Copyright ©

Kieler Meeresforschungen	Sonderheft **4**	Kiel 1978

Review of experiments on the chronic toxicity exerted by some pollutants on animal species from the Bay of Gdańsk[1]

F. Pautsch

Department of Biology, Medical Academy, 80–211 Gdańsk, Poland

Abstract

Subject of the experimental investigations are the effects of chronic toxicity exerted by the following pollutants of industrial origin on some animals from the Bay of Gdańsk: 1. phosphogypsum; 2. some detergents, i.e. a commercial product "SOLO", a mixture of nonionic and anionic surfactants (for household purposes), and an oil-spill remover Gamlen "CW" Solvent; 3. crude Kuwait oil and one of its derivatives, the fuel oil No. III. – The experimental animals are: *Crangon crangon L.*, *Rhithropanopeus harrisi* (*Gould*), the crucial carp (*Carassius carassius L.*), the pike (*Esox lucius L.*) and the perch (*Perca fluviatilis L.*). Beside these inhabitants of the Bay of Gdańsk, also carp fry is used in one of the experiments. – The pollutants mentioned above induce sublethal changes in: the enzymic system, the reproductive activity, embryonic and larval development. Additionally, degenerative changes in the ultrastructure of crustacean brain and pathological disturbances in function and structure of isolated mitochondria could be observed. The general conclusion is that chronic sublethal toxicity may severely affect or even destroy some marine ecosystems.

Zusammenfassung

Experimente über die chronische Toxizität einiger Schadstoffe auf Tierarten aus der Danziger Bucht

Gegenstand der experimentellen Untersuchungen sind die Folgen chronischer Toxizität folgender Schadstoffe auf einige Tiere der Danziger Bucht: 1. Phosphorgips, 2. einige Detergentien, z.B. das Haushaltsreinigungsmittel "SOLO", eine Mischung nichtionischer und anionischer oberflächenaktiver Substanzen und das Öldispersionsmittel Gamlen „CW" Solvent, 3. Kuwait-Rohöl und eine seiner Fraktionen, das Heizöl Nr. III. – Versuchstiere sind: *Crangon crangon L.*, *Rhithropanopeus harrisi* (Gould), die Karausche (*Carassius carassius L.*), der Hecht (*Esox lucius L.*) und der Barsch (*Perca fluviatilis L*). Neben diesen in der Danziger Bucht vorkommenden Arten wird in einem Experiment auch Karpfenbrut untersucht. Die oben erwähnten Schadstoffe verursachen subletale Änderungen des enzymatischen Systems, der Reproduktionsrate, der Embryonal- und der Larvalentwicklung. Darüber hinaus werden degenerative Veränderungen der Ultrastruktur im Crustaceenhirn und pathologische Funktions- und Strukturabweichungen an isolierten Mitochondrien beobachtet. Als übergeordnete Schlußfolgerung ergibt sich, daß subletale chronische Giftwirkung einige marine Ökosysteme schwer schädigen oder gar zerstören kann.

[1] This study was supported by the Polish Academy of Sciences as a part of project 10.2.

Introduction

The interest in the experimental approach to the problem of pollution in the sea is rapidly growing. The reason for this is that the elder, I dare say classic method, based on ecological observations made directly in the sea, does not fulfill all the demands of modern science. The lethal concentrations of toxicants may be roughly estimated by observations of ecosystems in nature, but precise data can be obtained only by laboratory experiments. On the other hand such results can be transferred to nature only with caution and they must be integrated with the results of the classic method. This is the only way to obtain precise information on the state of pollution in a given area.

The first experiments concerning the harmful effects of pollutants were based on measurements of their acute toxicity. This method may be useful, because it allows us to estimate the state of pollution by simple and quick tests.

A very good example of the fruitful interaction of the two methods is given by BYRNE and CALTER (1977). In experiments with the clam *Mercenaria* sp. it was shown that after exposure to the water-soluble fraction (WSF) of crude oil in concentration of 2 ppm only, the LC_{50} was reached in 10 days. According to ecological observations concentrations of similar range may occur in the sea as a consequence of oil-spills, as it was proved in the case of the Chevron Main Pass Block 41 (McAULIFFE et al., 1975). These concentrations may persist for a period longer than 10 days. So it was not only estimated, but exactly proved that oil-spills may be the cause of lethal acute toxicity.

In recent years more and more attention has been given to chronic toxicity. In the past this less spectacular effect of pollution has been overseen or neglected. Nowadays it is known that chronic toxicity, like a creeping disease, may display disastrous consequences. Among the first who emphasized the importance of chronic toxicity were GEORGE (1970) and SWEDMARK et al. (1971).

A good definition of this activity of pollutants is given by ROSENTHAL (1975). Sublethal effects of pollutants are defined as those pollutant-induced morphological, physiological and behavioural changes occuring at egg and larval stages that may reduce survival potential at a later stage. Here I would like to add sublethal effects on the physiological functions of adults which in the long range may be a threat to survival too.

In our experiments we tried to get information about the effect of some pollutants on 1. the enzymic system, as an important factor of metabolism, and on 2. reproduction, embryonic and larval development.

The latter problem is of special interest because of the many discrepancies of opinions as to the sensitivity of developmental stages to pollutants. Many authors observed higher sensitivity of these stages in comparison with adults (SWEDMARK et al., 1971 – in fish, bivalves and crustacea; WELLS, 1972 and WELLS et al., 1976 – in lobster larvae; LINDEN, 1976 – in *Gammarus oceanicus*; NICOL et al., 1977 – in *Mellita quinquiesperforata*). But a few authors are of different opinions. RENZONI (1973) claims that there are no differences of sensitivity between larvae and adults.

These differences come probably not only from the diversity of species used, but also from the different character of the tested pollutants. Beside these two main problems a few experiments of other character were additionally carried out.

Material and methods

The pollutants used in our experiments were of industrial origin. They belonged to three groups, that is: phospho-gypsum, then two commercial detergent products, i.e. a household preparation called ''SOLO'' and Gamlen ''CW'' Solvent, an oil-spill remover, and finally Kuwait crude oil with one of its derivatives, the Polish fuel oil No. III PS/c–96048.

Let me explain the choice of these toxicants. Phospho-gypsum (PG) is a waste product of the phosphate fertilizer industry. It is a mixture of compounds, some of them containing phophorus: Insoluble compounds (about 75%):

$CaSO_4.2H_2O$	96.1%
$Ca_3(PO_4)_2$	2.3%
$NaSiF_6$	1.0%
SiO_2	0.3%
CaF_2	0.2%
Al_2O_3	traces
Fe_2O_3	traces

Soluble compounds: (about 25%)

H_2SO_4	2.5%
H_3PO_4	2.0%
H_2SiF_6	0.1%
H_2O	95.4%

PG is known to have an eutrophication effect, but its toxicity has never been investigated before. PG does not actually pollute the Bay of Gdańsk, but it is produced in millions of tons yearly by industry located in the area and there have already been suggestions made to dump it into the waters of the Bay.

Such a procedure is used by some factories in other countries, but the hydrographic conditions of coastal waters there are very different from these in the Bay of Gdańsk. The latter area is extremely sensitive and dumping PG here could have catastrophic results. Therefore the masses of PG, stored now on land, represent a potential danger.

The importance of detergents as pollutants is growing in the Bay and the Vistula delta. This river carries large quantities of detergents, chiefly of household origin, from inland to coastal waters. Already in 1973–1974 the waters of the Dead Vistula, an arm of the main stream, contained detergents in concentrations ranging from 1.89 to 2.64 ppm (the differences caused by seasonal fluctuations) (DREWA et al., 1975).

''SOLO'' is a mixture of three surfactants, two nonionic and one anionic:

1. Alfenol 8 = alkylphenol ethoxylate (nonionic)	19.4%
2. Olbrotol 18 = Oleylethyl alcohol ethoxylate (nonionic)	4.85%
3. Detepon T = Triethanolamine salt of alkylbenzene sulphonate (anionic)	7.25%
4. Water	67.80%
5. Addenda	0.70%
	100.0

The composition of Gamlen ''CW'' Solvent is a secret of the producer.

The importance of oil in the Gdańsk region is increasing too, since a refinery has been established here. Although there are efficient installations to prevent pollution, the possibility of an occasional oil-spill, for instance during unloading tankers, cannot be excluded.

The fuel oil No. III, used beside crude Kuwait oil in some of the experiments, is a mixture of aliphatic hydrocarbons, the chains ranging from C14 to C20, with boiling temperatures between 251–308 °C.

Our primary interest concerned reaching purely scientific results, but we hope that these may be useful to ecologists too. This reasoning explains also the choice of the experimental animals used.

The Bay of Gdańsk is an area heavily threatened by pollution. Therefore almost all the used species are inhabitants of Polish coastal waters, mainly the Bay of Gdańsk itself. They are: the shrimp *Crangon crangon L.*, the crab *Rhithropanopeus harrisi* (*Gould*), the crucial carp (*Carassius carassius L.*), the pike (*Esox lucius L.*) and the perch (*Perca fluviatilis L.*). Only in one experiment carp fry was used.

