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The Propagation of Cod *Gadus morhua* L.

INGESTION OF BACTERIA BY COD (*Gadus morhua* L.) LARVAE

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ABSTRACT

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The uptake of Rhodamine-labelled bacteria by cod larvae was demonstrated using a fluorescent microscopy technique, which allowed a quick estimate of bacterial uptake and gut clearance. Live or heat-killed bacteria, isolated from cod-eggs, were rapidly ingested by larvae at yolk-sac and older stages. A substantial uptake could be seen after 15 min exposure, and eventually the bacteria became densely packed in the digestive tract.

Some morphological details in the developing gut of the cod larvae could be observed after ingestion of the fluorescent-labelled bacteria. In yolk-sac larvae, bacteria were bound in discrete areas in the midgut, indicating clustering of receptors or internal folding or segmentation of the gut. In older larvae, which had ingested bacteria or other cells, the hindgut was never emptied, not even after starvation. The results indicate that particles of undigested material are retarded in the hindgut, and that probably absorption by endocytosis takes place in this area.

The results reported in this paper point to a possible role for bacteria in the early life of cod larvae. Aspects discussed include the mechanism of uptake of bacteria, entrapment in the gut, digestion or elimination of bacteria by extracellular or intracellular processes, and the role of an indigenous microflora with respect to feeding strategies and health hazards of cod larvae.

INTRODUCTION

Cod larvae in their early life stages encounter a variety of particles, cells and prey organisms of different size and composition. The relative importance of these different food particles for successful growth and survival of the larvae is a matter of much debate, since the basic nutritional requirements and health hazards of the larvae are not understood in detail. This is reflected by the fact that in reports from feeding experiments with marine fish larvae the superiority of a live diet over dead or artificial food to sustain growth beyond metamorphosis is frequently stressed (Theilacker, 1980). Mainly because of their small size, little attention has so far been paid to bacteria, apart from obvious pathogenic ones. The total amount of bacteria in the sea may vary within wide limits ranging from a few hundred to about 10^5 bacteria/ml by direct counts (ZoBell and Feltham, 1938). Recent studies verify that even unpolluted sea-water may contain 10^5 bacteria/ml (i.e. Dundas, University of Bergen, personal communication, 1983).

ZoBell and Feltham (1938) have established the importance of bacteria as food for certain marine invertebrates. They demonstrated that the mussel, *Mytilus californianus*, could be maintained for several months on a diet consisting only of bacteria, and argued that bacteria might cumulatively play an important role for all marine animals by furnishing cell substances or essential micro-nutrients such as essential fatty acids, vitamins, minerals - and even enzymes. The ability of bacteria to grow rapidly on a variety of substrates may be an important factor, and since most marine bacteria are periphytes or epiphytes which grow attached to particles of organic matter, plankton or other solid surfaces, it is inevitable that the natural food available to fish larvae will also be abundant in bacteria. Not infrequently, the mass of bacteria attached to a particle of organic matter or plankton is greater than the mass of the substrate itself (ZoBell, 1936). Bacteria as food for marine animals has, since these early studies, been the subject of a number of investigations by, among others, Zhukova

(1963), Fenchel (1969), Reiswig (1975) and Rieper (1976).

Although digestive enzymes are present in the gut of cod larvae at the time of first food intake, it has been questioned whether cod larvae at an early stage have a digestive system capable of complete extracellular breakdown of ingested food particles (Dabrowski, 1979; Hogendoorn, 1980; Huse et al., 1982; Szlaminska, 1980) or whether they have to rely on partial intracellular digestion (Iwai, 1969; Noaillac-Depeyre and Gas, 1976; Stroband and van der Veen, 1981). The intracellular digestion of proteins by the rectal epithelial cells of larvae and juveniles of five teleost species has been described by Watanabe (1982). For invertebrates, the participation of both fixed and circulating phagocytes in the digestion of food particles is well established (Elston, 1980; Fletcher and Cooper-Willis, 1982; van der Knaap et al., 1981). If endocytosis, or intracellular digestion, is of importance for marine fish larvae, the surface characteristics of the food particles are likely to be critical.

In hatching units the bacterial count may be unexpectedly high (frequently 10^8 bacterial/ml or even above) but with no obvious detrimental effect on the fish larvae, Also, fish eggs, even from unpolluted sea-water, have a relatively high number of bacteria attached to their surface, as revealed by electron-microscopy (S. Lønning, University of Tromsø, personal communication, 1983). The possibility that this could imply the existence of an indigenous microflora, which could serve both to protect and immunize the fish, has not been investigated.

