Ann. Zool. Fennici 26:133–143. 1989

Survival, growth and disease of three-spined stickleback, *Gasterosteus aculeatus* L., brood exposed to bleached kraft mill effluents (BKME) in mesocosms

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Lehtinen, K.-J. 1989: Survival, growth and disease of three-spined stickleback, *Gasterosteus aculeatus* L., brood exposed to bleached kraft mill effluents (BKME) in mesocosms. — Ann. Zool. Fennici 26:133–143.

Three-spined stickleback, (*Gasterosteus aculeatus*), larvae were exposed in brackish water littoral model ecosystems to six different bleached kraft mill effluents during three consequtive half-year periods (1982–1984). Survival, weight increase, diseases and parasites were studied monthly during the experimental periods. The results from the experiments showed that conventional chlorine bleaching affected survival of young larvae. External treatment, introduction of oxygen pre-bleaching and increased substitution of active chlorine as chlorine dioxide decreased or eliminated mortality. The growth pattern of the larvae differed markedly with time and effluent type; externally treated BKME from conventional chlorine bleaching caused a slower growth, oxygen prebleaching (treated/untreated effluent) sequentially inhibited and stimulated growth. Increased use of chlorine dioxide did not cause any effect upon growth. Total body fat content and liver histological analysis indicated a positive correlation between affected growth and metabolic disturbances. The exposed fish were also shown to be more infested with parasites than unexposed fish.

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1. Introduction

Bleached kraft mill effluents (BKME) are complex mixtures containing both chlorinated and non-chlorinated organic substances (Holmbom & Lehtinen 1980, Mäenpää et al. 1968). The chlorinated substances are formed in the bleachery, especially when predominantly elementary chlorine is used in the bleaching process (Kringstad & Lindström 1984).

Softwood sulphate pulp is bleached in several, usually six, consequtive steps. In the first step (C-step), chlorine is traditionally used as a bleaching medium in combination with minor amounts of chlorine dioxide. The next step is an alkaline extraction (E) followed by a hypochlorite (H), a chlorine dioxide (D) and an alkaline extraction (E) step. The bleaching is finished using a final D-step. The complete sequence for a conventional chlorine bleaching is thus (C+D)EHDED. Several modifications (see Table 1 for examples), aimed at reduced emissions into the aquatic environment, exist today. The most important one is perhaps that using an oxygen (O) pre-bleaching step before the (C+D) step (Anon. 1985).

For many years, acute and sublethal effects on fish exposed to specific substances (both chlorinated and non-chlorinated) present in pulp mill effluents have been studied. In such studies effects of specific substances, i.e. chlorinated phenols and resin acids, have been obtained on fish (McLeay et al. 1987). However, despite the data obtained through work of this kind, it is still unknown if the same substances, or groups of substances, elicit an effect when organisms are exposed to complex, total mill effluents. It is generally assumed that the chlorinated matter emanating from pulp mills is the main fraction inducing ecotoxic effects in receiving waters. It is further assumed that a

Table 1. The different bleaching sequences tested between 1982–1984 and their codes used in the text. Use of C (Chlorine) and D (chlorine dioxide) for the respective CD-step given as per cent. For further information regarding the abbreviations used, see text under Introduction. Effluent treatment refers to treatment in an aerated lagoon.

Bleaching sequence	Effluent treatment	Code	Year
(C95+D5)EHDED	No treatment	Effl. 1	1982
(C87+D13)EDED	Aerated lagoon	Effl. 2	1983
O(C83+D17)EDED	No treatment	Effl. 3	1983
O(C85+D15)EDED	Partial aeration[a)]	Effl. 4	1983
O(84+D16)EDED	Pilot plant aeration	Effl. 5	1984
O(C52+D48)EDED	No treatment	Effl. 6	1984

[a)] Acid bleachery effluent to 80% not passing treatment in aerated lagoon.

Table 2. Daily production of pulp, and characteristics of waste water, of the mills tested. Washing loss = amount of cooking liquor carried over from digester to bleachery. BOD = Biochemical Oxygen Demand. COD = Chemical Oxygen Demand. t = tonnes of pulp produced.

	Production t/d	Waste water m^3/t/d	Washing loss (kg/t Na_2SO_4)	BOD kg/t	COD kg/t
Effl. 1	750	148	9–11	16–25	89–95
Effl. 2	770	100	16	11	83
Effl. 3	1000	54	6.7	12	52
Effl. 4	980	52	6.7	5.1	42
Effl. 5	1000	46	6.3	2.2	29
Effl. 6	1000	45	5.4	9.1	59

systematic reduction of chlorinated matter in the bleaching process would be environmentally beneficial (Anon. 1985).