The number of experimental and control animals, as well as the concentrations of the used pollutant will be given together with the description of the respective experiments.

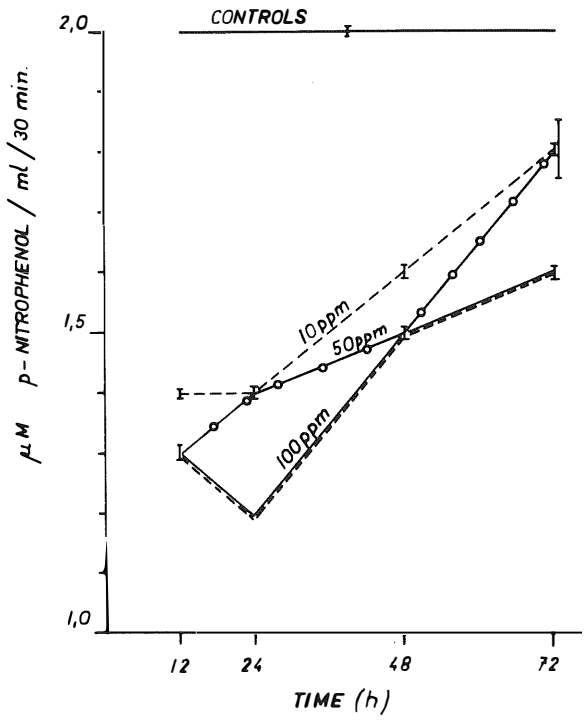

Figure 1

Acid phosphate activity in hemolymph of *Crangon crangon* after exposure to different concentrations of "SOLO", expressed in µM of p-nitrophenol / 30 min

Results

a) Enzymic system

Changes in the activity of the following enzymes could be detected: some lysosomal hydrolases, i.e. sulphatases, cathepsin D, acid phosphatase, and also in some other enzymes, such as tyrosinase, alanine aminotransferase and asparagine amino transferase.

In four series of experiments changes in the activity of hydrolases in crustacean hemolymph after exposure to "SOLO", crude oil and fuel No. III were observed. The hydrolases were: acid phosphatase and cathepsin D in *Crangon* (DREWA et al., 1977), arylsulphatases and acid phosphatase in *Rhithropanopeus* (DREWA et al., unpubl.).

Acid phosphatase in *Crangon* after exposure to "SOLO" or crude oil showed a decrease of activity until the 24th h of the experiment, later turning to a slight increase but without reaching the level in the controls (Fig. 1, Fig. 2). The behaviour of cathepsin D was different. After exposure to "SOLO" there was an initial increase

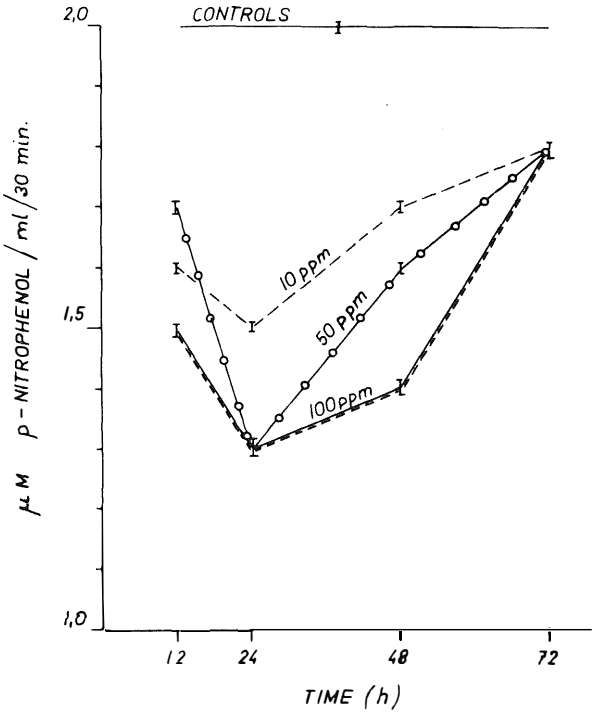

Figure 2

Acid phosphatase activity in hemolymph of *Crangon crangon* after exposure to different concentrations of crude oil, expressed in µM of p-nitrophenol / 30 min

of activity, later followed by decrease, but not reaching the level in the controls (Fig. 3). Exposure to crude oil caused an initial increase too, but after the first 24 h of the experiment there was a sharp dropping, far below the control level (Fig. 4). In each experiment groups of 42 animals were used.

In *Rhithropanopeus* after exposure to "SOLO" the activity of arylsulphatases A and B was significantly lower than in the controls (Fig. 5). Exposure to crude oil had a rather similar effect on these enzymes (Fig. 6). And a decrease of activity of acid phosphatase after exposure to "SOLO" or crude oil also could be observed (Fig. 7). In each experiment groups of 30 animals were used.

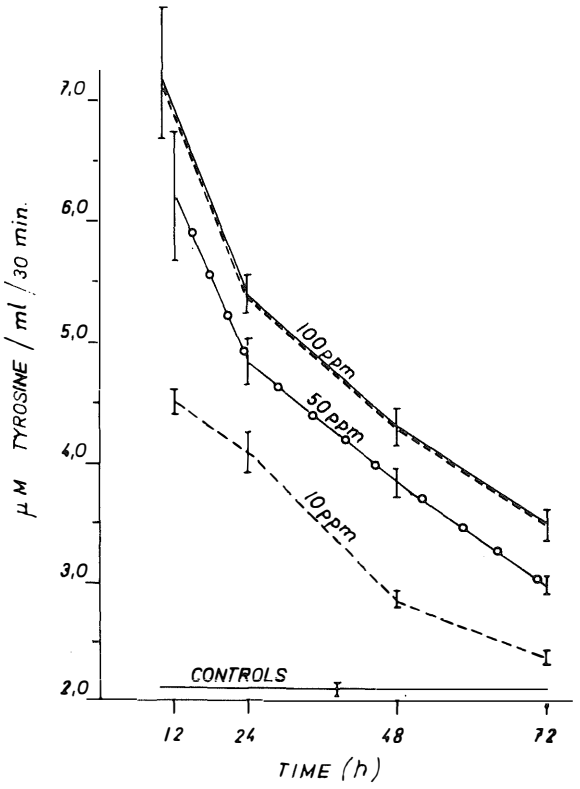

Figure 3

Cathepsin D activity in hemolymph of *Crangon crangon* after exposure to different concentrations of "SOLO", expressed in µM of tyrosine/ml / 30 min

In another series of experiments the effect of fuel oil No. III on the arylsulphatases and on cathepsin D in the striated muscle of the crucial carp was investigated. The following concentrations were used: 5, 6.6, 10, 13.3, 20, 33.3, 50, 100 and 200 ppm. To each of these solutions groups of 30 fish were exposed for 11 days. Number of the controls was the same. The lower concentrations (5–10 ppm) and the highest ones (100–200 ppm) were without significant effects. The medium concentrations were the cause of an activity increase, initially insignificant, later growing to a significant level. The maximum activity was reached at the 7th day (Table 1). The strongest effect was exerted by a concentration of 50 ppm (RUCZKAL-PIETRZAK, unpublished).

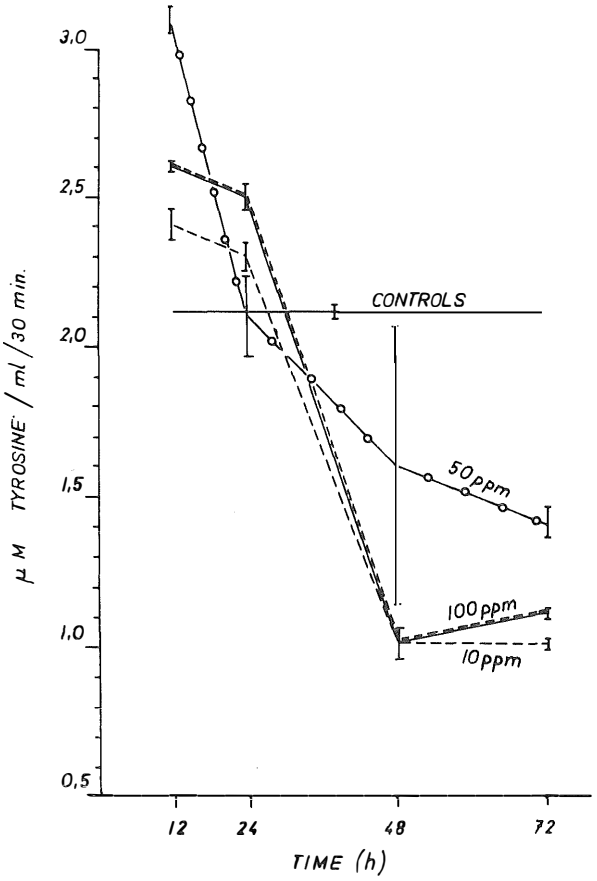

Figure 4

Cathepsin D activity in hemolymph of *Crangon crangon* after exposure to different concentrations of crude oil, expressed in µM of tyrosine/ml / 30 min