The present paper describes the ingestion of bacteria by cod larvae. The possible mechanisms of recognition, uptake, digestion or elimination are discussed, and the aim has been to focus some attention on the existence and biological significance of a larval microflora.

MATERIALS AND METHODS

Enrichment of bacteria from cod eggs

Cod eggs were collected from hatching units consisting of nets submerged in flowing sea-water at the Marine Biological Station, University of Tromsø, inoculated into culture flasks half-filled with *Vibrio* medium A (Olafsen et al., 1981) and incubated on a rotary shaker at 4°C.

In vivo labelling of bacteria with Rhodamine

An inoculum taken from the enrichment culture was grown in *Vibrio* medium A containing 25 mg/l Rhodamine 6G (BDH Chemicals Ltd) at 4°C. The bacteria were harvested by centrifugation (15000xg, 30 min) and washed in 2 % NaCl by repeated centrifugation until there was minimal coloration of the supernatant. The bacteria were then suspended in sea-water and used immediately.

In vitro labelling of bacteria with TRITC

An inoculum was taken from the enrichment cultures and grown in *Vibrio* medium A (2 l) on a rotary shaker at 4°C until well into the stationary phase, and harvested by centrifugation. The bacteria were washed once in 2 % NaCl by centrifugation, suspended in 2 % NaCl, heat-killed by warming at 60°C for 1 h, centrifuged and resuspended in 1 l 0.1 M carbonate buffer, pH 9.5. The heat-killed bacteria were then incubated with 500 mg of TRITC (tetramethyl-rhodamine isothiocyanate, Grade II, Sigma Chemical Co. Ltd) dissolved in 250 ml dimethylsulphoxide for 2 h (Bergqvist and Norberg, 1974). The resulting TRITC-labelled bacteria were washed extensively by repeated centrifugation in sea-water until no fluorescence could be detected in the supernatant. Bacteria treated in this way were brightly fluorescent, and were stored at -20°C until used. Frozen preparations must be freed from unbound fluorescent material by washing in the centrifuge prior to use.

Incubation of cod larvae with bacteria

Cod larvae were obtained from hatching tanks at the Marine Biological Station, University of Tromsø. Samples of larvae ranging from day 1 to day 20 post hatching were placed in small glass beakers kept at $4^{o}C$. The larval density was about 10 to 30 larvae in 100 ml sea-water. The larvae could be kept alive this way without changing the sea-water for at least one week (the maximum length of any experiment in this study).

A suspension of bacteria was added drop-wise to the larvae. No attempt was made in these experiments to accurately measure the numbers of bacteria added, but the concentrations were estimated to be about 10^{7} to 10^{8} bacteria/ml.

At intervals, cod larvae were transferred to fresh sea-water for 10 to 15 min to remove loosely attached fluorescence, and mounted for microscopical examination.

In some experiments cod larvae were offered a solution of TRITC-labelled glutaraldehyde fixed sheep red blood cells, a gift from Gustav Gaudernack, Institute of Medical Biology, University of Tromsø.

Microscopy

Light and fluorescent microscopy was carried out with a Leitz Orthoplan microscope equipped with Ploemopak vertical fluorescence illuminator and Leitz vario-orthomat camera system for automatic photomicrography. The larvae were mounted unfixed in sea-water for microscopical examination. Tiny blobs of modelling clay under the corners of the cover-glass prevented the larvae from being crushed. The films used were Kodak Ektachrome 160 (or 200) or Verichrome 1000.

RESULTS

Direct phase contrast microscopy of cod larvae and sea-water from hatching units revealed that the larvae were surrounded by massive amounts of bacteria, which were

especially abundant in the mucus layer covering the recently hatched larvae. No direct counts were made, but numbers were estimated by microscopy to be at least 10^7 to 10^8 bacteria/ml.

The uptake of live bacteria could be demonstrated following exposure of cod larvae to bacteria grown in the presence of Rhodamine 6G. However, in vivo labelling of bacteria by this method resulted in weak fluorescence which was quickly lost. Thus, once it was established that live bacteria could be ingested by cod larvae, the more sensitive method of using TRITC-labelled dead bacteria was preferred for the rest of the experiments.

The use of TRITC-labelled bacteria allowed sensitive and rapid measurement of ingestion by cod larvae (Fig. 1A-C). Unfortunately, the fixation methods for larvae tested so far gave serious background fluorescence, and the microscopy was therefore performed with unfixed larvae in sea-water. This method gives no interference from background fluorescence, but only allows 10 to 20 min examination before the larvae disintegrate.