In the Baltic both the feeding and reproduction of many fish species is highly concentrated in the shallow coastal waters. Fish populations of the Baltic are thus likely to be rather continuously exposed to BKME during their most sensitive stages, i.e. at spawning, hatching and early larval development (Haage 1975).

In a project, lasting several years the environmental impact of total BKME:s from mills using different bleaching processes was studied in mesocosms (Anon. 1985). Effects on mortality, weight increase and disease of the brood of the littoral three-spined stickleback, *Gasterosteus aculeatus* L., were included in this work parallel with physiological and biochemical effects on larger fish, *Salmo gairdneri* R., (Lehtinen et al. 1989). Effects on the ecosystem level were also included in the work (Rosemarin et al. 1989, Lehtinen et al. 1988).

In the present study, which constitutes part of the whole work performed using the model ecosystem technique, effects on brood of the stickleback, (*G. aculeatus*), exposed to total BKME:s are reported.

2. Methods and materials

Experiments were performed during three consequtive half-year periods (May–December) 1982–1984, using totally six different whole mill BKMEs (Table 1). The effluents were sampled at the mill during controlled process conditions. The mill processes, as well as the pulp quality, were strictly monitored before and during the effluent samplings. Relevant mill process and production data are given in Table 2.

After sampling, the effluents were immediately transported in 1.5 m^3 polyethylene containers to the Baltic Sea Laboratory in Karlskrona, Sweden, whereupon they were frozen (–30°C). Required amounts of effluent were thawed when needed, during the experiments.

Different amounts of effluents are produced per tonne of pulp in different mills. This results in varying *concentrations* of substances in the final effluent and thus also variations in tests performed if the same nominal waste water concentrations are compared. Most mills in this project had an effluent volume of 50 m^3 per tonne pulp (Table 2). Some had higher volumes, which had to be accounted for (lower nominal dilutions) when the effluent-concentrations were set in the experiments. The effluents were dosed at two dilutions, 2000 and 400 times respectively, based on a 50 m^3 effluent volume per tonne of pulp produced. Diaphragm type pumps (CFG, Prominent Electronic, Type A 201) were used for dosage.

In the experiments, 360 l fibre glass fish rearing tanks (Ewos, Södertälje) connected to the out-going water of the of larger 8 m^3 model ecosystem pools, were used (Fig. 1). In this study, emphasis is laid on studies of populations in smaller systems designed for detailed fish population studies (predation eliminated). In total, three controls (one per year) and 12 effluent doses (six effluents, two dilutions per effluent) were used during the three-year period.

Water temperature (Fig. 2) was monitored semicontinuously (hourly) by a computerized system throughout all the experimental periods.

The bottoms of the small tanks were covered with a layer of sand and two to three specimens of bladder-wrack were introduced as shelter. The model ecosystem method has recently been described by Rosemarin & Notini (1988). Thus, only a short description is given here. The keys to this technique are the long-term aspect, the large volume allowing for sub-sampling, system reproducibility (Rosemarin et al. 1989), the low realistic levels of pollutants used and that the systems are open to a flow-through of unfiltered raw seawater. The main experimental system consisted of 8 m^3 polyethylene lined pools supplied with continuous renewal 2.8 l/min of unpolluted brackish water (8‰), which was pumped from 8 m depth from Danmarks-

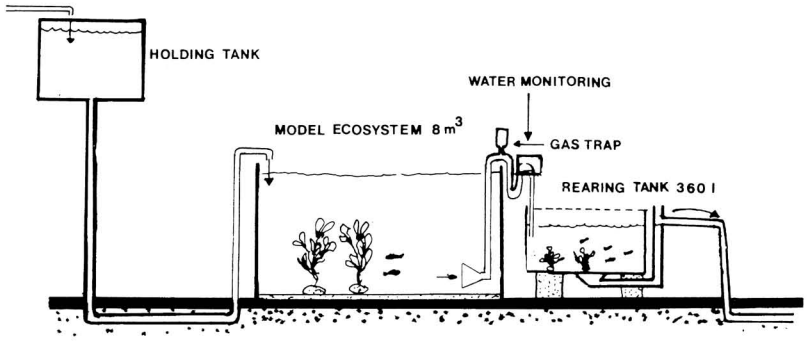

Fig. 1. Schematic illustration of the experimental set-up.

fjärden outside the laboratory in Karlskrona. Therefore, the incoming water temperature fluctuated along with the sea water temperature (Fig. 2). The temperature of the incoming water was always the same for all pools since the water was distributed via under-ground insulated tubes from the common holding tank. The insolation was identical for all pools (Rosemarin & Notini 1988). The small, 360 l tanks with stickleback young were thus connected with the larger systems, and items such as chemicals, food etc. were mediated via water from the mother systems.