Table 1

Activity of arylsulphatases and cathepsin D in the striated muscle of the crucial carp (*Carassius carassius*) after exposure to fuel oil No. III

Controls		5 ppm		6.6 ppm		10 ppm	
		Arylsulphatases, μM 4-NC/mg. protein					
340	34.1	320.4	43.2	346.8	43.2	307.5	40.1
		Cathepsin D, μM tyrosine/mg. protein					
340	34.1	453.0	87.5	457.7	100.2	500.0	82.6
13.3 ppm		**20 ppm**		**33 ppm**		**50 ppm**	
		Arylsulphatases, μM 4-NC/mg. protein					
390.6	24.4	749.8	160.7	690.5	75.6	901.0	124.6
		Cathepsin D, μM tyrosine/mg. protein					
721.1	99.3	743.0	163.5	777.6	43.8	1 337.7	129.5

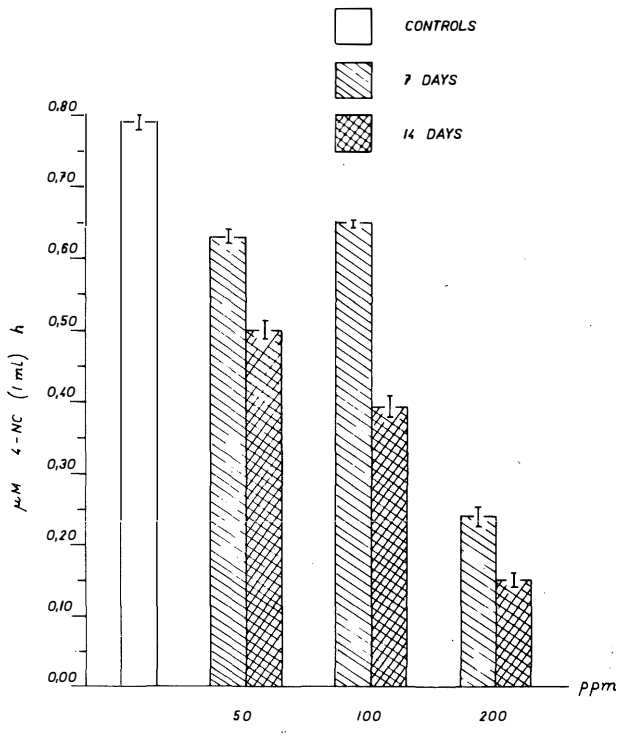

Figure 5

Activity of arylsulphatases in hemolymph of *Rhithropanopeus* after exposure to different concentrations of "SOLO" expressed in μM 4-NC/1 ml/h

In another experiment the activity of acid phosphatase, arylsulphatases, alanine aminotransferase and asparagine aminotransferase in the blood of carp fry after exposure to the anionic component of "SOLO", concentration 2.5 ppm, was registered. For testing of each of these enzymes groups of 10 animals were used, the number of the controls being the same. The activity of arylsulphatases showed a decrease, significant until the 6th day of the experiment (Fig. 8). As to alanine aminotransferase, there was an initial increase, turning later to decrease, significant at the end of the experiment (Fig. 9). The activity of asparagine aminotransferase and of acid phosphatase constantly decreased throughout the experiment (Fig. 10). Groups of 10 fishes were used in each experiment (PUCHACZ, unpublished).

In still another experiment changes of activity of tyrosinase in the hemolymph of *Crangon crangon* after exposure to PG solutions, concentrations 2.5 g/l and 5 g/l could be found. Time of exposure 72 h, number of experimental and control animals 10. There was a decrease of activity, more accentuated in the stronger solution (Fig. 11)(ZBYTNIEWSKI et al., 1973).

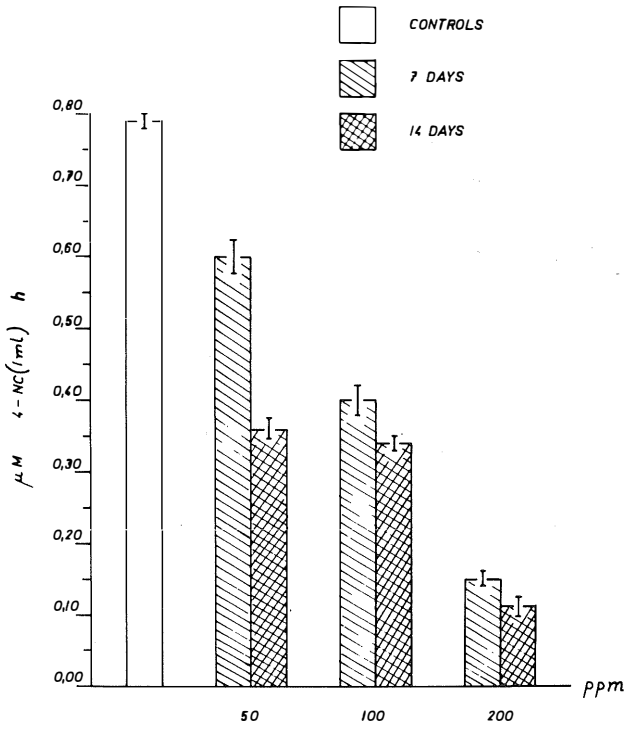

Figure 6
Activity of arylsulphatases in hemolymph of *Rhithropanopeus harrisi* after exposure to different concentrations of crude oil, expressed in µM 4-NC/1 ml/h

b) Reproduction and development

In five experimental series the effect of PG and of crude oil on vitellogenesis of *Rhithropanopeus* and of *Crangon* was observed.

Vitellogenesis in decapod crustaceans is under the control of the gonad inhibiting hormone (GIH). Decrease of activity or total absence of GIH results in accelerating gonad maturation. During maturation of the ovary the weight of the organ increases and a change of colour occurs. Immature ovaries are of pale hue, in later development turning to dark, a sign of growing yolk deposits in the maturing oocytes. These features allow a macroscopical estimation of the stage of maturing. In later experiments a gonad index (GI), allowing a more precise evaluation was used:

$$GI = \frac{\text{ovary weight} \times 100}{\text{body weight}}$$

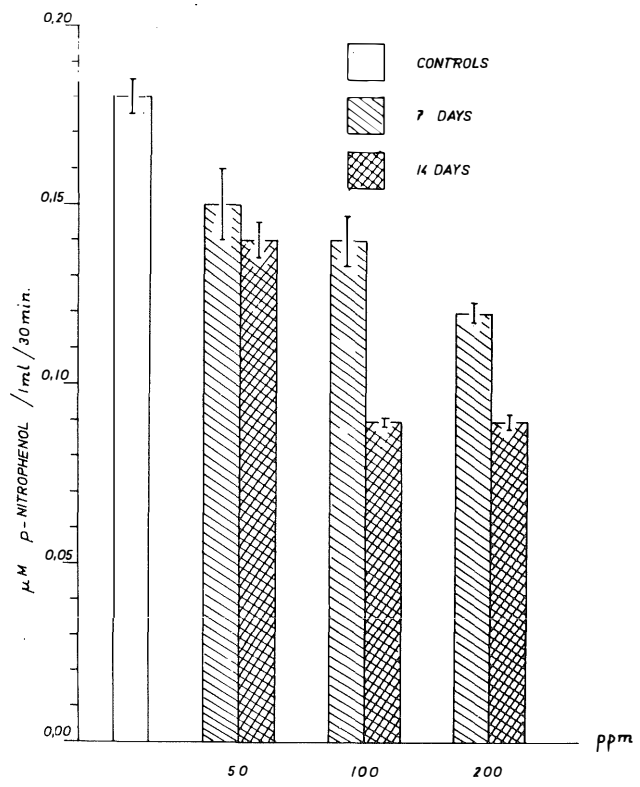

Figure 7

Activity of acid phosphatase in hemolymph of *Rhithropanopeus harrisi* after exposure to different concentrations of crude oil, expressed in µM of p-nitrophenol / 30 min

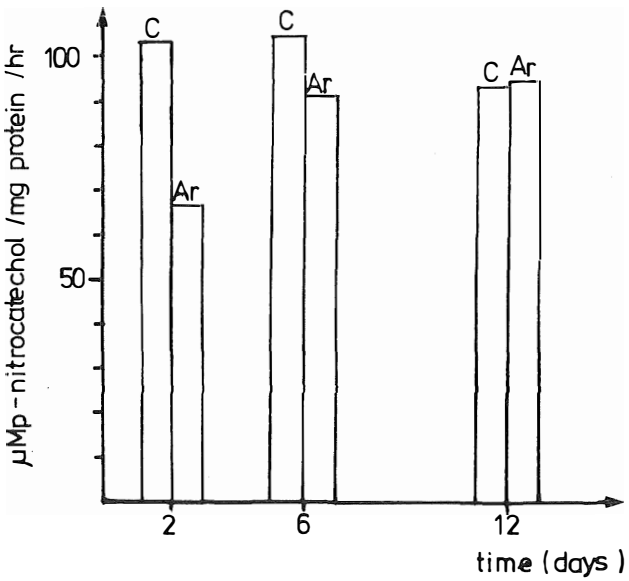

Figure 8

Activity of arylsulphatases in the blood of carp fry after exposure to anionic component of "SOLO", concentration 2.5 ppm expressed in µM 4-NC/1 mg protein/1 h, C-controls, Ar-arylsulphatases