Exposure of cod larvae to TRITC-labelled bacteria resulted in ingestion of the bacteria by all the age groups tested (from about 1 day to 2 weeks post hatching). Following 15 min inoculation of 3-4 day old larvae the midgut was brightly fluorescent, and after 1 h the gut was densely packed with bacteria (Fig. 1C). After a few days incubation, weak fluorescence could be seen to have spread throughout the body of the larvae, especially along the backbone. When the larvae were offered TRITC-labelled bacteria and then starved in filtered sea-water for several days, fluorescence in the fore- and midgut was reduced (but never eliminated), whereas the hindgut always remained fluorescent.

In some experiments bacteria were replaced by TRITC-labelled glutaraldehyde fixed sheep red blood cells. These fixed cells were used because their size (7 µm) was ideal for the study of endocytic processes in fish, they are relatively indigestible, and are used generally to study phagocytosis; also by tissue phagocytes in fish (Fletcher, 1982). Addition of the red cells to the water stimulated feeding behaviour of

the 18-20 day old larvae, and eventually the larvae were feeding actively on sedimented red cells lining the bottom of the tank. Microscopical examination revealed clusters of red cells trapped in the mucus layer around the mouth of the larvae. In larvae that had been exposed for more than 3 days to the red cells (which were then depleted), it was observed that the fluorescent material was predominantly present in the hindgut (Fig. 3). The same phenomenon was observed in larvae that, following incubation with red cells, had been starved for 2 days in filtered sea-water.

It was evident from these experiments that yolk-sac larvae readily ingested bacteria. However, a typical morphological difference was noted in the gut system of these larvae compared with older larvae, as the fluorescence seemed to be arranged in a characteristic pattern in the gut of the yolk-sac larvae (Fig. 2).

The presence of discrete fluorescent cells in the hindgut is shown in Fig. 4A. These structures were too big to be bacteria, thus indicating the presence of phagocytic cells. Unfixed microscopical preparations of intact larvae lasted only for about 15 min before the larvae disintegrated. Frequently, the section near the gut burst, as can be seen in Fig. 4B, which shows the release of fluorescent-labelled cells from the gut. The situation 15 min later, shown in Fig. 4C, indicated that the cells had spread in a manner similar to phagocytic cells.

As can also be seen from Fig. 4B, the gut contained an S-shaped configuration with strong fluorescence at the edges. This could frequently be observed in larvae during the late yolk-sac stage following ingestion of fluorescent-labelled bacteria, but its function is not known.

DISCUSSION

In these experiments it has been shown by fluorescent microscopy that cod larvae rapidly ingest bacteria, both at the yolk-sac and older stages. In yolk-sac larvae the bacteria

were bound in discrete areas in the midgut, indicating the presence of specific receptors or trapping mechanisms in these areas. In older larvae offered bacteria or other cells, and then starved, the remaining fluorescence was restricted to the hindgut, showing a well developed capacity for absorption by cells in this area. These observations pose the question of the role of bacteria in nutrition and health of young fish. By analogy, it could be argued that fish larvae had retained features from lower marine animals, such as the ability to entrap and digest food by endocytic processes (Fletcher and Cooper-Willis, 1982; Iwai and Tanaka, 1968; Watanabe, 1982).

The larvae offered bacteria seemed to thrive, but no attempt was made in these experiments to investigate the properties of bacteria as complete or complementary food for cod larvae, and so the possible importance of ingestion of bacteria by fish larvae can only be speculated on. When it is considered that even unpolluted sea-water may contain 10^5 bacteria/ml, an estimate of 10^7 bacteria/ml in patches surrounding the larvae is not unlikely. Taking the volume of each bacterium to be 1 μm^3, the bacterial biomass would be 10 mg/l sea-water. The wet weight of a larva is about 200 µg, which means that each larva, given a bacterial density of 10^7/ml, has to filter 2 ml of sea-water in order to ingest 10 % of the wet weight of its body. With the very short generation times (less than 1 h) observed for some of the bacteria associated with the hatching of cod larvae (T. Lien, University of Bergen, personal communication, 1983), this production potential could also be sufficient to help sustain growth at a very early stage, considering that the body weight of the larvae does not increase until they are about 14 days old (Hjelmeland et al., 1984).

Against this, it could be held that larvae are unable to actively take up particles of such small size as bacteria. In nature, however, bacteria tend to form clusters or associate with phytoplankton or detritus, and may constitute a substantial portion of such particles (ZoBell, 1936). Nevertheless, when the density of suspended bacteria is high they may make an essential contribution. Some bacteria may be taken up

passively with water intake in order to compensate for osmotic water loss, which is of the order of 2-10 % a day (Riis-Vestergaard, 1984). However, this is not sufficient to account for the substantial uptake observed in these experiments, and it is still questionable whether bacteria are just unspecifically trapped in the mucus, or recognized (agglutinated) by receptor-specific proteins. It has been suggested (Ellertsen et al., 1980) that the "green gut" sometimes observed in yolk-sac larvae is due to *Isochrysis* sp. being unspecifically trapped in the mucus layer, and then ingested.