Prior to the start of each years experiment, adult sticklebacks were caught at the end of May with a seine net in an unpolluted brackish water bay 30 km W of the laboratory. The newly caught fish were immediately transported to the laboratory and were kept in an 800 l glass-aquarium for at least one week. After this period each small tank was supplied with three males and females. In order to eliminate predation by birds, the tanks were covered with nets (10 mm mesh size).

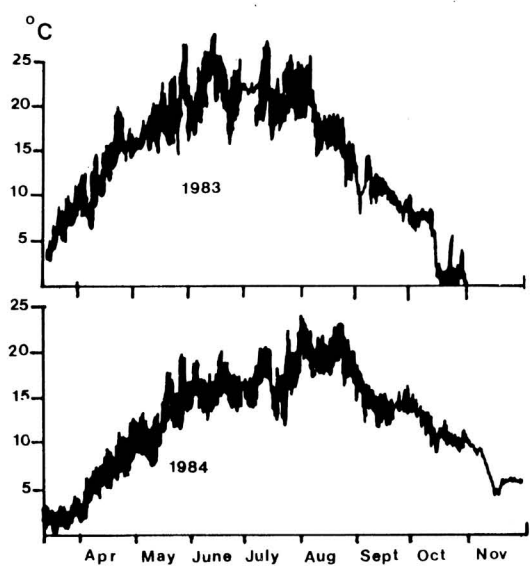

Fig. 2. Water temperature curves for the experimental periods 1983 and 1984. The curves are based on hourly computerized measurements.

The male fish showed territoriality and nest building activities a few days after introduction to the tanks, whereupon spawning took place. After larval hatching in late June, the parents were removed and the number of larvae was adjusted to 60 individuals per tank. Each mother mesocosm was supplied with 100 individuals from the respective small tank.

In the first experimental period in 1982, the procedure differed to some extent from the following ones, inasmuch that the larval populations were not adjusted to 60 and 100 in the small and large systems respectively. Instead three pairs of adult sticklebacks were introduced into the respective small and large system, whereupon survival and weight were compared with the control at the end of the experiment. This was because this experiment was the first using the stickleback as a test organism, and it was not known whether spawning and reproduction would be successful.

Effluent dosage was started on June 16th each year. Thus, hatching and early larval development were subjected to exposure.

After hatching and regulation of the populations in the small tanks, monthly observations on growth, mortality and diseases and parasites were made. At sampling, the water level of the tanks was lowered to a few centimetres, the bladder-wrack removed and the fish caught with a dip-net and placed in 10 l buckets with brackish water. The fish were counted and weighed *in vivo*. Ten fish from each population were randomly removed at every sampling and fixated in Bouin's solution for later parasitological and histological examination. The remaining fish were returned to their respective tank.

Two to three days later the fixated fish were transferred from Bouin's solution to 70% ethanol (Romeis 1968). The solutions were saved for a later count of possible loose parasites. The total number of ectoparasites was calculated by addition of loose and attached ones and related to mm^2 fish surface (calculated by total length × height × 2).

Counting was performed using an inverted (loose parasites) and a preparation (attached parasites) microscope. Parasites/ectocommensals occurring in very high numbers were estimated by a frequency scale using a rating of "no occurrence", "low", "medium" and "high" occurrence.

Table 3. Mortality (%) of stickleback larvae over the 5-month experimental periods. HD = high dose, LD = low dose.

Year	Control	Effl. 2		Effl. 3		Effl. 4		Effl. 5		Effl. 6	
		LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
1983	13	15	26.7	11.7	15	16.7	8.3				
1984	3.3							5	1.7	6.7	23

At the end of the experimental period in November, six to nine fish were further dehydrated in alcohol, embedded in paraffin, sectioned in 5µm sections (lateral, whole body sections) with a microtome (Reichert-Jung) and stained in hematoxylin-eosin-alcianblue (Romeis 1968). In this way, it was possible to detect both internal parasites, as well as histopathological changes. Total body fat content of the remaining fish not used for histology was measured at the end of the experiment, according to the method by Korn & Macedo (1973).

The fish fed on natural food introduced by the incoming water and on food produced within the experimental system. No systematic analysis of the food species present was made. Histological analysis of the fish at the end of exposure showed a similar stomach content (zooplankton) in all groups.

Statistics

Differences in weight gain per month were tested against the control using Student's *t*-test with Cochrans correction of the mean value.

3. Results and discussion

3.1. Mortality

In the first experimental period in 1982, the procedure differed somewhat from the following years regarding stocking of the systems with young fish (see methods and materials). Interpretation of the results from that experiment is not seriously affected when compared with the following ones, since clear-cut effects were obtained regarding mortality and diseases:

Conventional bleaching with chlorine (Effl. 1) caused acute mortality of the stickleback larvae after an exposure of about one week in 1:400 dilution effluent. The population of the tank receiving 1:2000 dilution effluent also died but apparently due to high infestations of *Gyrodactylus* sp. However, the reason for the infestation could have been an exposure related impairment of the general condition of the fish (Sniezko 1974). Compared with the control, the production of fish at the end of the experiment was 85–96% lower. At the end of the experiment in December 1982 the production in the corresponding mother ecosystem was very much alike, 83–88% lower than the control, indicating that the effect was exposure related.