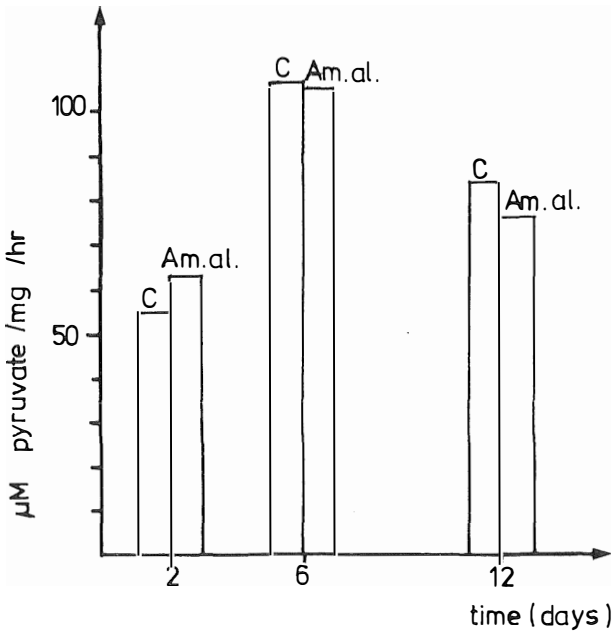

Figure 9

Activity of alanine aminotransferase in the blood of carp fry after exposure to anionic component of "SOLO", concentration 2.5 ppm, expressed in µM pryruvate/1 mg protein/1 h. C-controls, Am.al. – alanine aminotransferase

Experiments on female *Rhithropanopeus* were carried out in June, when the ovary maturation is going on and the level of GIH is relatively low. 20 females (the number of controls being the same) were exposed to PG solutions. The weight of experimental ovaries was lower in comparison with controls (Fig. 12), and their hue paler. The experimental ovaries showed also signs of degeneration.

In the ovaries of 20 normal female *Crangon crangon*, tested also in the time of intensive vitellogenesis, the changes after exposure to PG were similar, the only difference being the absence of degeneration (Fig. 13).

In other experiments the effect of PG on female shrimps with total lack of GIH was investigated. In shrimps and prawns such animals can be produced rather easily. The source of GIH is located in the eyestalk. So it is sufficient to lay a ligature at the basis of the eyestalk to eliminate any GIH activity. In a group of 20 females with ligated eyestalk maturing of the ovary also was delayed in comparison with the controls (Fig. 14). (PAUTSCH et al., 1975).

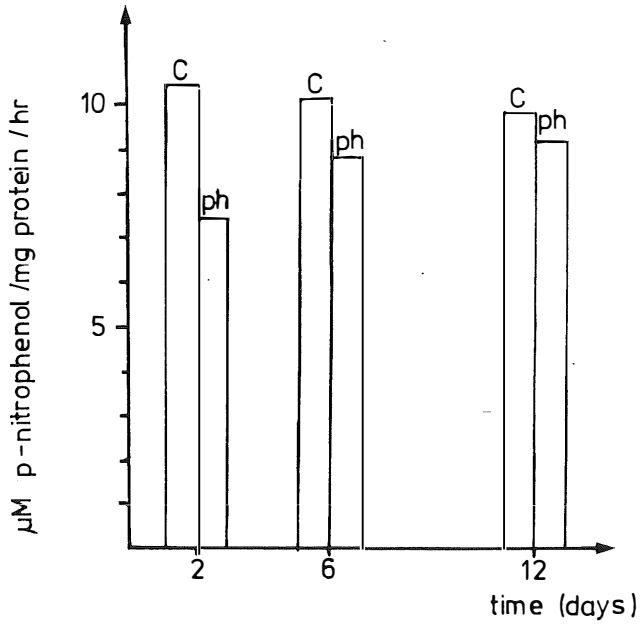

Figure 10

Activity of acid phosphatase in the blood of carp fry after exposure to anionic component fo "SOLO", concentration 2.5 ppm, expressed in μM p-nitrophenol/1 mg protein/1 h. C-controls, pH-acid phosphatase

In a later series of experiments changes in ovary maturation in *Crangon* after exposure to crude oil were observed. Only animals with ligated eyestalk were used, because from the above cited results the conclusion was drawn that animals totally lacking GIH activity are a more reliable experimental material, free of perturbances caused by eventual temporary changes of this hormone's level. In this case ovary maturation was delayed too (Table 2). In the experimental ovaries the oocytes were less numerous in comparison with the controls and of somewhat irregular shape (KLEK-KAWINSKA, unpublished).

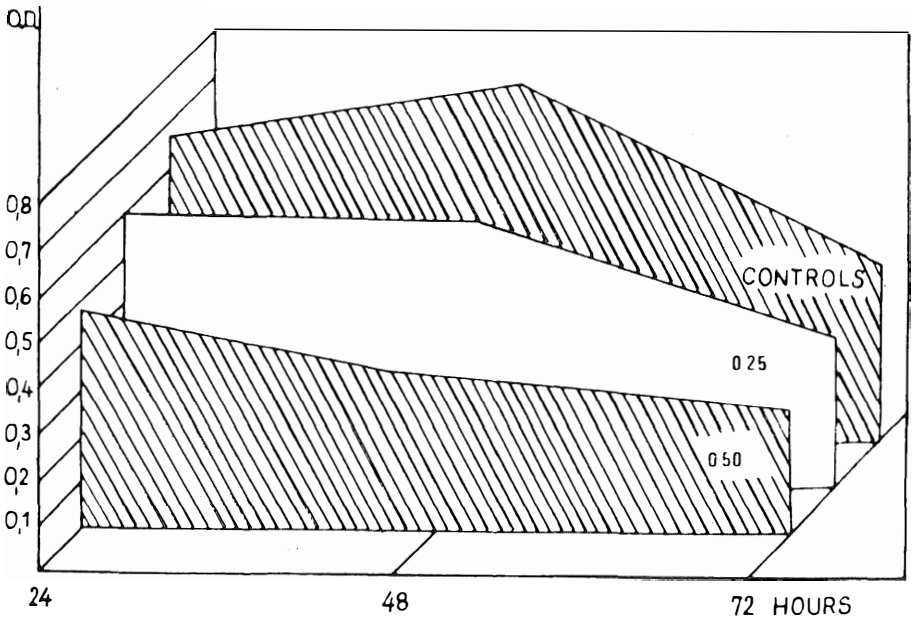

Figure 11

Activity of tyrosinase in hemolymph of *Crangon crangon* after exposure to PG, measured by increase of optical density of the system: 1 mg dopa + 4 ml 1/15 M phosphate buffer (pH 6.8) + 0.1 ml hemolymph, 420 nm, photometric cell 1 cm

Table 2

Crangon crangon, gonad indices in female experimental and control groups after exposure to crude oil, 19th day. Groups of 20 animals at the beginning of the experiment

	Number of control females	Mean GI	Number of experimental females	Mean GI
I	15	0.98	11	0.71
	14	1.04	9	0.96
	15	1.09	13	0.83
II experiment	13	1.00	13	0.88
	15	0.95	10	0.80
	15	0.99	12	0.94
	87	GI = 1.01	68	GI = 0.85

Effects of PG on the metamorphosis rate in decapodes were investigated in one experiment only. Exposure of a group of 20 *Rhithropanopeus* megalops to this pollutant resulted in an initial inhibition of metamorphosis, changing later to acceleration. In the controls metamorphosis proceeded steadily and uniformly (Fig. 15). There was no mortality in the solutions of lower concentration (1.25 g/l, 2.50 g/l), and a 30 % mortality in 5 g/l (PAUTSCH et al., 1975).

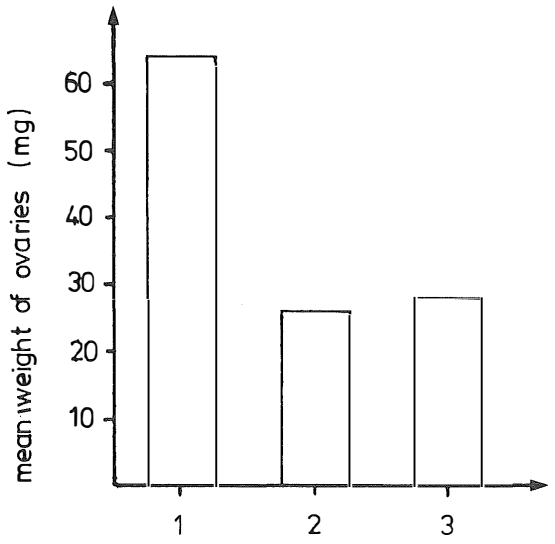

Figure 12

Mean weight of *Rhithropanopeus harrisi* ovaries after exposure to PG. Time of exposure 30 days (1: controls; 2: 1,25 g/l; 3: 2,5 g/l)

As to ontogeny of fishes, the effects on the embryonic development of the pike of representatives from all the used groups of pollutants were studied, i.e. of PG, fuel oil and Gamlen.

