THE ROLE OF FILTRATOR MOLLUSCS RICH IN CAROTENOID IN THE SELF-CLEANING OF FRESH WATERS

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Abstract

Large fresh-water bivalvian molluscs (*Unio, Anodonta*) are able to filter 20–40 l of polluted water in 24 h and to extract nutrient compounds from the filtered water. They can concentrate inedible organic and mineral residues as a detritus, which is degraded at the next stage of the aquatic biological system of self-cleaning. Molluscs play a fundamental part in the self-cleaning system.

Tolerance of the molluscs of low oxygen levels and the action of toxic agents and their ability to survive in anabiotic state under unfavourable conditions are important for the functioning of molluscs as a component of the biological self-cleaning system in polluted waters.

Molluscs with high carotenoid content are strongly resistant to environmental pollution. The population of these molluscs increases in response to pollution. On the other hand, the population of molluscs with low carotenoid content decreases when pollution increases.

Carotenoids together with haemoproteins and some respiratory enzymes form a special intracellular organoid (carotenoxysome) which is apparently capable of producing energy under conditions when mitochondria are not efficient. Carotenoids provide for an oxygen reserve in the carotenoxysome (by acting as accumulator of oxygen or of equivalent electron acceptors).

The preservation and cultivation of molluscs rich in carotenoid are very important aspects of positive human actions in the protection of the environment.

INTRODUCTION

At present the problems of biological self-cleaning of polluted waters are of great importance because the anthropogenic pollution of waters often exceeds permissible limits. The role of phytoplankton, zooplankton, micro-organisms and higher water plants in self-cleaning is well known. But far less is known about the outstanding role of molluscs in the self-cleaning of polluted waters and their possible use in the system of biomonitoring.

THE ROLE OF MOLLUSCS
IN THE CLEANING OF POLLUTED WATERS

The participation of bivalve filtrator molluscs in the cleaning of polluted waters is connected with the mode of their nutrition. The large bivalves, e.g. *Unio* and *Anodonta* can filtrate as much as 20–40 l of water per 24 h, extracting this way suspended particles of both organic and inorganic nature, as well as a number of organic compounds (Table 1). Filtered

TABLE 1

The Rate of Water Filtration by Some Bivalve Mollusc Species
in Relation to their Body (Shell) Length (ml per mm per h) at 16–17 °C

Species	Rate of water filtration	References	
Anodonta piscinalis	14.44 ± 0.70	Kondratiev	1963
Unio tumidus	11.90 ± 0.54	Kondratiev	1963
Unio pictorum	8.36 ± 0.42	Kondratiev	1963
Dreissena polymorpha	4.80 ± 0.80	Kondratiev	1963
Mytilus edulis	25.00 ± 60.00	Voskresensky	1948
Mytilus edulis	26.25 ± 32.72	Willemsen	1952
Cardium edule	15.48	Willemsen	1952

microorganisms and particles of organic nature enter the digestive system of the filtrator molluscs, while 'inedible' particles (including mineral oil drops and other pollutants) are ejected through the delivery syphon in the form of clods. This waste contains organic compounds together with wrapping slime and serves as a complex food for microorganisms. Microorganisms, in turn, serve as food for detrivorous animals, among them apparently some gastropod molluscs.

The efficiency of self-cleaning of polluted waters depends mainly on the quantity and activity of the filtrator molluscs because the ability of microorganisms to mineralize the organic waste and mineral oil depends on the preliminary concentration of these substances by molluscs (Fig. 1).

A number of experimental data allows us to judge the efficiency of the cleaning activity of the fresh-water molluscs. For example, 200 Unio having the average weight of about 70 g, were used for cleaning the water of the River Don which was polluted by anthropogenic waste in a quantity of 50 mg
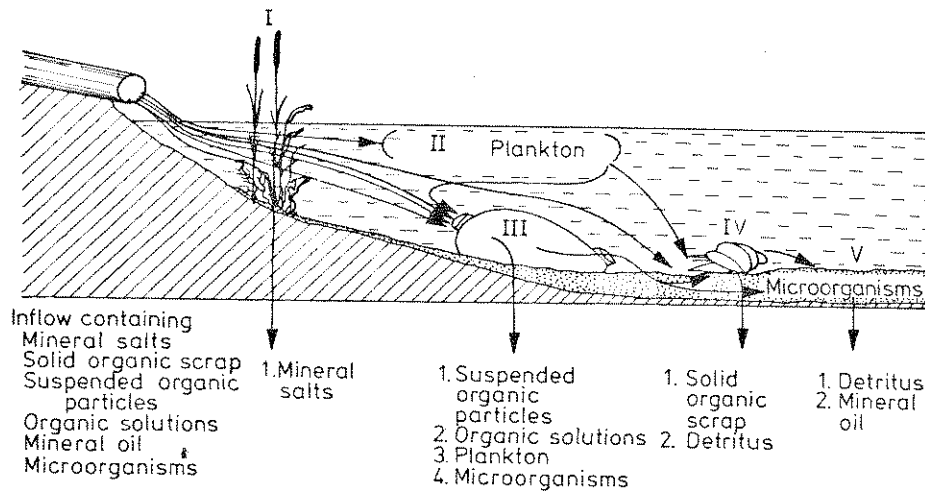