When mortality was studied in more detail in the following experiments in 1983 and 1984, considerably lower mortalities were seen within the populations exposed to alternative bleaching using oxygen pre-bleaching and/or external treatment (Effl. 2–6). Total mortality of fish exposed to Effl. 2–6 are given in Table 3. It can be seen from the table that only 1:400 dilution effl. 2 (conventional chlorine bleaching + aerated lagoon) and Effl. 6 caused a higher mortality (27 and 23%) than the control. However, the mortality in Effl. 6 was obviously due to accidental loss of fish larvae at the October sampling. No other significant effects on growth, histology (sticklebacks), physiology and biochemical parameters (rainbow trout) (Lehtinen et al. 1989), which could have been indicative for increased mortality due to toxicity, were seen.

3.2. Growth

As noted above, the growth of the fish differed from the control (Figs. 3–7). Growth was not recorded during the 1982 experiment due to lack of material. Conventional chlorine bleaching (Effl. 2) caused a slower, dose-related growth between July and September (Fig. 3). From this it can be concluded that the earliest stages after hatching of fish larvae are the most critical and that exposure to toxic substances in this effluent induce a lower fish production on a weight basis at dilutions down to at least 1:400, but even at dilutions around 1:2000 at internal process dilution premises given in Table 2.

Introduction of oxygen as a bleaching medium (Effl. 3) initially caused a strongly reduced (July–August) growth in both effluent dilutions (Fig. 4), whereupon a stimulation of the growth (in 1:400 dilution) occurred between August and September. The fish population in the 1:2000 dilution effluent was not

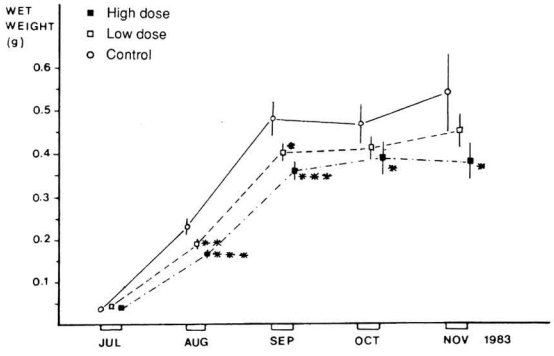

Fig. 3. Mean weight/month of the stickleback population exposed to Effl. 2. Bars indicate 95% confidence intervals. Initial n=60, final n=14(HD), 21(LD). Significance of differences from control: ***=P<0. 01, **=P<0. 05, *=P<0. 1.

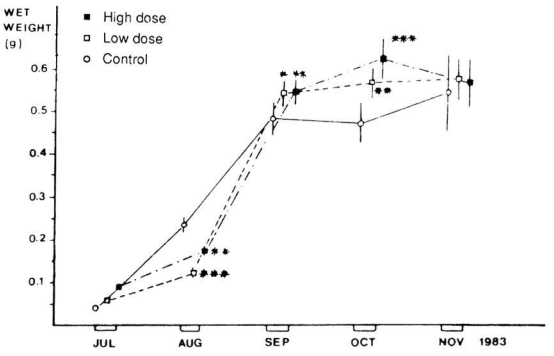

Fig. 5. Mean weight/month of the stickleback population exposed to Effl. 4. Bars=95% conf. int. Initial n=60, final n=25(HD), 20(LD). Symbols as in Fig. 3.

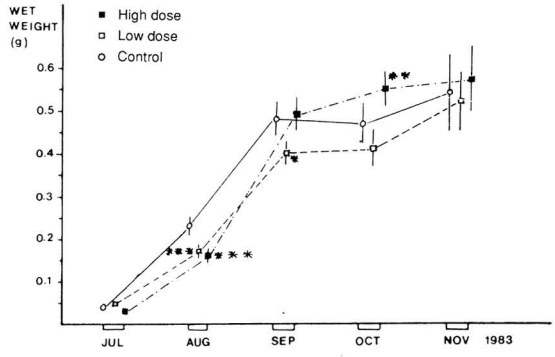

Fig. 4. Mean weight/month of the stickleback population ex posed to Effl. 3. Bars=95% conf. int. Initial n=60, final n=21(HD), 23(LD). Symbols as in Fig. 3.