Exposure to these substances resulted in 1. raising the death rate, and 2. accelerating the development. Principally the intensity of these induced processes was proportional to the concentration of the pollutant.

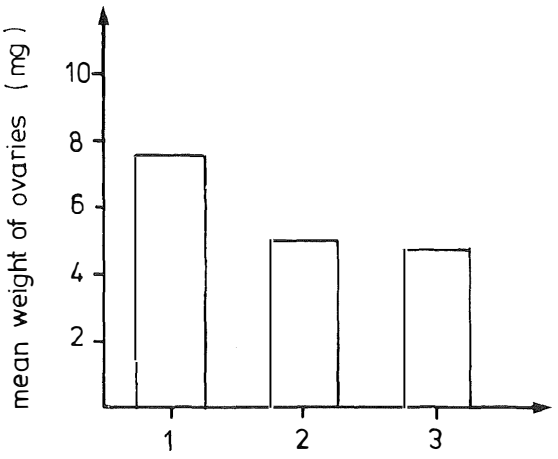

Figure 13

Mean weight of *Crangon crangon* ovaries after exposure to PG. Time of exposure 7 days (1: controls; 2: 1,25 g/l; 3: 2,5 g/l)

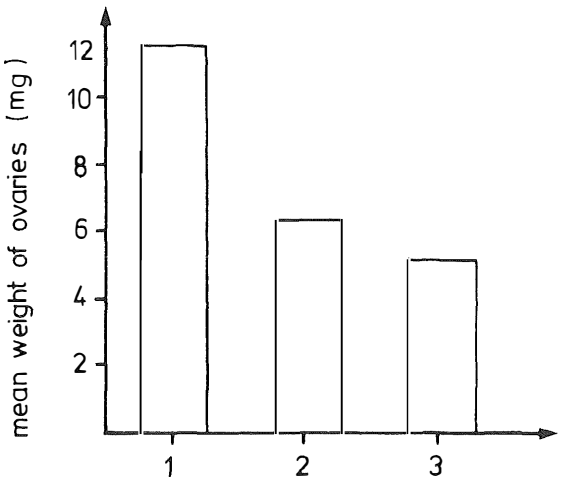

Figure 14

Mean weight of ovaries of *Crangon crangon* with ligated eyestalks after exposure to PG. Time of exposure 5 days (1: controls; 2: 1,25 g/l; 3: 2,5 g/l)

The highest grade of toxity was that of Gamlen, which even in a solution of only 1 ppm caused 100% of mortality. The last embryos survived to the end of gastrulation. At higher concentrations death occured earlier: at 10 ppm at the beginning of gastrulation, at 50, 100 and 200 ppm during cleavage.

As to PG and fuel oil the period critical for survival extended from the moment of fertilization to the end of gastrulation. PG was used in concentrations of 1.25, 2.5, 5 and 10 g/l, fuel oil in 1, 10, 50, 100 and 200 ppm. In a 200 ppm solution of fuel oil in a temperature of 10–13 °C for instance, only about 3% of the embryos survived this period, in 1 ppm – 37%. In the same temperature range PG in concentration of 10 g/l caused 100% of mortality within a few hours after fertilization, prior to the beginning of cleavage, in 5 g/l the same effect was reached at the fourth or fifth day in the final stage of gastrulation.

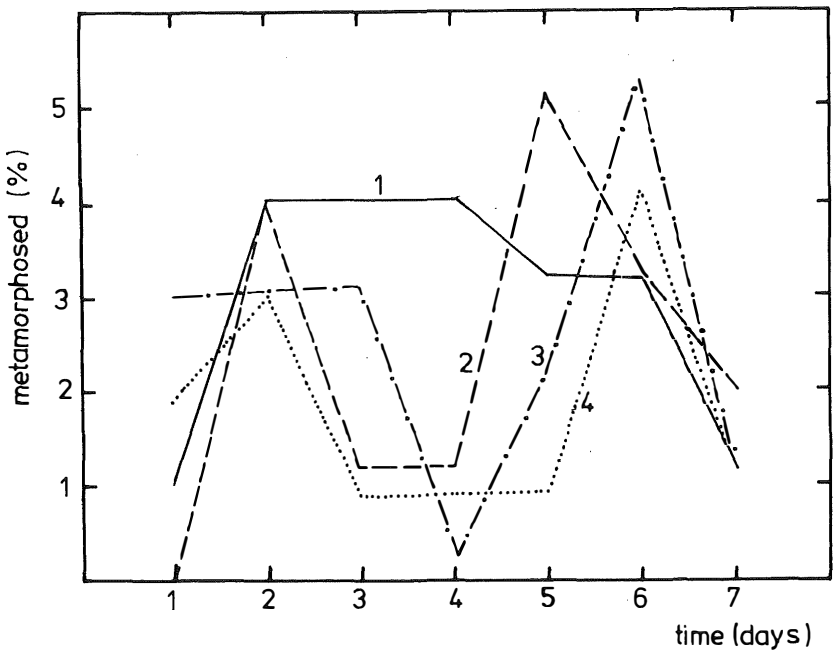

Figure 15

Percentage of metamorphosed *Rhithropanopeus harrisi* megalops after exposure to different concentrations of PG. 1 – controls, 2 – 1.25 g/l, 3 – 2.5 g/l, 4 – 5 g/l

In concentrations of 1.25 g/l and 2.5 g/l the percent of mortality during the critical period was 87%, and 90% respectively. In the controls too the highest rate of mortality occurred in this time (27%) (DĄBROWSKA, unpublished). Later, in both controls and experimental animals the mortality was rather low. In fuel oil it never reached 100%.

Among the control eggs (temperature 10–13°C), hatching of larvae occured in nearly 60% of the initial number. In eggs exposed to PG or fuel oil the percentage of hatching larvae was reduced. In a PG concentration of 1.25 g/l hatching occured in

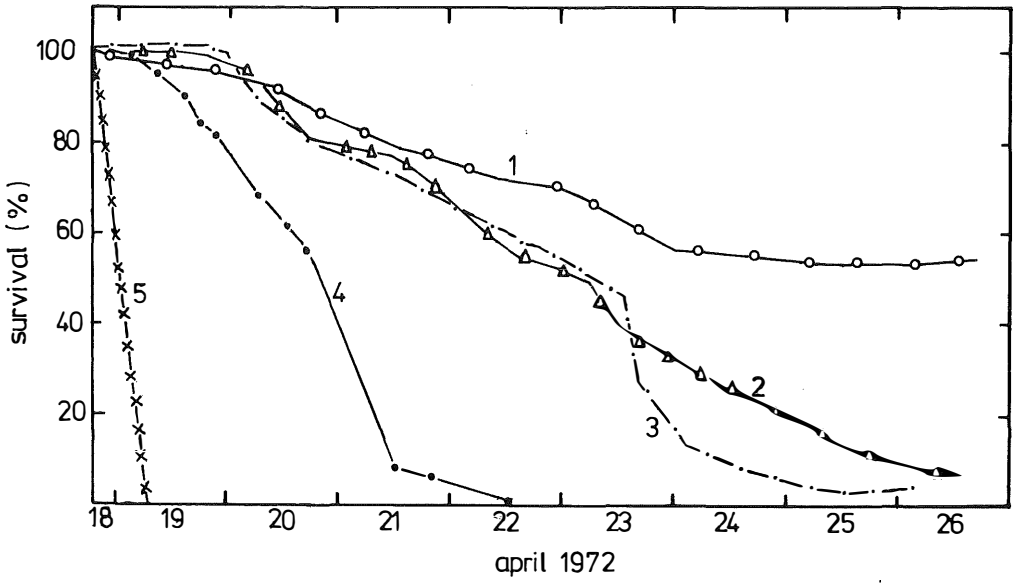

Figure 16
Survival of fertilized pike eggs exposed to different concentrations of PG. 1 – controls, 2 – 1.25 g/l, 3 – 2.5 g/l, 4 – 5 g/l, 5 – 10 g/l

