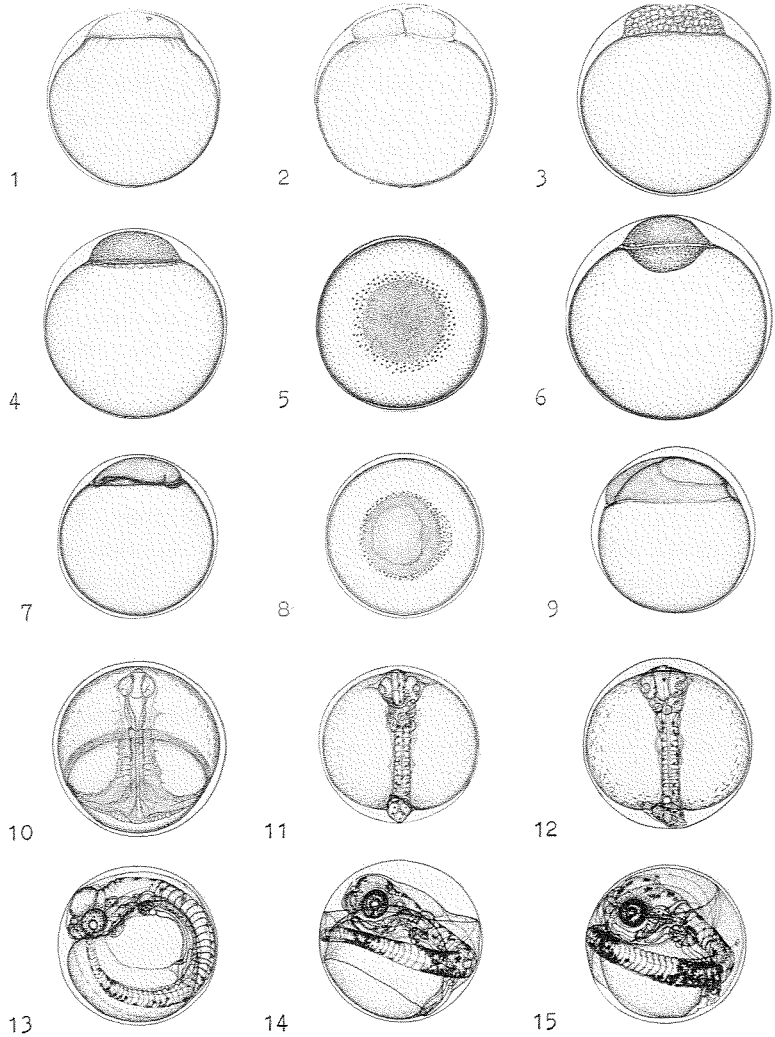

Fig. 1. The embryonic development. Stage I (1), II (2-3), III (4-6), IV (7-8), V (9-11), VI (12-13) and stage VII (14-15).

for synchronous cell division to appear is two, which is characteristic of boreal species. In the process of cleavage the adhesive properties of the blastomere membrane change at regular intervals and thus determine the resistance of the eggs to mechanical and other effects at different stages of cytotomy.

Stage III. Blastulation. This is characterized by embryonic disc differentiation into provisional structures: the surface cover layer (or periderm), the multinuclear (periblast) and the embryonic material proper; the inner cells are not adhesive, but they form lobopodii. This stage lasts for the six intervals of synchronous cleavage divisions. This stage is critical in embryogenesis, and the late blastula stage is the most sensitive to environmental influences.

Stage IV. Gastrulation. The inner cells show adhesive properties and the power of active movement. They are divided into epi- and hypoblast. Epiblast cells are attached to periderm while hypoblast cells migrate from the centre toward the peripheral zone. The embryonic disc gets flattened, and an embryonic ring appears; this is an external sign of the gastrulation process being completed. Epiboly of periblast and periderm follows gastrulation.

Stage V. Organogenesis. Hypoblast followed by epiblast then converge on the body axis of the future embryo. The axial organs are differentiated, the process of epiboly accompanying it.

Stage VI. Embryo mobile state. The resulting tissues and organs begin to perform definitive functions.