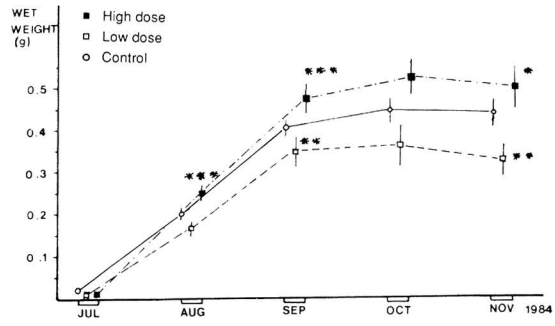

Fig. 6. Mean weight/month of the stickleback population ex posed to Effl. 5. Bars=95% conf. int. Initial n=60, final n=29(HD), 27(LD).

stimulated during this period. The reasons behind this are not clear. Possibly the growth may have been affected by a *Micro-* or *Myxosporidean* infection (Möller & Anders 1986) observed both visually and histologically in the present work.

Partial treatment of effluent from bleaching with oxygen (Effl. 4) induced the same growth response pattern, i.e. an initial inhibition followed by stimulation (Fig. 5). In this case the stimulation persisted until October in the 1:400 dilution effluent, whereafter the mean weight of the remaining fish decreased to some extent.

Effluent from the same bleaching sequence treated in a pilot aerated lagoon (Effl. 5) stimulated the growth of the fish in the 1:400 dilution effluent until the October sampling, whereas the group treated with 1:2000 dilution effluent caused a slower growth during the three first months of exposure (Fig. 6). The reasons for this are unknown. Histologically some *Micro-* or *Myxosporidean* cysts were seen in the vis-

Table 4. Total body fat content (%) of the stickleback larvae at the end of the experiments in November each year. Analyses were made from composite samples of 7–15 fishes. HD = high dose, LD = low dose.

Year	Control	Effl. 2		Effl. 3		Effl. 4		Effl. 5		Effl. 6	
		LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
1983	2.1	1.8	2.9	2.3	3.5	4.4	5.5				
1984	1.8							3.7	5.2	2.3	1.4

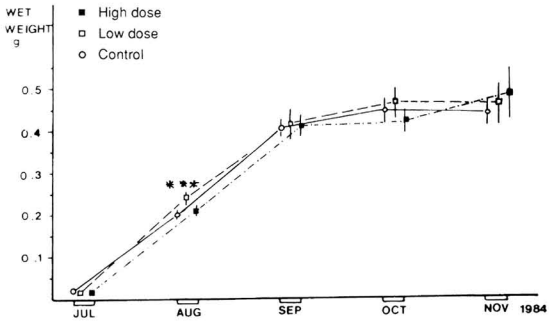

Fig. 7. Mean weight/month of the stickleback population exposed to Effl. 6. Bars = 95% conf. int. Initial $n = 60$, final $n = 16$(HD), 26(LD). Symbols as in Fig. 3.

ceral mass of these fish. The infection was not as predominant as for Effl. 3, however. Possibly some other parasitic or pathological agents undetected by routine histological technique might have affected the growth of this group of fish. Water temperature cannot be considered as the reason behind slower growth since there were no diffrences between the different groups of the respective years.

Untreated BKME from oxygen prebleaching and a 50% substitution of the active chlorine as chlorine dioxide in the C+D stage (Effl. 6) had the least impact on fish growth, with only a slight stimulatory effect in the low dose group at the August sampling (Fig. 7). There were no other differences recorded at the following three samplings.

The alternating inhibition and stimulation of growth noted for Effl. 3 and 4, might be signs of disturbed metabolic pathways in the fish. This assumption is further supported by the results from the total body fat analyses (Table 4) and the liver histology (see below). The fat analyses showed that the fish exposed to Effl. 3, 4 and 5 contained considerably more fat than the control fishes, even though the weight did not differ significantly from the control at the November sampling. Moreover, especially the fish exposed to high dose of Effl. 4 appeared thin compared with control fish.

The effluent from conventional chlorine bleaching (Effl. 2) did not cause significant alterations in fat content. In other studies both increased (McLeay & Brown 1979), as well as decreased (Stoner & Livingston 1978), growth in fish exposed to BKME has been noted. Depending on the time of exposure and type of effluent, changes in body protein and fat content were observed in these studies. Also Oikari et al. (1985) reported metabolic disorders in rainbow trout exposed to simulated pulp mill effluents for 30 days. These authors found increased muscle lipid and decreased muscle protein levels in the exposed fish. The results in the present study may be considered to reflect similar disorders in the exposed sticklebacks. Stoner & Livingston (1978), on the other hand, obtained decreased total lipid content, reduced condition factors and increased protein content, combined with reduced growth, in fish also exposed for 30 days to BKME. The type of bleaching was not reported, but it may be assumed that it was a conventional chlorine bleaching.