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Mineral salts
Solid organic scrap
Suspended organic particles
Organic solutions
Mineral oil
Microorganisms

1. Mineral salts

1. Suspended organic particles
2. Organic solutions
3. Plankton
4. Microorganisms

1. Solid organic scrap
2. Detritus

1. Detritus
2. Mineral oil

Fig. 1. The interrelation of higher water plants (I), phyto- and zooplankton (II), microphagous filtrator molluscs (III), detrivorous molluscs (IV) and microorganisms (V) in the biological self-cleaning of natural aquatic systems

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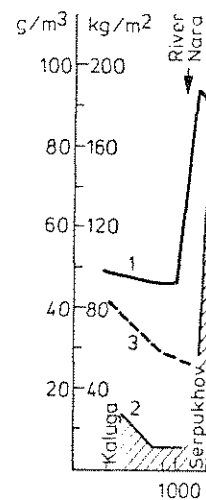dry weight of o
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per l of suspended particles. The molluscs filtered 3 m³ water in 24 h and after that the quantity of suspended particles was 0.2 mg per l (Skadowsky 1961). This means that one mollusc removed 99.5 per cent of the particles from 15 l water each day.

Naturally, in more polluted waters the rate of cleaning decreases. The molluscs *Unio* and *Anodonta* kept in the water of the Irtish-Karaganda Canal filtered 5 m³ of water per 24 h. The quantity of suspended particles decreased from 562 mg per l to 238 mg per l, while the amount of dissolved organic compounds decreased from 395 mg per l to 254 mg per l (Bervald). Thus the molluscs removed 58 per cent of the suspended particles and 35 per cent of dissolved organic compounds, i.e. 1.5 kg of suspended particles and 0.7 kg of dissolved organic compounds per day. A total amount of 63 kg dry weight of organic matter was precipitated in a container in 27 days. During the experiment the average weight of one mollusc increased from 219 to 245 g, i.e. by 26 g. The whole weight of the molluscs in the container increased by almost 3 kg.
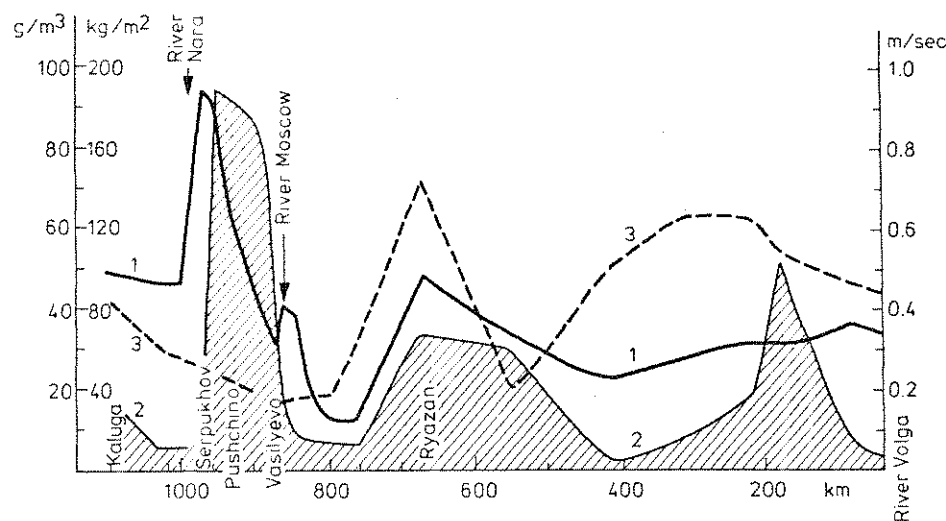

Fig. 2. The quantities of pollutants, in g per m³ (curve 1), of the molluscan biomass, in kg per m² of river-bed cross-section (curve 2), and the rate of water flow, in m per sec (curve 3) along the River Oka in 1959 (after Shadin 1964)

The role of molluscs in the cleaning of polluted natural waters can be seen from the investigations carried out on the River Oka (Shadin 1964*a*). Near Kaluga, the number of filtrator molluscs is not very large and the pollution of the water (50 g of suspended particles per 1 m³) along a distance of about 80 km remains practically the same (Fig. 2, curve 1). Near the town of Serpukhov, the pollution of the River Oka sharply increases, reaching 95 g of suspended particles per 1 m³. Eighty km below Serpukhov, the density of large filtrator molluscs (*Unio* and *Anodonta*) reaches 150 specimens per m² of bottom, and water pollution decreases by 70 per cent; the level is about 30 g of suspended particles per 1 m³ of water at the inflow of the River Moscow into the River Oka. Thus, owing to the high density of filtra-

tor molluscs (Fig. 2, curve 2), the River Oka is cleaned of industrial and other wastes entering the river at Serpukhov at a distance of 50 km. Unfortunately, the same cannot be stated about the River Oka below the town of Kaluga, because the number of filtrator molluscs is not large enough there.