The characteristic pattern observed in the gut of yolk-sac larvae after ingestion of fluorescent - labelled bacteria (Fig. 2A) possibly reflects morphological or biochemical changes taking place at this stage. Thus, in yolk-sac larvae discrete areas of the gut seem to be equipped to trap or bind bacteria, possibly due to folding, segmentation or clustering of receptors.

At about the time when the yolk-sac is depleted, the hindgut is demarcated from the midgut by a structure visible under the microscope. The hindgut, and possibly also the rear part of the midgut, seems to be the area where particles are trapped. This was demonstrated by the fact that the glutaraldehyde fixed red blood cells, which should be very resistant to extracellular breakdown, were predominantly bound in this area even in starving fish (Fig. 3A). The same was true for bacteria, although they were also bound, or were partly digested, in the midgut. However, when fish were starved following intake of fluorescent-labelled bacteria, the fluorescence was always most predominant in the hindgut. This could imply that the hindgut is especially equipped for trapping particles which are not completely digested in the midgut. These results are in agreement with those of Iwai and Tanaka (1968), who described the posterior gut epithelium of the halfbeak (*Hemiramphus sajori*) larva as consisting mainly of peculiar columnar cells with acidophilic granules which were implicated in the absorption and probably ingestion of protein. In marine and other invertebrates it has been demonstrated that fixed phagocytic cells play an important role in food

uptake and digestion (Elston, 1980; Fletcher and Cooper-Willis 1982; van der Knaap et al., 1981). Furthermore, the release of fluorescent cells from the bursting gut of cod larvae that had been offered TRITC-labelled bacteria is indicative of endocytic processes, although it is not conclusive that intact bacteria were trapped by these cells. Microscopy of thin sections of the gut system of such larvae may help to reveal this.

Nevertheless, even if the natural abundance of bacteria and their uptake by fish larvae is recognized, a high bacterial density in conjunction with hatching fish automatically leads to a fear of disease. The use of antibiotics is then frequently recommended, even though it is reported that pathogens such as *Vibrio anguillarum* (Rødsæther et al., 1977; Olafsen et al., 1981) and *Aeromonas hydrophila* (Gatesoupe, 1982) may be isolated from healthy fish, and that their pathogenic behaviour is most likely triggered by alterations in the host - pathogen relationship caused by environmental or other factors. In warm-blooded animals the beneficial role of an indigenous microflora serving both to protect and immunize the animal, and which is often inherited through birth, is appreciated. The possibility that the skin mucus microflora of fish may be assigned a similar role, and may be passed on through the egg microflora, deserves further attention. The skin mucus of fish is generally assigned a defensive role (Fletcher, 1982; Hjelmeland et al., 1983) and it is suggested that it is an active part of the immune system of the fish (Bradshaw et al., 1971). The presence of antibodies in the mucus has been demonstrated (Fletcher and Grant, 1969) and it is known that fish may be immunized through the skin by immersion (Egidius et al., 1982). Thus, it seems evident that the microflora surrounding the fish larvae may be assigned a more complex function than is usually admitted.

The problems associated with mass rearing cod larvae will not be fully understood and eliminated until the nutritional requirements and health hazards are revealed in more detail. In this context an understanding of the role of bacteria during early larval life is clearly of importance.

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REFERENCES

Bergqvist, N.R. and Norberg, R., 1974. Determination of specific antibody content of fluorescent conjugates by radioimmunoabsorption. J. Immunol. Meth., 5: 275-282.

Bradshaw, C.M., Richard, A.S. and Sigel, M.M., 1971. IgM antibodies in fish mucus. Proc. Soc. Exp. Biol. Med., 136: 1122-1124.

Dabrowski, R.K., 1979. The role of proteolytic enzymes in fish digestion. In: European mariculture society, No. 4. Cultivation of fish fry and its live food, pp. 107-126.

Egidius, E., Andersen, K., Clausen, E. and Raa, J., 1982. Bath-vaccination against vibriosis. Dev. Comp. Immunol., Suppl. 2: 193-196.

Ellertsen, B., Moksness, E., Solemdal, P., Strømme, T., Tilseth, S. and Øiestad, V., 1980. Some biological aspects of cod larvae (*Gadus morhua* L.). Fisk Dir. Skr. Ser. HavUnders., 17: 29-47.