These studies show that the growth response of the fish exposed to Effl. 2 is in good accordance, since growth reduction (as for Effl. 3 and 4) was seen during the first 30 days of exposure. The stickleback larvae fed upon natural food occurring in the test-pools. It cannot be fully elucidated whether food intake, or food availability was the reason to the growth responses obtained. However, apart from results from other experiments referred to above on metabolic disorders, it may be noted that the fish biomass was very low at the beginning of the experiment, i.e. at the

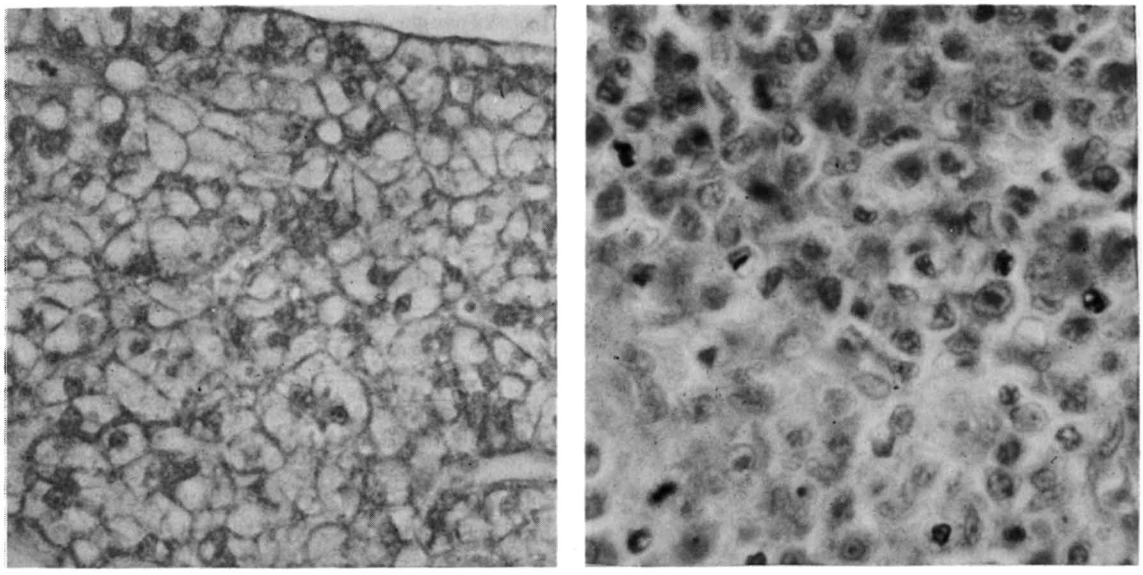

Fig. 8. Liver tissue (left) from a fish exposed to the high dose (400 times dilution) of Effl. 2. Compared with the tissue (right) from a control fish, extensive vacuolization with shrunken nuclei is seen. Also note the diffuse area in the centre of the vacuolized tissue. Hematoxylin-Eosin-Alcian blue (Romeis, 1969). ×360.

observation in July, roughly 3 g/small pool and 5 g/mesocosm pool. This low fish biomass and the period of slower growth as compared with control groups, coincided with a period when production is high in the aquatic ecosystem, and also considering that stickleback biomasses of up to 150 g/mesocosm pool have been reported by Rosemarin & Notini (1990), lack of food for the small biomasses mentioned above cannot be considered as probable.

In view of this, food or lack of food, does not seem to be the reason behind the effects noted. It must be remembered, on the other hand, that an increased energy demand, or an impaired food conversion capability, due to exposure to BKME:s cannot be ruled out (Stoner & Livingston 1978). It is interesting to note in this connection the results of Sandström et al. (1987) from their studies on growth and gonadal development in fish from BKME receiving waters. The authors reported high length increase, high condition factors and impaired gonadal development. High growth was related to a disturbed energy allocation pattern, from gonadal development to development of other tissues. It can, however, in the light of the present results and those of Stoner & Livingston (1978), McLeay et al. (1979), Larsson et al. (1985) and Oikari et al. (1988), be questioned whether this was the only explanation of their results.

3.3. Liver histology

When liver tissue from fish exposed to Effl. 2 were examined, extensive alterations were seen. The cytoplasm showed poor stainability, and regions with membraneous damage were frequent. Nuclear pycnosis was also observed (Fig. 8). Fish exposed to effluents from mills using oxygen prebleaching (Effl. 3, 4 and 5) displayed different structural features as compared with the former group. Here the hepatocytes contained vacuoles of round, smooth shape (Fig. 9). The occurrence was extensive with little or no nonvacuolized tissue present in the preparations.

The results obtained from fish exposed to Effl. 2 are in accordance with previous ones obtained from preliminary mesocosm experiments and from the field using flounder, *Platichtys flesus* L., as testorganism (Lehtinen et al. 1984). Bylund and Björklund (pers. comm.) recently discovered similar hepatic tissue reactions in fish caught outside the discharge area of a Finnish pulp mill.