Stage VII. The embryo is about to hatch. The process is characterized by the fact that provisional larval organs are ready to function and the secretory activity of the hatching glands is enhanced. In the epithelium of the provisional embryonic organs (head sinus and the fin folding of protopterygoid) chloride cells are differentiated. The tissues of the embryo, the head sinus and protopterygoid foldings greatly increase their water content, while the eggs lose their positive buoyancy.

Different stages of the early larval period are shown in Fig. 2. The subperiod of prolarval development is characterized

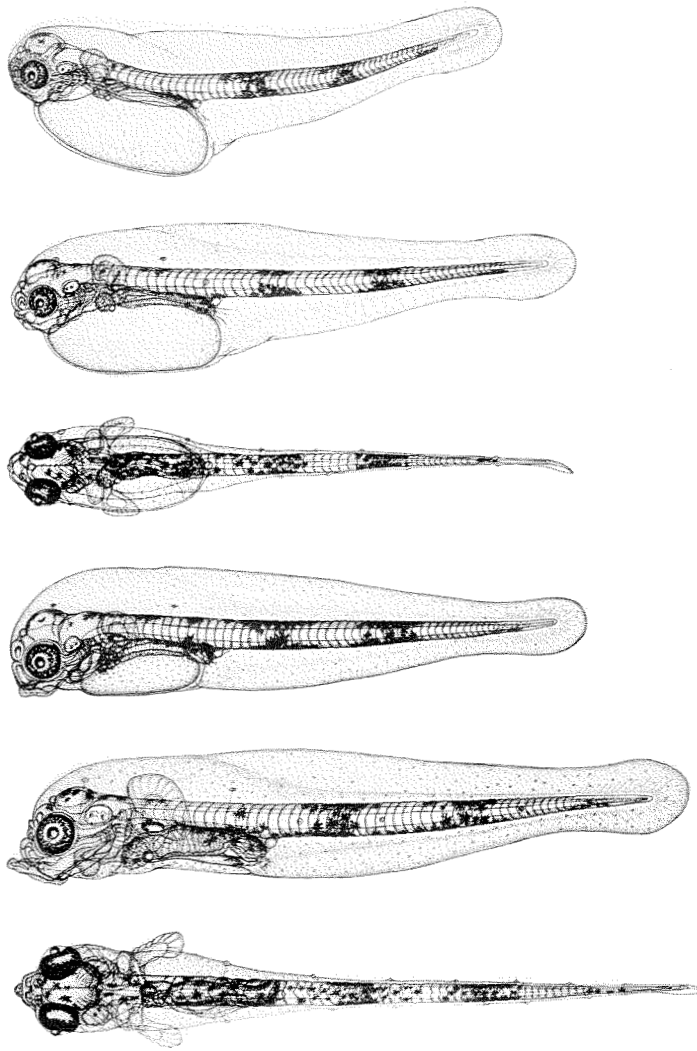

Fig. 2. The larval development. Stage VII (1, after hatching), VIII (2-3), IX (4) and stage X (5-6).

by endogenous feeding. On hatching the prolarvae are usually about 4.2-4.4 mm long, the body being 1.5 times shorter than the tail section, the former having 11-12 muscular segments, the latter 38-39. The morphological structure and the nature of prolarval pigmentation are similar to those in other populations and subspecies of the cod (Meek, 1924; Fridgeirsson, 1978; Thompson and Riley. 1981). Prolarvae keep close to the water surface, and are indifferent to light.

Stage VIII. The onset of blood cell circulation. Blood cells differentiate from the endothelium of the vessels. The swim-bladder is filled with gas. The prolarvae are orientated "belly downwards", and show a positive reaction to light.

The digestive tract of prolarvae is not fully differentiated into sections typical of the adult. It is a straight narrow tube which is morphologically divided into 2 sections: a short oesophagus and a long intestine (Fig. 3A). The liver is double-lobed, the parenchyma being loose. The gall bladder, situated between the lobes, opens into the intestine. The epithelial layer of the oesophagus is formed by flat cells arranged into 1-2 rows. The mucous cells in the epithelium of the digestive tract are absent, while the mucous membrane of the intestine is represented by a single row of cylindrical epithelium (Timeiko, 1978).

The subperiod of larval development proper begins with the stage IX of mixed (exogenous-endogenous) feeding. The larvae are about 5.0 mm long and 2/3 of the yolk has been resorbed. The mouth is open and the jaws can move to seize the food.