Elston, R., 1980. Functional morphology of the coelomocytes of the larval oysters (*Crassostrea virginica* and *Crassostrea gigas*). J. Mar. Biol. Ass. U.K., 60: 947-957.

Fenchel, T., 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated protozoa. Ophelia, 6: 1-182.

Fletcher, T.C., 1982. Non-specific defence mechanisms of fish. Dev. Comp. Immunol. Suppl. 2: 123-132.

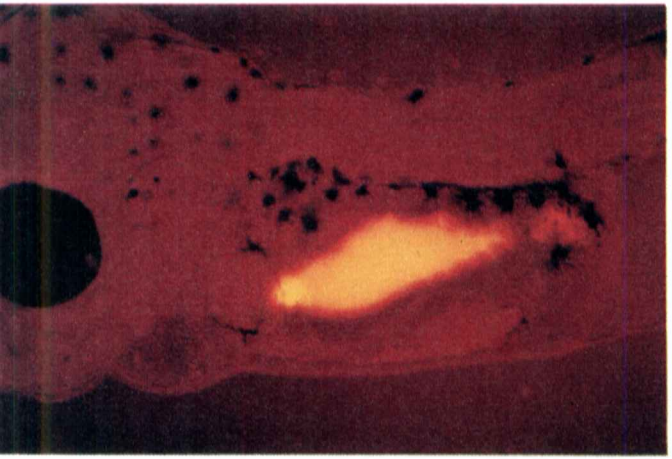
Fletcher, T.C. and Grant, P.T., 1969. Immunoglobulins in the serum and mucus of the plaice *Pleuronectes platessa* (L.). Biochem. J., 115: 65.

Fletcher, T.C. and Cooper-Willis, C.A., 1982. Cellular defence systems of the mollusca. In: N. Cohen and M.M. Sigel (Editors), The reticuloendothelial system, Vol. 3., Plenum Publishing Co., pp. 141-166.

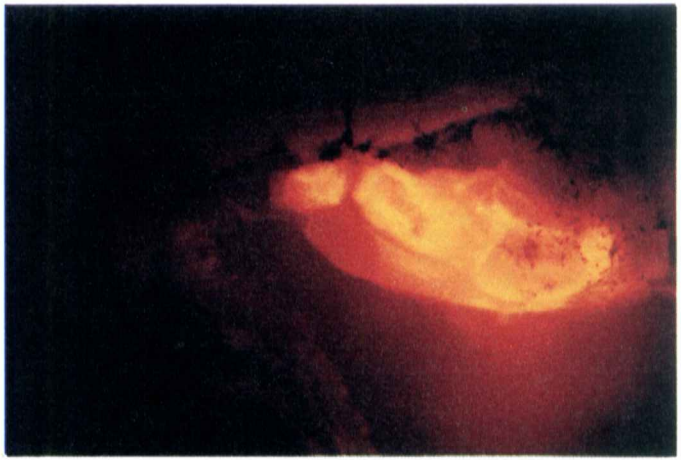
Gatesoupe, F.J., 1982. Nutritional and antibacterial treatments of live food organisms: The influence of survival, growth and weaning success of turbot (*Scopthalmus maximus*).