Comparing the results of investigations carried out on the River Oka in 1923–1924 and 1959 (Shadin 1964b) it can be seen that the level of pollution had increased in the course of 35 years, especially below Serpukhov. Together with an increase in pollution at some places in the river, a sharp increase in the number of the filtrator molluscs *Unio* and *Anodonta* was recorded. For example, below Serpukhov in the region of the town of Pushchino, the biomass of molluscs increased from 0.75 kg to 183 kg per 1 m^2 of river-bed cross-section, which is an almost 200-fold increase. The density of *Unio* and *Anodonta* in some places reached 158 specimens per m^2 of bottom. The density of the gastropods *Viviparus viviparus* also increased to 594 specimens per m^2 (Table 2). It should be noted that increased pollution

TABLE 2

Population of Molluscan Species (in Number of Specimens per m^2 of Bottom at Two Stations on the River Oka in 1959) (Shadin 1964)

Species	Pushchino	Vasilyevo
Unio pictorum	60	26
Unio tumidus	72	46
Anodonta piscinalis	26	—
Anodonta anatina	—	24
Viviparus viviparus	594	—
Sphaerium rivicola	54	—

causes a considerable growth of bivalves like *Unio* and *Anodonta*. Other species such as *Sphaerium solidum* and *Pisidium supinum* become unstable with the increase of water pollution, and their number declines sharply. In some places of the river they have entirely disappeared (Shadin 1964b).

THE PHYSIOLOGICAL MECHANISMS UNDERLYING THE TOLERANCE
OF MOLLUSCS TO ENVIRONMENTAL POLLUTION

It can be seen that only those filtrator molluscs are capable of cleaning water which are resistant to pollution, including the increase of toxic agents, mineral salts and a decrease in the oxygen content of the environment.

Among the physiological mechanisms providing for the high resistance of molluscs to unfavourable environmental conditions one should be particularly emphasized: this is the ability of the molluscs to fall into anabiosis when the rate of metabolism in the cells sharply declines and, consequently, the oxygen uptake from the environment also decreases. Under these conditions, bivalves close their valves tightly, stop their filtrating activity, and there is a decrease in oxygen consumption (Salánki 1965, 1968).

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It is apparently anabiosis that enables molluscs to survive for a long time in hypoxic conditions (sometimes for several days). Five specimens of *Mytilus galloprovincialis* were placed in a hermetic vessel (one litre volume). It was shown (Karnaukhov et al. 1977a) that when the oxygen concentration was decreased, the rate of oxygen consumption of the molluscs also decreased. After 10 h, the oxygen consumption rate was 1/10, while after 30–40 h, 1/100 of the initial value. Under these conditions, the oxygen consumption rate was 0.05 ml O_2 per h per 100 g tissue. Data in Fig. 3 show that the molluscs placed in the hermetic vessel fell into anabiosis within 6–10 h. Their oxygen consumption rate was slowed down when about 20 per cent of the initial oxygen level was still present in the vessel. In the anabiotic state the molluscs consumed this oxygen slowly and died after 7 days, when oxygen concentration became zero.

Fig. 3. Change of oxygen concentration in hermetic vessels (1 l volume) containing 5 specimens each of *Mytilus galloprovincialis* in pure sea water (curve 1) and in water polluted by 1 ml of mineral oil (curve 2)
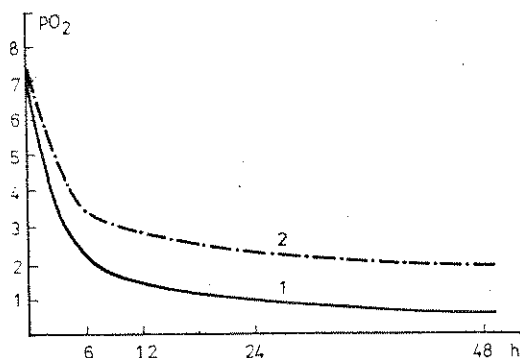
If 1 ml of mineral oil is added into the hermetic vessel, molluscs fall into anabiosis more quickly, at an oxygen content of 40 per cent in the vessel. In this case molluscs can survive up to 12 days. It is interesting to note that *Mytilus galloprovincialis* can survive in oxygen-free water only for 8 hours (Zs.-Nagy and Ermini 1972).

The anabiotic state of the molluscs is analogous to mammalian hibernation. It is characterized by a decrease in all metabolic processes, including the synthetic activity of cells and the rate of ATP consumption in molluscan tissues. Apparently, this was the cause of the high ATP concentration measured in molluscan tissues (Zs.-Nagy and Ermini 1972) under hypoxic conditions.

THE ROLE OF CAROTENOIDS IN OXIDATIVE METABOLISM. CAROTENOXYSOME

It is evident from the physiological mechanism of the resistance of the animals to low oxygen levels and to toxic agents that some molecular mechanisms must exist that provide energy for cells, first of all for nerve cells, in hypoxic conditions. Carotenoids are considered to be part of such a molecular mechanism (Karnaukhov 1969a, 1973a).

Microspectral (Karnaukhov et al. 1966, Karnaukhov 1971) and chemical (Karnaukhov et al. 1970, Andreyev et al. 1971) investigations of the giant

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neurones of the mollusc *Lymnaea stagnalis* have allowed us to establish that the yellow and orange colour of the giant neurones (Fig. 4) is caused by carotenoids and haemoproteins localized in cytoplasmatic granules (Fig. 5) with specific ultrastructural organization (Karnaukhov and Varton 1971). These granules have been called 'cytosomes' by Nolte et al. (1965) who found some respiratory enzymes in them.