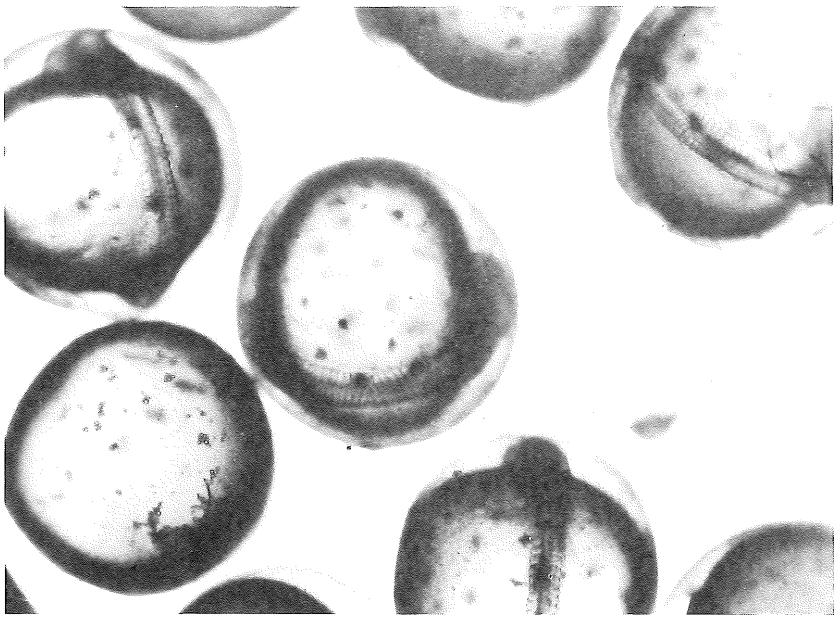

Figure 17
Control pike embryos, stage of fish-like appearance, 153 h after fertilization

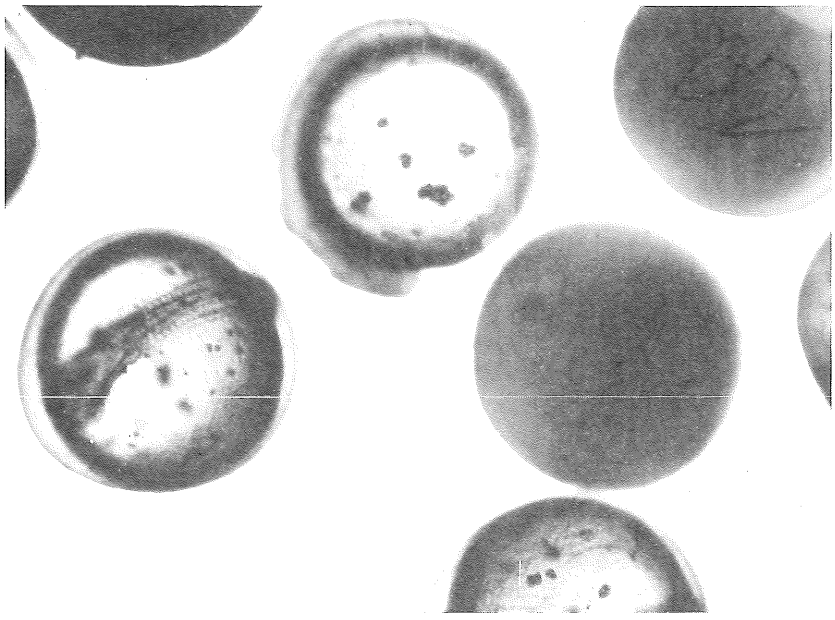

Figure 18
Pike embryos after exposure to crude oil, concentration 200 ppm, stage of fish-like appearance, 129 h after fertilization

about 7 % of the initial number of eggs, in 2.5 /l respectively in about 1 % only (Fig. 16). Fuel oil seems to be less toxic. In a concentration of 1 ppm the percent of hatching was only slightly reduced, reaching 54%, the respective figures being 20% for 10 ppm, 12% for 50 ppm, 4% for 100 ppm and 3% for 200 ppm.

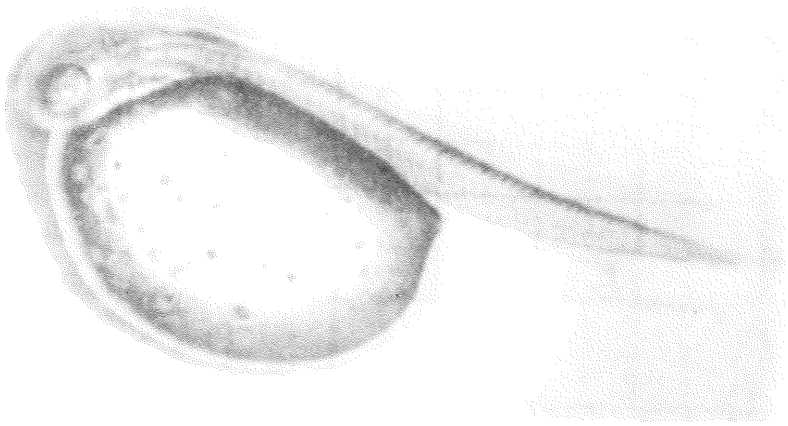

Figure 19
Freshly hatched pike larva, control, 214 h after fertilization, body length 7.7 mm

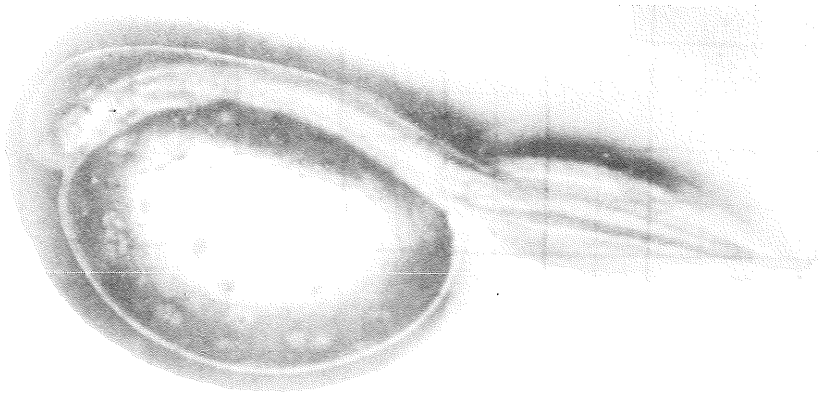

Figure 20
Freshly hatched pike larva, after exposure to PG, concentration 1.25 g/l, 197 h after fertilization, body length 7.1 mm

In the controls the development until hatching lasted 8 days. In all the eggs, experimental and controls, the cleavage began at the same time, i.e. 8 h after fertilization. But later differences in the speed of development arose. In both the PG and oil solutions cleavage was shortened by 4 h in comparsion with the controls (1.25–5 g/l of PG, 1–200 ppm of oil), gastrulation by 12 h (1.25 g/l and 2.5 g/l of PG, 1–200 ppm of oil), the first fish-like appearance (Fig. 17, Fig. 18) and the ''eyed egg'' stage by 6 to 24 h (proportionally to the concentration of the solutions), the prehatching stage by 16 to 17 h in oil solutions, by 8 to 20 h in PG solutions. The total period of development was shortened even in the lower concentrations. After exposure to fuel oil it was reduced by 6 to 11 h, after PG by 5 to 17 h. In each of the described experiments groups of 100–150 eggs were used.

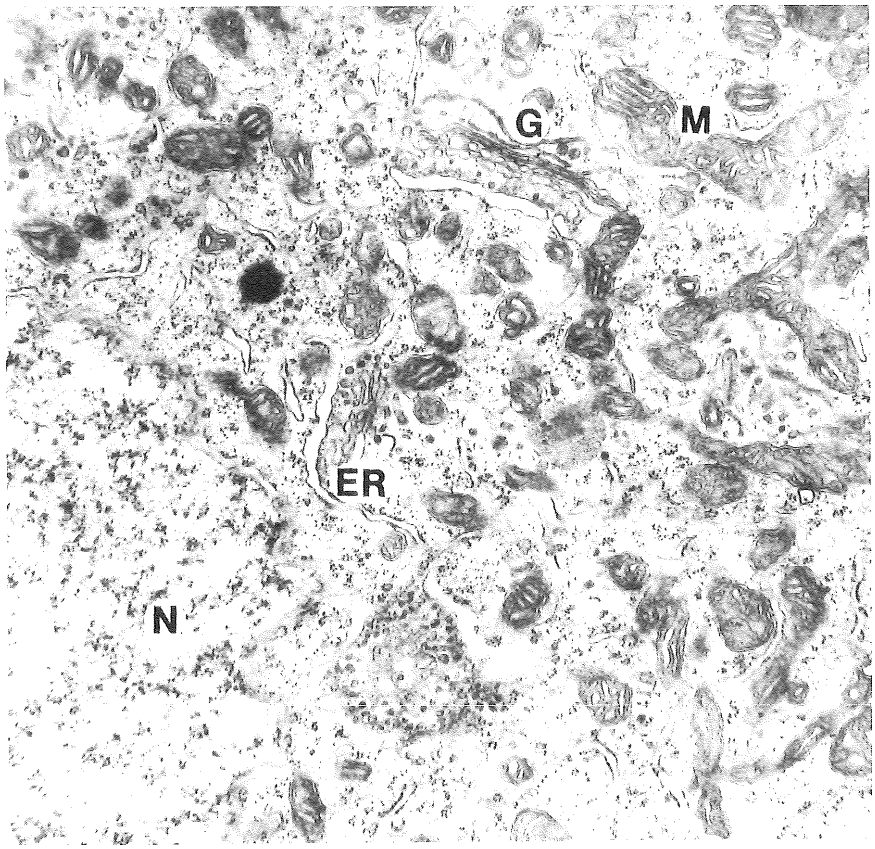

Figure 21

Part of the cytoplasm of a normal neurosecretory cell from the brain of *Rhithropanopeus harrisi*. M – mitochondria, ER – endoplasmic reticulum, G – Golgi apparatus, N – nucleus. 22,750 x. (Laboratory of Electron Microscopy, Head Dr. T. WRZOŁEK)

The size of experimental freshly hatched larvae was reduced in comparison with the controls. After exposure to 1.25 g/l of PG for instance, the difference of body length in comparsion with the controls was 0.6 mm. (Fig. 19, Fig. 20) (PAUTSCH et al., 1975).