Coun. Meet. Int. Coun. Explor. Sea, 1982 (F:10), 1-14, 3 Figs and 7 Tables (Mimeo.).
Hjelmeland, K., Christie, C. and Raa, J., 1983. Skin mucus protease from rainbow trout, *Salmo gairdneri* Richardson, and its biological significance. J. Fish Biol., 23: 13-22.
Hjelmeland, K., Huse, I., Jørgensen, T., Molvik, G. and Raa, J., 1984. Trypsin and trypsinogen as indices of growth and survival potential of cod (*Gadus morhua* L.) larvae. In: E. Dahl, D.S. Danielssen, E. Moksness and P. Solemdal (Editors), The Propagation of Cod *Gadus morhua* L., Flødevigen rapportser., 1, 1984: 189-202.
Hogendoorn, H., 1980. Controlled propagation of the African catfish, *Clarias lazera* (C. & V.). III. Feeding and growth of fry. Aquaculture, 21: 233-241.
Huse, I., Opstad, I., Klungsøyr, L. and Walther, B., 1982. Endocrine and nutritional factors affecting the first food uptake by cod larvae. Coun. Meet. Int. Coun. Explor. Sea, 1982 (F:17), 1-7, 5 Figs (Mimeo.).
Iwai, T., 1969. Fine structure of gut epithelial cells of larval and juvenile carp during absorption of fat and protein. Arch. Histol. Jap., 30: 183-199.
Iwai, T. and Tanaka, M., 1968. The comparative study of the digestive tract of teleost larvae - III. Epithelial cells in the posterior gut of the halfbeak larvae. Bull. Jap. Soc. scient. Fish, 34: 44-48.
Noaillac-Depeyre, J. and Gas, N., 1976. Electron microscopic study on gut epithelium of the tench (*Tinca tinca* L.) with respect to its absorptive functions. Tissue & Cell, 8: 511-530.
Olafsen, J.A., Christie, M. and Raa, J., 1981. Biochemical ecology of psychrotrophic strains of *Vibrio anguillarum* isolated from outbreaks of vibriosis at low temperature. Zbl. Bakt. Hyg., I. Abt. Orig. C2: 339-348.
Reiswig, H.M., 1975. Bacteria as food for temperate-water marine sponges. Can. J. Zool., 53: 582-589.
Rieper, M., 1976. Investigations on the relationships between algal blooms and bacterial populations in the Schlei Fjord (Western Baltic Sea). Helgolander wiss. Meeresunters., 28: 1-18.
Riis-Vestergaard, J., 1984. Water balance in cod eggs. In: E. Dahl, D.S. Danielssen, E. Moksness and P. Solemdal (Editors), The Propagation of Cod *Gadus morhua* L., Flødevigen rapportser., 1, 1984: 87-103.
Rødsæther, M.C., Olafsen, J., Raa, J., Myhre, K. and Steen, J.B., 1977. Copper as initiating factor of vibriosis (*Vibrio anguillarum*) in eel (*Anguilla anguilla*). J. Fish Biol., 10: 17-21.
Stroband, H.W.J. and van der Veen, F.H., 1981. Localization of protein absorption during transport of food in the intestine of the grasscarp *Ctenopharyngodon idella* (Val.). J. Exp. Zool., 218: 149-156.
Szlaminska, M., 1980. A histochemical study of digestive enzymes in pike larvae. Fish. Mgmt., 11: 139-140.
Theilacker, G.H., 1980. Rearing container size affects morphology and nutritional conditions of larval jack mackerel, *Trachurus symmetricus*. Fish. Bull., U.S., 78: 789-791.

van der Knaap, W.P.W., Sminia, T., Kroese, F.G.M. and Dikkeboom, R., 1981. Elimination of bacteria from the circulation of the pond snail *Lymnea stagnalis*., Dev. Comp. Immunol., 5: 21-32.
Watanabe, Y., 1982. Intracellular digestion of horseradish peroxidase by the intestinal cells of teleost larvae and juveniles. Bull. Jap. Soc. Sci. Fish., 48: 37-42.
Zhukova, A.I., 1963. On the quantitative significance of microorganisms in nutrition of aquatic invertebrates. In: C.H. Oppenheimer (Editor), Symposium on marine microbiology, D.D. Thomas, Springfield, Illinois, pp. 699-710.
ZoBell, C.E., 1936. Periphytic habits of some marine bacteria. Proc. Soc. exp. Biol., N.Y., 35: 270-273.
ZoBell, C.E. and Feltham, C.B., 1938. Bacteria as food for certain marine invertebrates. J. Mar. Res., 1: 312-327.