The fat content in the fish exposed to Effl. 3, 4 and 5 suggest that the vacuoles contained fat and that the fish had high fat deposits not accessible for use in the energy metabolism (Link et al. 1984, Levine & Reihardt 1983). Interestingly, if the growth data, fat content and liverhistological results are connected

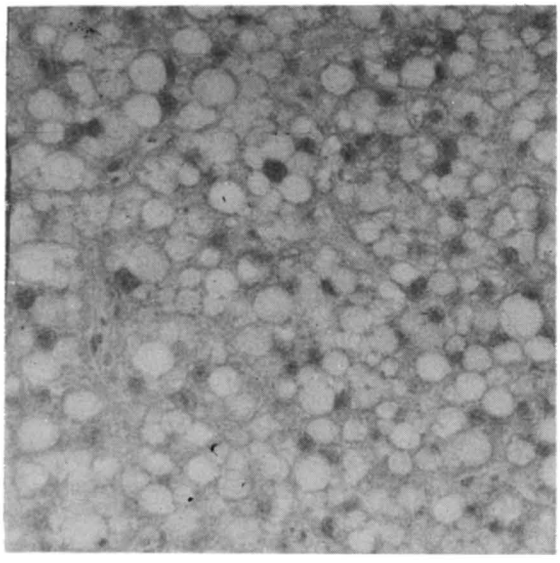

Fig. 9. Liver tissue from a fish exposed to the high dose (400 times dilution) of Effl. 3. Note the round, smooth intracellular vacuoles. Heimatoxylin-Eosin-Alcian blue. ×360.

Table 5. Formation of total organic chlorine (TOCl) as kg/tonne pulp and the corresponding formation of chlorinated phenolic compounds as g Cl/tonne pulp in the different bleaching sequences tested. Cl-P=chlorophenols, Cl-P=chloroguaiacols, Cl-Cat=chlorocatecols.

	TOCl	Cl-P	Cl-G	Cl-Cat
Effl. 1	>5 *	1.2–1.7	5.7–6.9	–
Effl. 2	2.3	<0.01	3.4	3.0
Effl. 3	2.7–3.1	0.4	2.9	–
Effl. 4	2.6	<0.01	1.5	<0.01
Effl. 5	1.8	0.2	0.6	6.3
Effl. 6	2.0	0.5	2.5	4.2

Table 6. Concentrations of chlorinated phenolic compounds (µg/l) in the total BKMEs tested.

Sequence	Cl-P	Cl-G	Cl-Cat
Effl. 1	16	82	–
Effl. 2	19	85	24
Effl. 3	15	100	360
Effl. 4	20	74	495
Effl. 5	14	46	500
Effl. 6	14	130	375

with results from parallel studies on physiological and biochemical effects on rainbow trout (*S. gairdneri* R.) exposed to the same effluents (Lehtinen et al. 1989), a possible interrelated effect pattern is discernible. In the rainbow trout work, the activity of the MFO (Mixed function oxidase) detoxification enzyme 7-ethoxyresorufin-O-deethylase (EROD) was measured. Effl. 2 caused an inhibition of the enzyme, whereas effl. 3, 4 and 5 stimulated it.

Apart from its detoxifying function the MFO-system also functions as a regulator of endogenous substances such as steroid hormones in fish (Payne et al. 1987). It may thus be speculated that competitive mechanisms between xenobiotic substances and steroids at some stage may induce abnormal hormonal levels in fish, which in turn may lead to secondary effects on growth, reproduction and fat metabolism.

During detoxification of xenobiotic substances, formation of intermediate reactive metabolites may occur, which may be more toxic to the organism than the parent substance (Buhler & Williams 1988, Lech & Bend 1980). Such substances are known to react irreversibly with membrane components at sites where detoxification takes place (Parke 1981) sometimes destroying the detoxification system (Levine & Reinhardt 1983). In the present study, there was an indication that Effl. 2 was more cytotoxic than the other effluents causing membrane damage in the hepatocytes. The reason for an assumption that Effl. 2 was more toxic than the other effluents is seen from Table 2. From the table it can be seen that the washing loss was higher than for the other effluents. This certainly meant that toxic substances such as resin acids contributed to the effects noted for Effl. 2. Resin acids have previously been shown to inhibit enzymes involved in detoxification processes (Oikari & Nakari 1982).

The significance of the effects noted on growth, fat content and liver histology of Effl. 3 and 4 is not known. Organisms do have some capability to adapt to polluted conditions (Tana et al. 1988). This field is highly neglected and no stringent information in this connection is available at the moment.