Beside the experiments concerning enzymes and development, two other ones were carried out.

After a 10 days exposure of adult *Rhithropanopeus* to PG, severe symptoms of brain degeneration could be observed. In a solution of 5 g/l of an initial group of 20 crabs only 7 survived. These animals showed characteristic behavioural disturbances. Their movements were incoordinate and sluggish. Their brains were reduced to an amorphous mass without any histological structure. After exposure to a con-

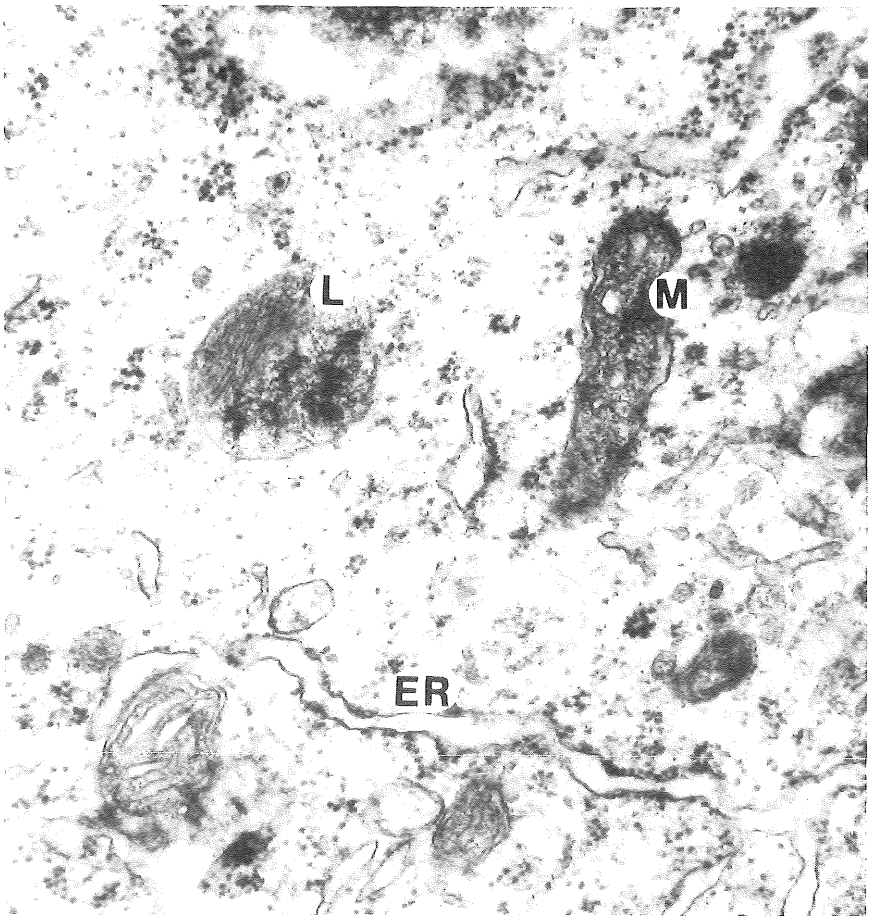

Figure 22

Part of the cytoplasm of a neurosecretory cell from the brain of *Rhithropanopeus harrisi*, after exposure to PG, concentration 1.25 g/l. M – mitochondria, ER – endoplasmic reticulum, L – autophagosome. 45,000 x. (Laboratory of Electron Microscopy, Head Dr. T. WRZOŁEK)

centration of 1.25 g/l the histological and the fine structure of the brain could be examined. In the perikaryon of normal brain neurosecretory cells for instance, organellae such as mitochondria, endoplasmic reticulum, Golgi apparatus are typically developed, with no abnormal changes (Fig. 21) (PAUTSCH et al., 1973). In the respective organellae in brain neurosecretory cells of experimental animals symptoms of high-grade degeneration appeared (Fig. 22). In the mitochondria the electron-density was significantly increased. The mitochondrial cristae were short and angular in shape. But the most convincing evidence of advanced degeneration comes from the presence of autophagosomes. These structures, surrounded by a membrane, contain lamellar and granular fragments – remnants of structures impossible to identify.

PG solutions affect also the functions and the structure of isolated mitochondria of the perch. Solutions of 187 and 312 ppm uncouple the oxidative phosphorylation in the mitochondria, this in turn inhibiting ATP synthetase. Beside this, PG in the same concentrations causes swelling of the mitochondria, which means serious damage (PAUTSCH et al., 1975).

Discussion and Conclusions

Any attempts to give an interpretation of all the experimental results must be tentative only, because of their great diversity.

As to PG, it is known from our earlier experiments that in higher concentrations ranging up to 15 g/l it displays a considerable acute lethal toxicity for some brackish water animals species. The sensitivity grows in the order: molluscs, amphipods, brachyuran decapods, fish, polichaetous annelides, natantian decapods (PAUTSCH et al., 1975). The results presented in the present paper prove that PG in concentrations ranging from 1.25 g/l to 5 g/l produces chronical sublethal intoxication. These seem to be rather high concentrations, but if we take into account that the main toxic agents of PG, the fluorine compounds Na_2SiF_6 and CaF_2, constitute only about 4.6% of the whole product, we come to the conclusion that in a 5 g/l solution for instance, the toxic agents are of a concentration of about 230 ppm only. And it should be stressed that after an experimental dump of PG in the Bay of Gdańsk, locally even higher concentrations appeared (CISZEWSKI, 1971).

As to the chronic toxicity of detergents there are many publications. The most widespread interpretation of their biological activity is that as surfactants they dissolve in the lipids of biological membranes, enhancing their permeability. This holds true especially in the case of lysosomes, whose membranes are particularly sensitive to detergents. In consequence of the labilization of these membranes the lysosomal hydrolases are released into the cytoplasm (HELENIUS and SIMONS, 1975). Probably this holds true also for other enzymes, which may change their activity, reacting to changes of other membranes, including the cell membrane itself.

It is not easy to explain why in consequence of treatment with detergents the activity of some enzymes (especially the hydrolases) increases in some cases (cathepsin D in crustacean hemolymph, cathepsin D and arylsulphatases in fish muscle), in others decreases (arylsulphatases and acid phosphatase in crustacean hemolymph). A plausible explanation for an increase could be the simple fact that the hydrolases, being inactive when enclosed in the lysosomes, become active after release into the cytoplasm. But this probably helds true in the case when the concentration of the enzyme is rather low. In case of a high enzyme level, a kind of feedback reaction may be evoked, resulting in the appearance of inhibitors of the enzyme's activity. There may be specific inhibitors (DREWA et al., 1977), or even the detergent

itself can take over this function (BOYER, 1970). Comparable observations were made by BROWN Jr. (1976), who found that the allantoinase of the polychaete *Eudistylia vancouveri* is inhibited by mercury ion in a concentration of 22×10^{-8}M, but enhanced at lower concentrations of this metal.

The well-known toxicity of petroleum hydrocarbons appeared in our experiments mainly as a chronic one. It may be assumed that this sublethal activity is linked with the metabolism of petroleum hydrocarbons. In crustaceans their uptake is from food or swallowed water (CORNER et al., 1976; LEE et al., 1976). In fish their way goes through the gills (LEE et al., 1972). In crabs about 20–50% of the hydrocarbons passes the alimentary tract and is excreted without changes. The remaining part is metabolized to highly polar metabolites, the main site of these processes being the hepatopancreas (LEE et al., 1976). These metabolites, or naphthalenes mainly, are somewhat more water-soluble than their parent compounds and therefore can more easily be excreted (LEE et al., 1976; CORNER et al., 1976). In fish the petroleum hydrocarbons are metabolized in the liver. The metabolites are transferred to the bile and excreted. Water-soluble metabolites can be excreted also with urine (LEE et al., 1972).