The digestive tube increases in size while the intestine cavity is markedly expanded and the rectum is being formed. (Fig. 3B). Peristaltic waves can be seen passing along the gut.

Generally the digestive tract of the prolarval cod is presumptive in structure: the stomach and pyloric caecae are absent while mucous and goblet cells characteristic of epithelial oesophagus and intestinal structures are not yet formed.

Research into the activity of acid enzymes proteinase and α-amylase in eggs and larvae, starting from the stage of cleavage up to external feeding (Fig. 4), show that throughout

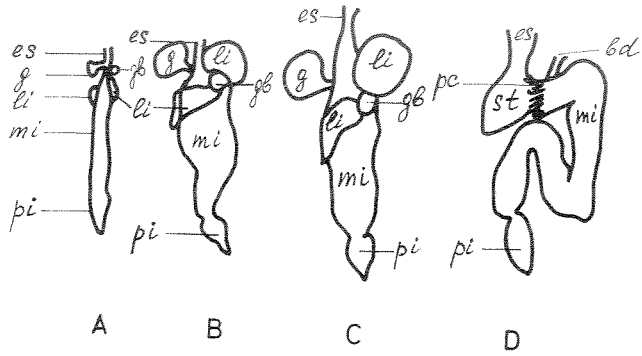

Fig. 3. Diagrammatic outlines of the digestive tract of cod, A) newly hatched larvae, B) prelarvae at mixed feeding, C) larvae after absorption of yolk, D) juvenile cod. Abbreviations: bd - bile duct, es - oesophagus, g - gas bladder, gb - gall bladder, li - liver, mi - middle intestine, pi - posterior intestine, pc - pyloric caecae, st - stomach.

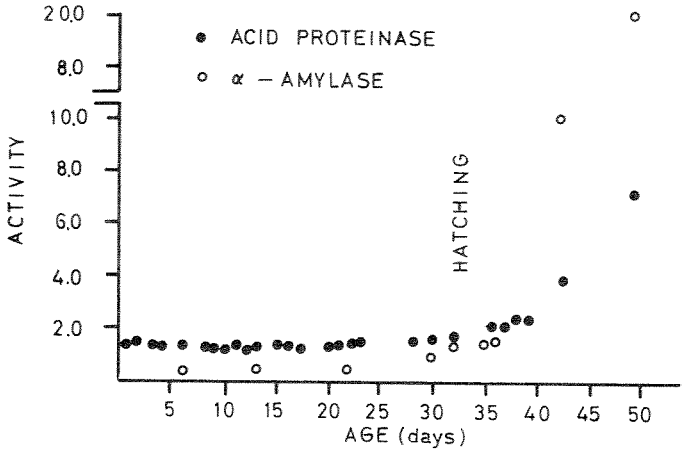

Fig. 4. The activity of acid proteinase and α-amylase in eggs and the cod larvae.

the period of embryogenesis the activity level of these enzymes remains practically constant. Before hatching their activity increases by 1.5 as compared with the cleavage period. A marked increase in the activity is observed at the mixed feeding stage (IX), acid proteinase by three times and amylase by nine times. Consequently, the stage of mixed nutrition in the cod is characterized by an onset of activity of pepsin-like acid proteinase and amylase in the digestive tract.

Stage X. Complete exogenous feeding. The larval length is 5.5, the yolk being completely resorbed. Diffuse yellow pigment appears in the larval body. The structure of the digestive tract remains almost unchanged, with only growth of its sections. It is only in the juvenile period that the digestive tract is markedly differentiated into oesophagus stomach, middle intestine and rectum (Fig. 3D).

There is a continued increase in the activity of acid proteinase and amylase, which is due to the increase in activity of these enzymes in the digestive system. This has been shown by analyses of enzyme activity in isolated digestive tracts and

TABLE 1

The activity of acid proteinase and α-amylase in the digestive tract of cod larvae

	Acid proteinase (pH 3,3) (mkm/g/h)			α-amylase (mg/g/h)		
	whole larvae	digestive tract	trunk (without tract)	whole larvae	digestive tract	trunk (without tract)
Stage of mixed nutrition (5th day after hatching	3,7 3,8	6,0 5,5	2,4 2,4	11,3 11,7	15,6 15,2	
Stage of complete exogenous nutrition (12th day after hatching	6,9 6,2	15,0 16,4	2,5 2,4	20,1 19,8	25,1	2,7 2,8

bodies of cod larvae at stage 0 of both mixed and complete external nutrition (Table 1).