The absorption spectra of these granules (cytosomes) show certain changes in the neurones of molluscs in the anaerobiotic state as well as in response to inhibitors of oxidative metabolism (Karnaukhov 1968, 1969b, 1971) suggesting that carotenoids participate in the oxidative metabolism. Based on these findings, we have put forward a hypothesis on the function of carotenoid-containing granules in the cells (Karnaukhov 1969a, 1970, 1971, 1973b). According to this hypothesis, carotenoids may act as electron acceptors, and, together with haemoproteins, form a system of intracellular oxygen reserve (accumulator) in the cytosomes (Fig. 6). Thus, cytosomes can provide energy for the cell when the rate of oxygen penetration into the tissue is low.

The electron-acceptor and electron-donor properties of the conjugated double-bond chain of carotenoids (Pullman and Pullman 1963) allow it to connect an oxygen molecule in place of a (central) unsaturated double bond with the help of a haemoprotein. The decrease of double bonds in the conjugated chain of carotenoid leads to the loss of its colour. The colourless, oxygenated carotenoid may serve as an electron-acceptor equivalent of molecular oxygen and can be considered analogous with the oxidized form
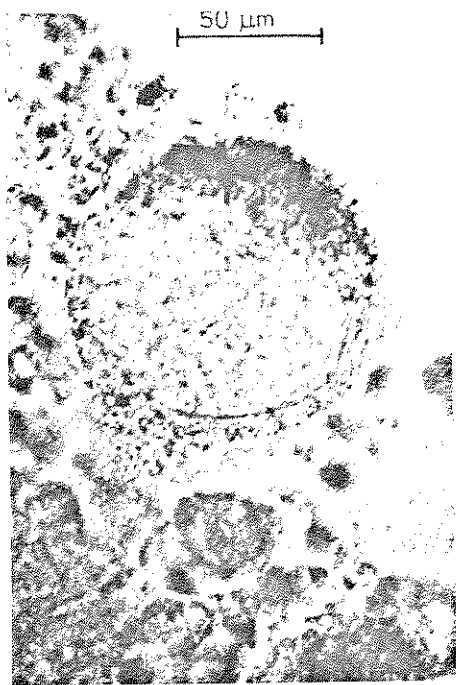

Fig. 4. Neurones of *Lymnaea stagnalis*

Fig. 5 a and
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Fig. 5 a and *b*. Ultrastructural organization of carotenoxysomes (cytosomes) in *Lymnaea stagnalis* neurones

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of the well-known cytochrome oxidase ($a + a_3$) of the mitochondrial respiratory chain. Carotenoids can accumulate (or concentrate) oxygen (or electron-acceptor equivalents of oxygen) in resting cells under conditions of low-rate oxygen penetration (in hypoxic condition). When the cells enter an active phase under these conditions, the rate of their oxygen consumption (solid line 1 in Fig. 7a) becomes higher than the rate of oxygen penetration from the environment (base line 2 in Fig. 7a). The oxygen deficiency arising in this situation is relieved by oxygen released from the carotenoid intracellular accumulator (Fig. 7b) and carotenoids once more assume their colour. When the cell returns to rest, the oxygen reserve in the carotenoid intracellular accumulator can be restored (Karnaukhov 1973a).

Such granules rich in carotenoid (Fig. 6) are characteristic not only of the molluscan cells (cytosomes; Nolte et al. 1965, Zs.-Nagy 1967, 1971, Zs.-Nagy and Kerpel-Fronius 1971b, Karnaukhov and Varton 1971), but also of plant cells (carotenoid plasts; Matienko and Chabanu 1973), of the cells of warm-blooded animals and also of man (lipofuscin granules; Björkerud 1963, Karnaukhov 1973b, Karnaukhov et al. 1972, Karnaukhov and Fedorov 1977). Therefore they might be considered universal energy providing organoids of cells, phylogenetically older than mitochondria. Therefore we

Fig. 6. Interrelation between the respiratory chains of mitochondria and of carotenoxysomes

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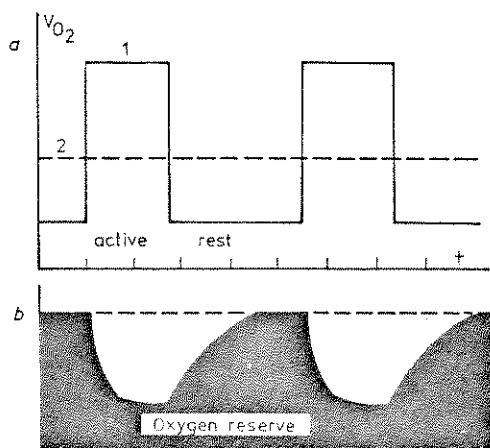homogenate but
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Fig. 7a. Scheme of interrelation between the rate of oxygen consumption of the cell at rest and in the active state (solid line 1) and that of oxygen penetration into the cell from media (broken line 2) under hypoxic conditions. *b.* Intracellular oxygen reserve (or its electron-acceptor equivalent) bound by carotenoid

have suggested the introduction of the term *'carotenoxysome'* for general use (Karnaukhov 1976).