A



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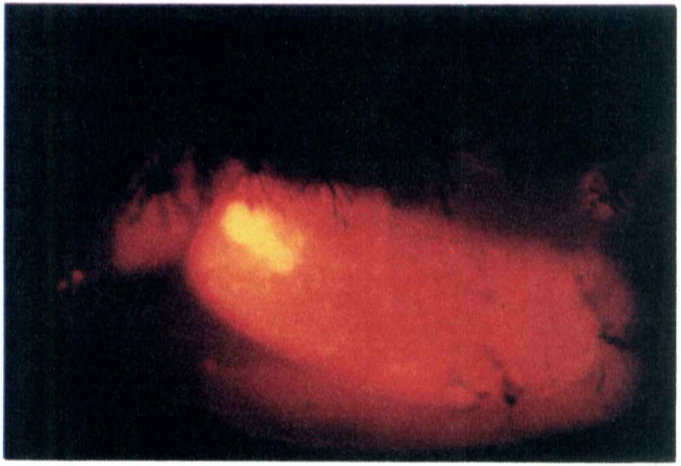
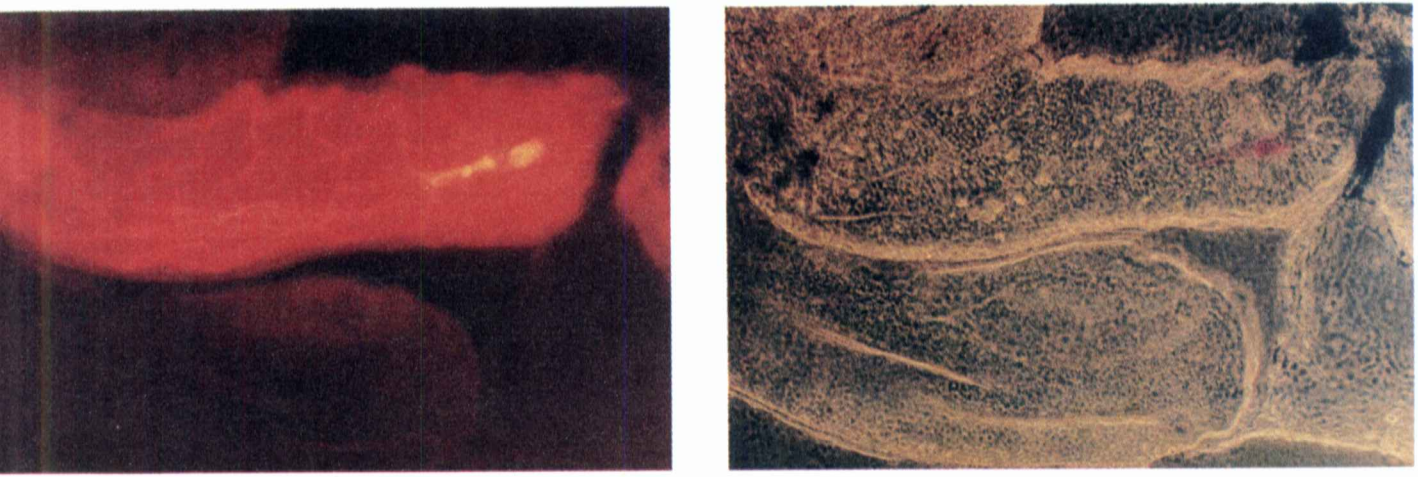
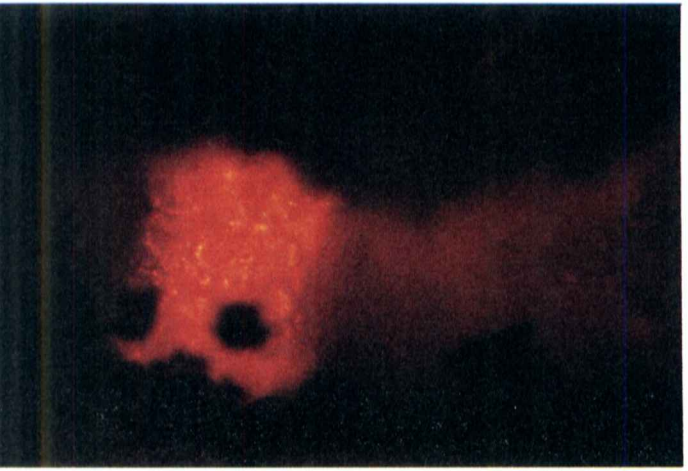


Fig. 1. Photomicrograph of a 3 - 4 day old cod larva offered TRITC-labelled bacteria for 1 h (C), 1 day (B) and 2 days (A), respectively; demonstrating the uptake of fluorescence in the digestive system. A, combined fluorescence and phase contrast; B, C, fluorescence. Leitz NPL Fluotar 10/0.3 (A, B) and 16/0.45 (C); Kodak Ektachrome 200.



A B

Fig. 2. Photomicrograph of a 3 - 4 day old yolk-sac larva offered TRITC-labelled bacteria for 1.5 h (hindgut to the right). A, fluorescent and B, phase contrast microscopy of the same section of the digestive system. Note typical pattern of fluorescence in the midgut. Leitz NPL Fluotar 16/0.45; Kodak Verichrome 1000.