Of course all this cannot explain in detail the mechanism of chronic toxicity of petroleum hydrocarbons seen in our experiments. Only assumptions can be made. The opinion of MORROW (1974) is that these hydrocarbons may act altering the permeability of cell membranes, a.o. in the gills of fishes. DREWA et al. (unpublished) extend this supposition to biological membranes in general. If this assumption is right, there would be in part an explanation of changes in enzyme activity after exposure to petroleum hydrocarbons.

Differences of sensitivity between juvenile and adult forms could be observed in our experiments only in *Rhithropanopeus*. The megalops were much more sensitive to PG than the adults. In adults there was no mortality after a 4 days exposure to PG concentrations ranging from 1.25 to 5 g/l, whereas in the megalops the last concentration was the cause of rather high death rate (30 %).

As to the development of the pike, the relative low toxicity of fuel oil No. III is probably caused by its content of 3 heavy hydrocarbons, which are less toxic than the light ones.

An acceleration of development as a consequence of pollution was observed also by KINNE et al. (1967) in developing herring eggs exposed to an industrial waste, composed mainly of sulfuric acid and other sulfuric compounds. MORROW (1974) observed a significant mortality in young coho and sockeye salmon after exposure to crude oil at concentrations > 500 ppm. LINDEN (1976) obtained larvae of *Gammarus oceanicus* of decreased growth after exposure to crude oil. The larvae showed also morphological and behavioural abnormalities. Similar observations were made by WILSON (1976) as an effect of dispersants (100 parts/10^6) on young fish embryos.

In spitè of the many unclear points of this discussion, some more general conclusions should be drawn, even if sceptical minds might accept them as a kind of working hypothesis only.
I quite agree with KLEEREKOPER (1975), that at a relatively short distance from the focus of pollution a rapid dilution of the pollutant's concentration occurs. Therefore most populations with great locomotory ability, especially fish, never encounter acute toxic concentrations. Nevertheless, they remain in areas where chronic toxicity is at work.

If this pollution level is maintained for a longer period, including several generations of a sensitive species, the consequences may lead to very dangerous situations.

Returning once more to our own results, it may be supposed that continuous damage of lysosomes and mitochondria, which organellae are of basic importance for the normal work of enzymic systems, must result in severe abnormalities of metabolic processes. As to mitochondria, also the uncoupling of oxidative phosphorylation and inhibition of ATP synthetase should be emphasized.

The rate of reproduction will decrease with each new generation and finally may be reduced to zero. Larvae with abnormalities, possibly caused by induced mutations, will appear in growing numbers.

And last not least, let us mention the partial or total destroying of the brain, a highly developed organ in decapods, necessary for normal behaviour and function of the neurosecretory system.

Based on these data, even without taking into account similar statements made by other authors concerning other pollutants, I hope to have given sufficient arguments supporting the view that chronic sublethal toxicity can severely damage or even destroy marine ecosystems.

References

BOYER, P. D.: The Enzymes. London – New York: Academic Press. vol. IV., pp.450–496 (1970)

BROWN JR., G. W.: Effects of polluting substances on enzymes of aquatic organisms. J. Fish. Res. Board Canada **33**, 2018–2022 (1976)

CISZEWSKI, P.: Telewizyjny i nurkowy zwiad terenowy dotyczacy określenia wpływu zrzutu fosfogipsu na życie denne Zatoki Gdańskiej (Television and diving exploration of the effect of a phospho-gypsum dumping on the benthos in the Bay of Gdańsk) In: Wpływ zrzutu fosfogpsu na zycie denne Zatoki Gdańskiej (Effect of a phosphogypsum dumping on the biocenosis of the Bay of Gdańsk), pp. 129–158. Sopot: Polskie Tow. Geofizyczne (in Polish, 1971)

BYRNE, C. J. and J. A. CALTER: Effect of water-soluble fractions of crude, refined and waste oils on the embryonic and larval stages of the Quahog clam *Mercenaria* sp. Mar. Biol. **40**, 225–231 (1977)

CORNER, E. D. S., R. P. HARRIS, C. C. Kilvington and S. C. M. O'HARA: Petroleum compounds in the marine food web: short-time experiments on the fate of naphtalenes in *Calanus*. J. Mar. Biol. Ass. U.K. **56**, 121–134 (1976)

DREWA, G., Z. ZBYTNIEWSKI and F. PAUTSCH: Seasonal changes in the level of detergents in the brackish water of the Dead Vistula and the Bay of Gdańsk. Merentutkumislait. Jul,./Havsforskningsinst. Skr. No. **239**, 105–108 (1975)

DREWA, G., Z. ZBYTNIEWSKI and F. PAUTSCH: The effect of detergent ''SOLO'' and crude oil on the activities of cathepsin D and acid phosphatase in hemolymph of *Crangon crangon* L. Pol. Arch. Hydrobiol., **24**, 279–284 (1977)

GEORGE, J.D.: Sublethal effects on living organisms. Mar. Pollut. Bull. **1**, 107 – 109 (1970)

HELENIUS, A. and K. SIMONS: Solubilization of membranes by detergents. Biochim. Biophys. Acta **415**, 29–79 (1975)

KINNE, O. and H. ROSENTHAL: Effects of sulfuric pollutants on fertilization, embryonic development and larvae of the herring, *Clupea harengus*. Mar. Biol. **1**, 65–82 (1967)

KLEEREKOPER, H.: Effects of sublethal concentration of pollutants on behaviour. Pacific Science Association: Thirteenth Pacific Science Congress: Record of Proc. vol. **1**, 43 (1975)

LEE, R. F., C. RYAN and M. L. NEUHAUSER: Fate of petroleum hydrocarbons taken up from food and water by the blue crab *Callinectes sapides*. Mar. Biol. **37**, 363–370 (1976)

LEE, R. F., R. SAUERHEBER and G. H. DOBBS: Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. **17**, 201–208 (1972)

LINDEN, O.: Effects of oil on the amphipod *Gammarus oceanicus*. Environmental Pollution **10** (4), 239–250 (1976)

McAULIFFE, C. D., R. D. SMALLEY, W. M. GROOVER, M. WELSH, W. S. PICKLE and G. E. JONES: Chevron Main Pass Block 41 oil-spill: chemical and biological investigations. In: Proc. of a Joint Conference on Prevention and Control of Oil-Spills, pp. 555–556, (1975)

MORROW, J. E.: Effects of crude oil and some of its components on young coho and sockey salmon. U.S. Environmental Protection Agency. Office of Research and Development. Ecological Research Series EPA-660/3-73-018, (1974)

NICOL, J. A. C., W. H. DONAHUE, R. T. WANG and K. WINTERS: Chemical composition and effects of water extracts of petroleum on eggs of the sand dollar *Melitta quinques perforata*. Mar. Biol. **40**, 309–316 (1977)

PAUTSCH, F., I. CISZEWSKA and T. ŻAWROCKA-WRZOŁKOWA: Ultrastructure of neurosecretory cells in the brain of the crab *Rhithropanopeus harrisi* (Gould). Folia. histochem. cytochem. **1**, 347–348 (1973)

PAUTSCH, F., A. BOMIRSKI, T. DĄBROWSKA, G. DREWA, E. KLĘK-KAWIŃSKA, L. ŁAWIŃSKI, J. TARZYŃSKA-KLEINEDER, ZB. ZBYTNIEWSKI, T. ŻAWROCKA-WRZOŁKOWA, I. CISZEWSKA, T. DOMINICZAK, E.F. SKORKOWSKI, J. SZLACHCIC-NAJDROWSKA and K. TUROBOYSKI: Studies of the toxity of phospho-gypsum. Pol. Arch. Hydrobiol. **22**, 449–476 (1975)

RENZONI, A.: Influence of crude oil, derivatives and dispersants on larvae. Mar. Pollut. Bull. **4**, 9–13 (1973)

ROSENTHAL, H.: Sublethal effects of pollutants on marine fish eggs and larvae. Pacific Science Association: Thirteenth Pacific Science Congress: Record of Proc. vol. **1**, 73 (1975)

SWEDMARK, M., B. BRAATEN, E. EMANUELSON and A. GRAMNO: Biological effects of surface activaegents on marine animals. Mar. Biol. **9**, 183–201 (1971)

WELLS, F. G.: Influence of Venezuelan crude oil on American lobster larvae. Mar. Pollut. Bull. **3**, 105 (1972)

WELLS, F. G. and J. B. SPRAGUE: Effects of crude oil on American lobster (*Homarus americanus*) lavae in the laboratory. J. Fish. Res. Board Canada **33**, 1604–1614 (1976) (1976)

WILSON, K. W.: Effects of oil dispersants on the developing embryos of marine fish. Mar. Biol. **36**, 259–268 (1976)

ZBYTNIEWSKI, Z. and F. PAUTSCH: Effect of phospho-gypsum on the protein and alpha-amine nitrogen level and on the tyrosinase activity in the hemolymph of *Crangon crangon*. Oikos. Suppl. **15**, 232–235 (1973)