The system of terminal oxidation localized in the carotenoxysomes is similar to the mitochondrial substrate–NADH system. The mitochondria are connected with carotenoxysomes by a specific regulatory mechanism whereby the system of the carotenoxysomes is not active during normal mitochondrial activity. At low oxygen levels or if mitochondrial cytochrome oxidase $(a + a_3)$ is inhibited by KCN, mitochondria lose their activity, and the NADH oxidation system localized in carotenoxysomes becomes activated.

The existence of such a relationship has been proved by studying the respiration of the nerve tissue homogenate of the mollusc *Lymnaea stagnalis* (Petrunyaka et al. 1974). The homogenate contained both mitochondria and carotenoxysomes. KCN was found to inhibit the respiration of the homogenate (though it did not block respiration completely). Addition of NADH sharply increased (7–8 times) the oxygen consumption rate (Fig. 8a). The effects observed did not depend on the sequence of introducing the substrate (NADH) and the inhibitor (KCN). Addition of NADH to the fresh homogenate incresaed the oxygen consumption rate. This effect can be abolished by sodium amital, a specific mitochondrial inhibitor, while by adding KCN, a sudden rise in the respiration rate can be observed again (Fig. 8b). The degree of KCN action on NADH oxidation is dependent on KCN concentration (Fig. 8c).

Increase of the absorption bands of carotenoids in the absorption spectra of carotenoxysomes was found in living molluscan neurones under the influence of KCN. After treatment with sodium amital this effect was not observed (Karnaukhov 1969b, 1971, 1973a). This refers to the relationship between KCN-stimulated NADH oxidation and carotenoxysomes.

The NADH oxidizing ability of the homogenate when the respiratory chain of mitochondria is blocked by KCN testifies to the presence of a special NADH-dehydrogenase in the carotenoxysome structure (Fig. 6). Stimulation of NADH oxidation by adding KCN (Fig. 8a, b) was observed only in fresh homogenate but was absent after a day-long storage in a refrigerator at 4 °C, or when it was heated up to 90 °C. When typical mitochondrial sub-

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strates, such as α-ketoglutaric acid or succinic acid, were used, addition of KCN to the homogenate resulted in respiratory inhibition, which is quite usual for mitochondria.
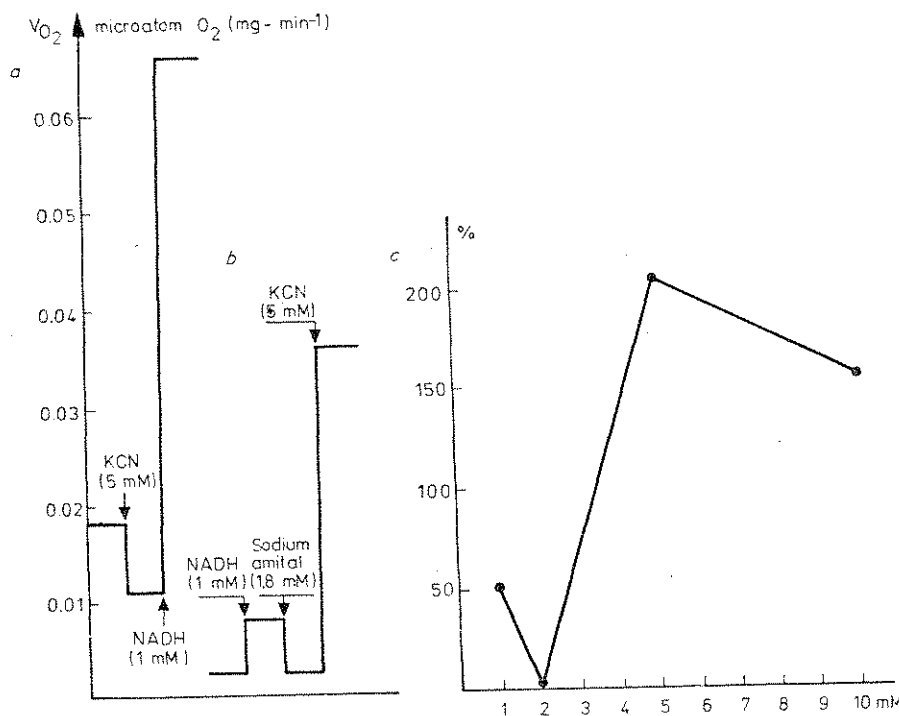

Fig. 8a, b. Action of KCN (5 mM), sodium amital (1.8 mM) and NADH (1 mM) on the rate of oxygen consumption of molluscan (*Lymnaea stagnalis*) nerve tissue homogenate blocks. *c.* Change of NADH oxidation as a function of KCN concentration

The experimental data obtained show that the carotenoxysomes are capable of oxidizing substrates and apparently of producing energy when mitochondria fail to function due to oxygen deficiency or suppression by inhibitors. This conception is in accordance with the results obtained for energy-dependent Sr^{++} accumulation in molluscan nerve cells (Zs.-Nagy and Kerpel-Fronius 1970a). It has been shown that under normal aerobic conditions, Sr^{++} is accumulated mainly in the mitochondria and, to a lesser degree, in the cytosomes (carotenoxysomes). Under anaerobic conditions the situation is reversed: Sr^{++} accumulation is observed mainly in cytosomes (carotenoxysomes), while mitochondria show very weak activity.