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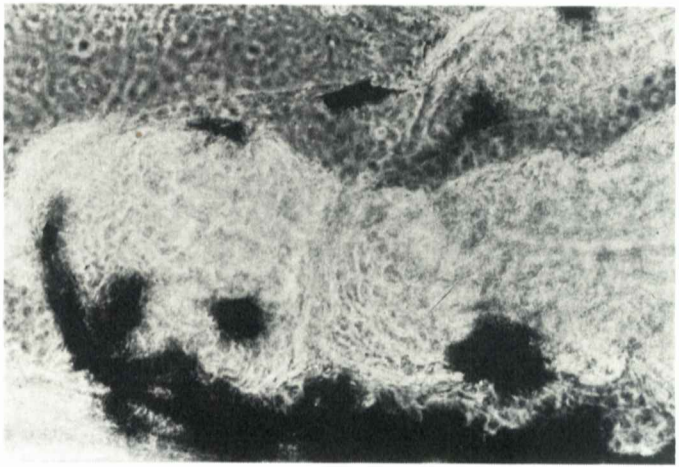
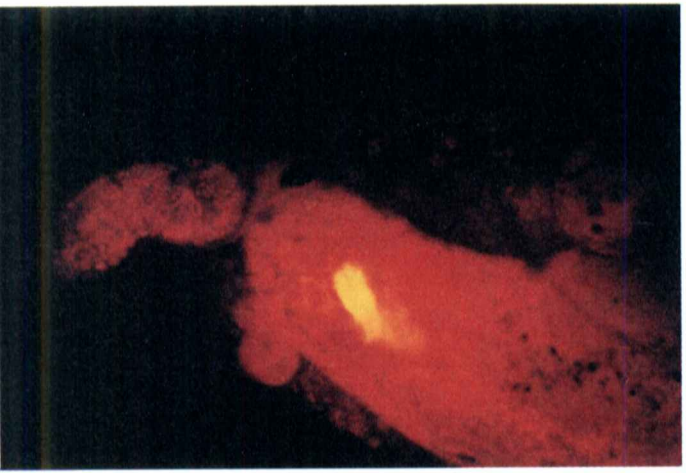


B

Fig. 3. Photomicrograph of the hind- and mid-gut of an 18 - 20 day old cod larva offered TRITC-labelled glutaraldehyde fixed sheep red blood cells for 3 days. A, fluorescent and B, phase contrast microscopy of the identical part of the digestive system, showing that the fluorescent material is restricted to the hindgut (left). Note the demarcation between the hind- and mid-gut. Leitz NPL Fluotar 25/0.55; Kodak Ektachrome 160.



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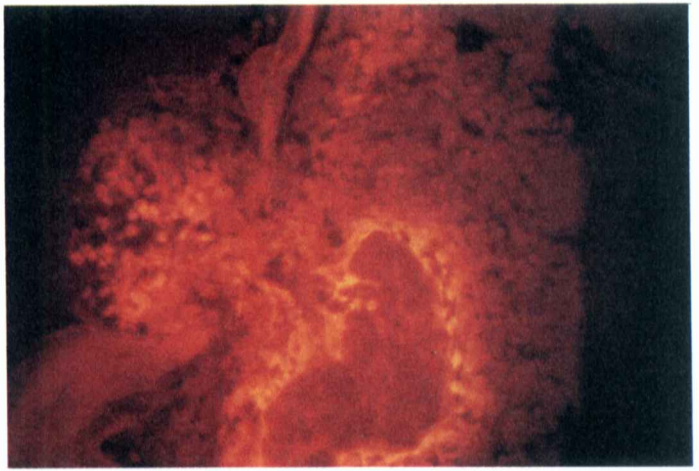

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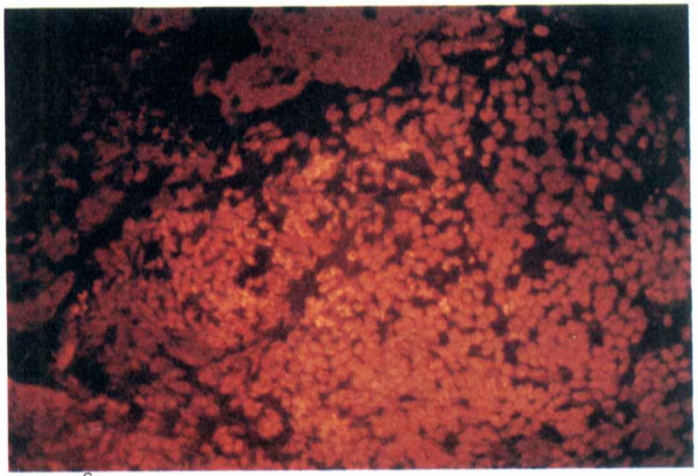

C

Fig. 4. Photomicrograph of the mid- and hind-gut (A) and section of bursting gut (B, C) in a 3 - 4 day old cod larva offered TRICT-labelled bacteria for 2 days. Note the intact cells in the hindgut (A), and the fluorescent cells exuding from the gut (B), followed by the situation in the same magnification 20 min later (C). Leitz HPL Fluotar 160/0.45 and 250/0.55; Kodak Ektachrome 160.