It may be seen that the activity of acid proteinase and amylase in the intestine is several times higher than that in the larval body with the digestive tract removed.

Data on the development of the digestive system give us every reason to believe that it achieves its functional level before the completion of yolk resorption.

Thus the digestive processes of larvae are peculiar in the sense that food organisms pass quickly along the oesophagus and are accumulated in the intestine, where digestion and absorption take place. The function of digestive glands is probably partly taken over by epithelial cells through intracellular and contact digestion.

The morphological features described here and the peculiaritites of morphogenetic processes are, however, only valid only under a definite set of external conditions. For the cod, as for many other species of boreal fish, one of the most effective abiotic factors affecting early ontogenesis is temperature. Since epiboly, gastrulation, axial convergency and differentiation of the axial organs in the embryo are relatively independent processes, the ratio of the rates of these processes differs with temperature (Makhotin and Pavlov, 1981).

Other processes are less obviously dependent on temperature. For example, at 1.5°C melanin appears in promelanophores, when there are 16 pairs of somites in the embryonic body, while at 5°C this occurs with 12 pairs. At 1.5°C the melanophores start branching when there are 30-32 myotomes in the embryonic body and the tail part has begun to segment, while at 5°C this occurs when there are 22 myotomes in the body and before the tail bud differentiates.

At low temperatures somites are first to contract; at high temperatures the heart contracts before the somites.

One of the best known responses to temperature variation is the change in duration of development. This is easily seen by comparing the time of embryogenesis in different populations of the species in the area. The reduced period of embryonic

development at higher temperatures is due not only to the accelerated rate of morphogenetic processes, but also to the disproportionate reduction of mainly stages VI and VII of development; this is reflected in the lower degree of organization of embryos developing at higher temperature as shown in Table 2.

Thus in the cod, as well as in other species of fish prolarvae developing at different temperatures hatching may occur at different stages with a different degree of organization of organs and different sizes of the body. This should produce an impact upon their integration with the environment and future life.

In general, the nature of differences in the degree of prolarval organization at hatching under different temperature

TABLE 2

Effect of temperature on development

Incubation temperature of +6°C	Incubation temperature of +1°C
prolarvae are smaller (on the average 4.2 mm)	prolarvae are larger (on the average 4.9 mm)
hydrosinus underdeveloped	normal development
head (section) is not separated from the yolk sac	the head section is separated, the completely formed gills and jaws begin to function shortly after hatching
eyes are slightly pigmented, little melanin pigment is present, guanine almost absent (no "metallic glitter")	eyes are intensly pigmented ("metallic glitter" present)
vertical stripes of melanin on the body are absent	melanophores are aggregated in vertical stripes in the tail section of the body
the digestive system is represented by the tube and rudimentary liver	a higher degree of complexity of digestive system is observed, the liver being divided into two lobes with a complex structure

conditions is defined both by the changes and the peculiarities of morphogenetic processes and the changes in the parameters of exchange processes of the developing eggs. This is associated to a great extent with the specific nature of the utilization of yolk supplies for growth and development under different temperature conditions, i.e. the redistribution of substances in the embryo-yolk system and the regulation of this process by temperature factors.

It has been shown (Novikov and Kuftina, 1982) that the increase in embryonic protein at the initial stages of embryogenesis is reduced to a minimum. A marked increase in protein begins with organogenesis, followed by intensive growth up to the moment of hatching.

Generally protein increase of cod embryos, as well as other fish, is exponential (Novikov et. al., 1982). At the same time some regularities in change of protein (increase curve Fig. 5A) can be observed with change in temperature.

With the onset of organogenesis, when the temperature rises in the "warm" series, there is a more marked increase in the protein of the embryos, the higher the temperature, the more marked is the correlation. However, towards the beginning "of the functional state of organ formation" (the onset of heart beat, etc.) the growth rate slows down and the embryos of the "cold" series catch up with the "warm" series embryos at this particular stage.