The above considerations allow us to suppose that the high resistance to environmental pollution is characteristic of mollusc species with high carotenoxysome content, which thus contain large amounts of carotenoids in their tissues.

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(1) *Flexopecten ponticus*
(2) *Donax trunculus*
(3) *Gouldia minima*
(4) *Polititapes aurea*
(5) *Pitar rudis*
(6) *Chemellea gallina*
(7) *Acanthocardia tubercu*
(8) *Cerastoderma glaucum*
(9) *Mytilus galloprovincia*
(10) *Tritia reticulata*
(11) *Cerithium vulgatum*

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THE ROLE OF CAROTENOIDS IN THE TOLERANCE
OF MOLLUSCS TO ENVIRONMENTAL POLLUTION

Our conception was tested experimentally by studying the concentration of carotenoids in the body of several species of Black Sea molluscs with different degrees of tolerance of environmental pollution. Pollution is accompanied by a decrease of dissolved oxygen and by an increase of different respiratory inhibitors in the water (Karnaukhov et al. 1977a, b). We had access to the data on quantitative and qualitative changes of littoral animals in the Black Sea, collected over many years at the Novorossiisk Biological Station and at the Institute of South Seas Biology (Zernov 1913, Milovidova 1966a, b, 1975). The effect of oil pollution was particularly interesting from the point of view of elaborating biodegradation methods for cleaning polluted waters (Mironov 1973, Milovidova 1975).

The most important results of the study are shown in Table 3 and Fig. 9, listing different mollusc species in order of their tolerance of environmental pollution. The degree of tolerance is expressed by a system of four grades elaborated on the basis of observations on the bottom biocenosis of the Black Sea littoral (Zernov 1913, Milovidova 1966a, b, 1972).

Group I includes the pure-water inhabitants: *F. ponticus* and *D. trunculus*, which have been living recently only on the shore (sea coast). In the past these species used to live in large numbers in Sevastopol Bay and the middle part of Novorossiisk Bay (Zernov 1913). This is proved also by the large number of empty shells of these species found on the bottom of the bay. At that time the water's oxygen deficit amounted to 1.8 mg O_2 per l in the Novorossiisk Bay. The increasing pollution and silt accumulation had led to the gradual disappearance of these species from the bay; now they only live near the bay mouth.

TABLE 3

Comparison of Some Black Sea Mollusc Species as Regards Their Degrees of Tolerance of Environmental Pollution and the Carotenoid Concentration in Their Bodies

Species	S_{10}	T	N	Carotenoid concentration in mg per 100 g tissue (wet wt)	
				Total	U nsaponifiable
(1) *Flexopecten ponticus*	—	1	21	trace	trace
(2) *Donax trunculus*	—	1	28	1.32	0.33
(3) *Gouldia minima*	—10	II	46	0.01	trace
(4) *Politilapes aurea*	— 7.6	II	20	0.08	0.06
(5) *Pitar rudis*	— 6.6	II	20	0.24	0.20
(6) *Chemellea gallina*	— 2.8	II	30	0.42	0.08
(7) *Acanthocardia tuberculata*	+ 2.5	III	7	1.71	1.62
(8) *Cerastoderma glaucum*	+ 2.1	III	11	2.06	0.92
(9) *Mytilus galloprovincialis*	—	III	117	2.00	0.70
(10) *Tritia reticulata*	+ 2.0	III	571	2.05	0.90
(11) *Cerithium vulgatum*	—	III	19	2.60	1.38

$S_{10} = k(n_0/n_{-10})^k$: Population change during 10 years.
n_0 and n_{-10} Number of specimens per 1 m² of bottom recently and 10 years earlier, respectively; $k = 1$ if $n_0 \geq n_{-10}$ and $k = -1$ if $n_0 < n_{-10}$.
T: Degree of tolerance of environmental pollution.
N: Number of specimens studied.

Species which populate sea areas with moderate pollution belong to group II. In 1967 the water oxygen deficit in these areas was 2.04–2.34 mg O_2 per l (Milovidova 1972). The degree of tolerance of these species, *G. minima, P. aurea, P. rudis,* and *C. gallina* (see Table 3), was established on the basis of the decrease of population from 1960 to 1969, since pollution had been increasing during this period of 10 years. The decrease in population number with an increase in pollution is characteristic of the species of group II (3–6 in Fig. 9*b*).
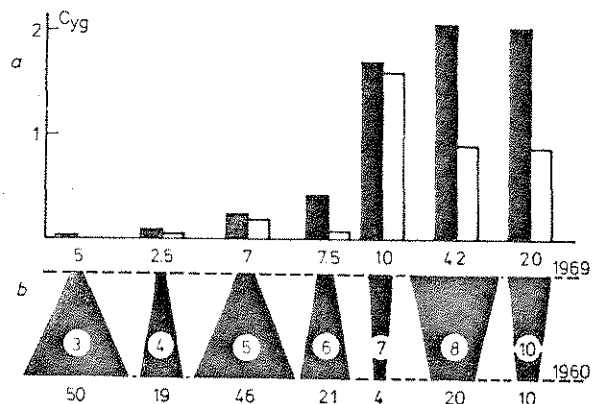
Fig. 9a. Total (black blocks) and unsaponifiable (white blocks) carotenoid concentrations (in mg per 100 g wet weight of tissue) in molluses. *b.* Change of the population of molluscan species (No. = number of specimens per 1 m² of bottom) in response to pollution which increased from 1960 to 1969 in the Novorossiisk Bay. For the serial numbers indicating molluscan species see Table 3