It is not known which substances caused effects in the present study. However, decreasing TOCl (Table 5) values gave smaller effects. Chemical analyses on the levels of chlorinated substances did not give any information as to their possible role as effects-causing substances, since Effl. 5 and 6 causing the least impact on fish had equally high levels as those causing the highest impact (Table 6). Tana (1988) showed that

Table 7. Different types of parasites and lesions noted in sticklebacks exposed to Effl. 1, 1982. – = none, + = slight, ++ = intermediate, +++ = high.

	Control	LD	HD
Trichodina SPP.	+	+++	+++
Apiosoma	+	++	++
Gyrodactylus	+	+++	+++
Hemorrhagic eyes	–	+	++
Fin rot	–	–	+

chlorinated phenols alone and in combination with resin acids, and resin acids alone influenced a conjugating enzyme (UDPGT) differently. Chlorinated phenols stimulated the enzyme, resin acids and chlorinated phenols together caused an alternating stimulation and inhibition. Resin acids alone inhibited the enzyme.

It is, therefore, evident that different answers may be obtained depending on the moment of observation and length of exposure and (possibly) the relative concentrations between stimulatory and inhibitory substances when BKMEs are studied. Thus, increased knowledge on how central biochemical mechanisms are regulated, or affected, in fish under polluted conditions, is needed.

3. 4. Parasites and diseases

Occurrences of a parasite or disease in fish is the result of an interaction both between the environment, the parasite or disease and the fish. It is known from epidemiology that infectious agents cause outbreaks of diseases in the host if the environmental conditions are right (Sniezko 1974).

In the first experiment in 1982 the fish populations exposed to Effl. 1 were heavily affected in the low dose treatment. In this group *Apiosoma* and *Trichodina* spp. and the highly pathogenic *Gyrodactylus* sp. were seen. The remaining fish were infested by ectoparasites in the high dose treatment also (Table 7). The eyes of some fish were infected by *Diplostomum* or showed periferal hemorrhages of the eyes. Fin rot was also seen in fish from the large mesocosm pools during the course of the experiment. During the 1983 and 1984 experiments no acute infestations of *Gyrodactylus* were noted. On the other hand, the ectocommensal *Apiosoma* was frequent, especially in fish from the high dose treatments of all effluents, whereas control fish were only sporadically infested. *Trichodina* spp. infestations were also seen, but it was not possible to state whether they occurred due to exposure or not, since control fish had a higher incidence/mm^2 in 1983 than exposed fish. However, a possible temporal relationship between high incidence of *Apiosoma* and a low or non-parasitic state of *Trichodina* cannot be ruled out. In work by Lom (1973), the idea that *Trichodina* may under some circumstances exist either as a pure ectocommensal or as a parasite was discussed. Under the parasitic condition the parasite would feed on the epidermal mucus layer as previously indicated by Lehtinen et al. (1984) using flounder, *Platichtys flesus* L., as experimental animal.

In general it can be noted that exposure to BKMEs increased the incidence of parasitic/ectocommensal infestations and that the lowest incidence aside of the control, occurred in the low dose treatments of Effl. 5 and 6.

The mechanisms behind higher incidences of parasites and diseases in exposed fish are not clear, but there exist several possibilities how diseases may break out under polluted conditions (Wedemeyer 1969). One plausible mechanism could be related to events connected with the detoxification system and hormonal imbalance. Several authors (Fries1981, Johansson-Sjöbeck et al. 1978, Weinreb 1958) have reported immunosuppressive effects in fish mediated through increased levels of corticosteroids and ACTH under physiological stress. Mayer et al. (1978) observed that channel catfish exposed to toxaphene had thinner mucous and epidermal strata than unexposed ones and that the effect was counteracted by ascorbic acid. Andersson et al. (1988) found an increased liver ascorbic acid level in perch, *Perca fluviatilis*, caught from a BKME receiving area. The increase may have been a result of an enhanced demand of ascorbic acid for detoxification or wound healing processes. Thus, in the present work it cannot be ruled out that the mucous and epidermal layer could have been affected by such mechanisms, favouring the sessile *Apiosoma* against *Trichodina*.

4. Conclusions

The results from the present work show that BKMEs affect survival and growth, as well as prevalence of diseases and parasites. In the short term perspective, conventional chlorine bleaching (treated and untreated effluent) affects both survival and

growth of fish larvae. Introduction of oxygen prebleaching, with or without external treatment and high substitution (50%) of active chlorine as chlorine dioxide diminish or eliminate mortality. Untreated or treated effluent from the mill using O_2-bleaching still induces abnormalities of growth in fish, whereas 50% chlorine dioxide in the CD-step and O_2-prebleaching eliminate also this effect.

Acknowledgements. The study was financed by the Environmental Research Foundation of the Swedish Pulp and Paper Association and by the Swedish Ministry of Agriculture, through the Swedish Environmental Research Institute (IVL).

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Received 29.XII.1988
Revised 31.VIII.1989
Printed 24.XI.1989