As is known, with the rise of the temperature the rate of protein synthesis increases and from this standpoint the increase in embryonic mass is quite consistent. What is the cause of the subsequent slowing of growth? One of the plausible explanations is that because of the specific features of morphogenetic processes the supply of nutritive yolk substances to the embryo cells is restricted. Due to this, all metabolic processes should be affected mainly at the expense of those yolk supplies which were carried off by the migrating cytoplasm at the moment of blastodisc formation (Lents and Trinkaus, 1967; Trinkaus, 1973; Yurovitskii, 1973). It is this limited supply of energetic and plastic substances that determins maximum embryo size, while variation in temperature accelerate

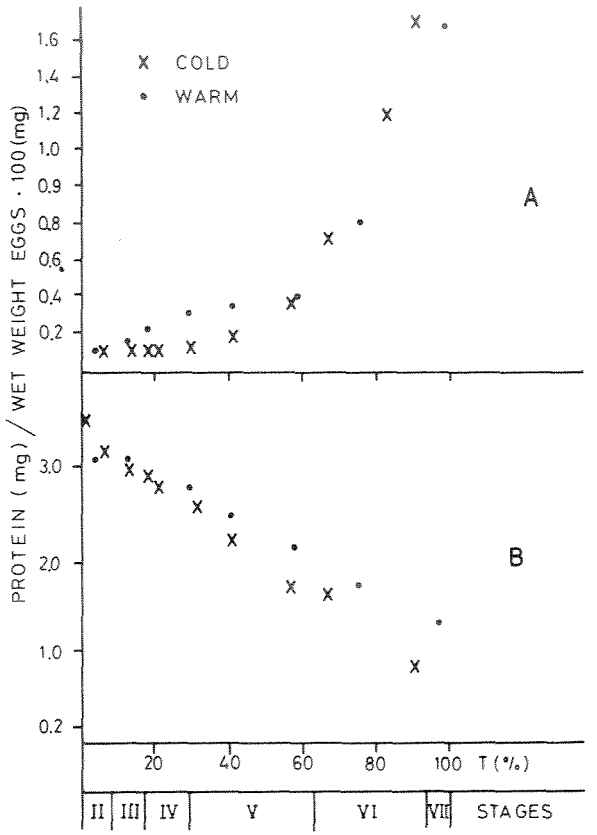

Fig. 5. Growth mass embryo (A) and yolk decrease (B).

or slow down its utilization.

Later, starting from the onset of heart beat and circulation of plasma in the blood vessels, the temperature influences on embryonic protein seem paradoxical at first sight. The embryos in the "cold" series are greater in protein content, than those in the "warm" series. However, when estimating the rate of protein growth per 24 h day it turns out that the growth rate (including the initial stage) is in direct proportion to the temperature - the higher the temperature, the higher the growth

rate (Novikov and Kuftina, 1982). The smaller final amounts of protein mass of the embryos in the "warm" series are the result of disproportionate changes in the rates of the two processes under different temperatures - on the one hand, the protein synthesis rate and, correspondingly, the growth in the embryonic protein content and on the other hand, the development rate,(i.e. changes in the duration of development). With the increase in temperature the reduced development duration occurs at a greater rate than does the increase in protein content.

Against the background of curvilinear dependence of protein increase on temperature, the decrease in yolk supply is practically rectilinear (Novikov and Kuftina, 1982) (Fig. 5B). But at hatching the remaining yolk supply is greater in the embryos of the "warm" series. It has also been established that the activity of proteolytic enzymes which catalyse yolk resoption in the egg and which is practically constant during embryogenesis, varies with the rise in temperature (Timeiko, 1979), and that it increases as the temperature rises. Thus, in this case again, the differences in the final amount of yolk at hatching are caused by disproportionate changes in the rates of the two processes: the increase in resorption rate of yolk, on the one hand, and the decrease in the development duration prior to hatching, on the other, when the incubation temperature is increased.

From the foregoing, it may be assumed that the initial stage of qualitative morpho-physiological differences observed in adult species and populations starts with the embryonic and larval period of development (Soin and Novikov, 1977).

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SECTION II

Larvae. Feeding, growth and behaviour

CHAIRMEN

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