Group III includes species inhabiting the strongly polluted area of the bays, where water oxygen deficit was 2.64–2.94 mg O_2 per l in summer 1967 (Milovidova 1972). An increase of the population parallel with increasing pollution is characteristic of the species *A. tuberculata, C. glaucum,* and *T. reticulata* (7, 8, 10 in Fig. 9*b*). It must be noted that the data presented in Fig. 9*b* refer to a definite region in the central part of Novorossiisk Bay, where water oxygen deficit changed from 1.9 to 3.1 mg O_2 per l during 1960–1969. Sanitary protective measures taken in the bay in 1968–1969 had led to a sudden decrease in the pollution rate. This resulted in a decrease of the water oxygen deficit and a gradual recovery of bottom biocenosis. Therefore, the data showing the molluscan populations in this transition period are not considered in the present report.

The mollusc species studied can be definitely classified into three groups on the basis of their different degrees of tolerance of environmental pollution accompanied by a decrease in dissolved oxygen concentration and by an increase in various toxic substances. Comparison of the data presented in Table 3 and Fig. 9 shows that the species of Groups II and III differ consid-

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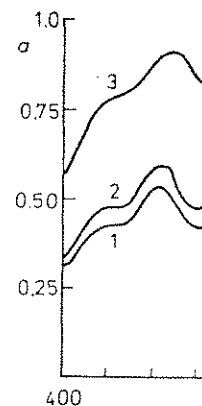*Fig. 10.* Visible light abs
petroleum) from equal an
(*a*) and *Tritia reticulata* (
3, 4) bays. Ord

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erably as regards the carotenoid concentrations in their bodies. A low concentration of carotenoids is characteristic of group II, the population of which decreases as pollution increases. At the same time and at the same place, the species of group III are characterized by a high carotenoid concentration (0.7–1.62 mg unsaponified carotenoids per 100 g wet weight) in their bodies. These species respond to increased pollution by increasing their population.

It is believed that the existence of such a strong correlation between the degree of molluscan tolerance of environmental pollution and the carotenoid concentration in their bodies supports our hypothesis that carotenoids are involved in the adaptation of the animal to hypoxic conditions. However, the existence of species like those included in group I shows that other environmental factors, e.g. silt accumulation, may have similar effects.

It must be stressed that the specific carotenoid concentrations presented in Table 3 and Fig. 9a were found in animals obtained from relatively pure areas of the sea. At the same time, the species of group III (*C. glaucum*, *T. reticulata*) taken from strongly polluted areas had higher carotenoid concentrations (Fig. 10). Probably this is due to the adaptation of animals to a higher degree of environmental pollution.

Analysing the data of Table 3 and Fig. 9, it is necessary to point out that species Nos 3 to 8 belong to the order Venerida. The species *G. minima*, *P. aureus*, *P. rudis*, *C. gallina* of the family Veneridae are characterized by low carotenoid concentration and by low tolerance to environmental pollution, while *A. tuberculata* and *C. glaucum*, belonging to the same order but to the family Cardiidae, have high carotenoid concentration and high tolerance to environmental pollution as well.

According to the hypothesis formulated earlier (Karnaukhov 1969a, b, 1970, 1971, 1973a, b) the utilization of carotenoid conjugated double bonds for oxygen accumulation results in the loss of colour of carotenoid. On the contrary, the withdrawal of the accumulated oxygen from the colourless
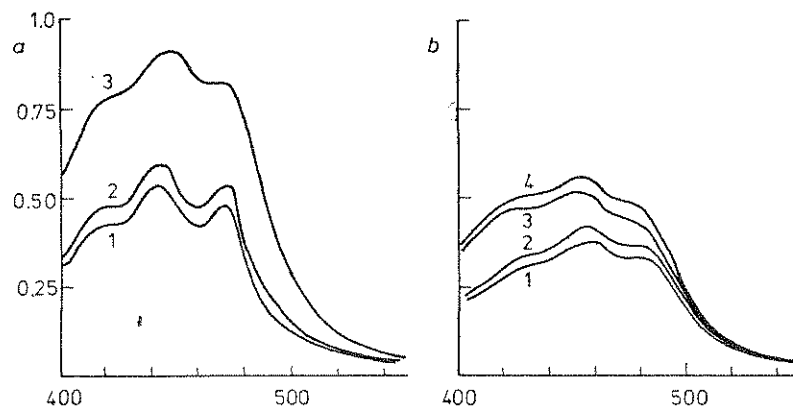

Fig. 10. Visible light absorption spectra of unsaponifiable carotenoid extracts (light petroleum) from equal amouts (P = 1 g; V = 5 ml) of tissues of *Cerastoderma glaucum* (a) and *Tritia reticulata* (b), which inhabit clean (curves 1, 2) and polluted (curves 3, 4) bays. Ordinate: optical density; abscissa: wavelength in nm

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carotenoid under hypoxic conditions is accompanied by reconversion of the carotenoid into coloured form with characteristic three-band absorption spectra in the visible region.

This allows us to expect an increase in the coloured carotenoid concentration in the body of molluscs having been kept under hypoxic conditions.

To test this hypothesis, *Mytilus galloprovincialis* was studied. 250 specimens obtained from a moderately polluted area of the sea were placed in an aquarium with slow perfusion of sea water polluted by mineral oil. Under these conditions the molluscs remained in an active state (the valves were open), but they died after 50–60 h. Concentration of carotenoids in the animals was measured before, and 6, 24 and 48 h after the beginning of the experiment. The carotenoids were extracted from 20 specimens simultaneously, and the experiment was repeated four times for each point. The data obtained are presented in Fig. 11. The most remarkable increase in carotenoid concentration was detected in the first 6 h of the oil treatment and hypoxia. After 48 h carotenoid concentration reached 6–10 mg per 100 g tissue (i.e. 3–4 times higher than the initial one) and then the molluscs died within 2–3 h.

From the time of survival of *Mytilus* in oxygen-free water (8 h) (Zs.-Nagy and Ermini 1972b) and assuming that molluscs are in anabiotic state under these conditions (the rate of oxygen consumption is 0.05 ml per h per 100 g wet tissue), the intracellular reserve of oxygen (or its electron acceptor
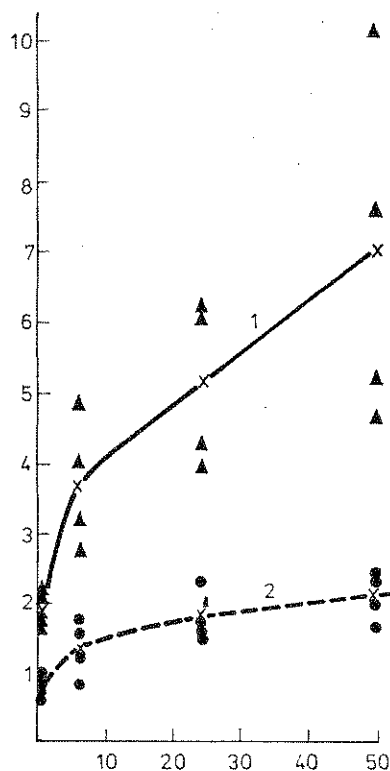
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Fig. 11. Change of average concentration (in mg per 100 g wet weight) of total (curve 1) and unsaponifiable (curve 2) carotenoids in the body of *Mytilus galloprovincialis* under the action of slow perfusion (hypoxia) of mineral-oil-polluted sea water. The dots are data of the pooled tissue of 20 specimens. Abscissa: time of perfusion in h

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equivalent) required for survival can be calculated. The intracellular reserve of oxygen (or its equivalent) for 8 h survival is 0.4 ml O_2 per 100 g tissue $(1 \times 10^{19}$ molecules $O_2)$. Taking into account that 1 mg carotenoid contains 1.2×10^{18} molecules, and assuming that one carotenoid molecule can bind one oxygen molecule, it is clear that 8.3 mg of carotenoids per 100 g tissue is needed for *Mytilus* to survive for 8 h in oxygen-free water. This calculated value is close to the total carotenoid concentration (7.4 mg per 100 g tissue) which has been measured in molluscs kept in polluted water for 48 h (curve 1 in Fig. 11), when the intracellular oxygen reserve was not completely exhausted.

In case of fresh-water molluscs, too, it can be expected that their tolerance of environmental pollution is determined by the carotenoxysomes in their cells and, consequently, by the high carotenoid content in their tissues. The presence of the carotenoxysomes (cytosomes) in *Anodonta cygnea* L. cells was repeatedly demonstrated by investigators at the Tihany Biological Research Institute (Zs.-Nagy 1967, Zs.-Nagy and Kerpel-Fronius 1970*a*, *b*). They also showed that the carotenoid content in the tissue of the ganglia of *Anodonta cygnea* reaches up to 10 mg per 100 g wet weight (Lábos et al. 1966).

In addition, it must be remembered that carotenoids are not uniformly distributed in the animal's tissues and cells. A higher carotenoid concentration is characteristic of the nerve tissue. For example, the carotenoid concentration in the nerve tissue of the fresh-water snail *Lymnaea stagnalis* is 30–40 mg per 100 g wet tissue. Apparently, the carotenoid system for intracellular accumulation of oxygen (or of its electron acceptor equivalent) is an exclusive property of tissues and cells which are more significant for the animal's survival.

THE USE OF MOLLUSCS IN THE PROTECTION OF WATER BODIES

In conclusion, the fundamental role of the molluscs for the self-cleaning of polluted waters must be emphasized again. Among the actions directed towards the preservation of the environment the recultivation of molluscs, particularly of those rich in carotenoid in waters where they have been killed by pollution, is one of the most urgent tasks. Further, these molluscs need strong protection where they still survive; and, finally, experiments have shown that molluscs rich in carotenoid may be used as components of an artificial biocenosis with high resistance to pollution as well as for the cleaning of polluted waters.

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