FISHERY BOARD OF SWEDEN



# INSTITUTE OF FRESHWATER RESEARCH

## DROTTNINGHOLM

### Report No 54

LUND 1975
CARL BLOMS BOKTRYCKERI A.-B.

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## Contents

# Muscle and Blood Lactate in Juvenile *Salmo salar* Exposed to High $pCO_2$

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## I. INTRODUCTION

Transitory acidosis in salmon parr due to hypercapnia brought about by a sudden increase in $pCO_2$ in the ambient water has been described by HÖGLUND and BÖRJESON (1971). According to earlier observations by HÖGLUND and HÄRDIG (1969) young salmon also respond to a raised environmental $pCO_2$ by transitory agitated swimming and hyperventilation. HÖGLUND and BÖRJESON (*op. cit.*) found no statistically significant increase in the blood lactate content during this initial phase of carbon dioxide induced hyperactivity. No determinations of muscle lactate were performed, as no valid sampling technique suitable for assay of carbohydrate metabolites in fish muscle *in vivo* was known by the authors at that time. Since then the sampling technique developed by WOLLENBERGER *et al.* (1960) has been adapted by BÖRJESON and FELLENIUS [1] (to be published) for accurate sampling of fish muscle. The present paper describes the use of this method for measurement of the lactate contents in body muscle from salmon parr during the agitation caused by a high external $pCO_2$.

## II. MATERIAL AND METHODS

### Test fish

Second-summer parr (*Salmo salar* L.) weighing 25—40 g were brought from the salmon hatchery of the Fishery Board of Sweden, Älvkarleby, to Uppsala two months before the experiments. The fish were kept in our laboratory in a 1 000 litre aquarium with streaming water (1—3 m/min) and acclimatized to the aerated Uppsala tap water (for quality, see Table 1). The fish were fed automatically twice a day with pellets (Salmon Grove, size 4, Astra-Ewos AB, Södertälje, Sweden).

### Experimental

The fishes were placed in the test aquarium (HÖGLUND and HÄRDIG 1969, Fig. 2, p 84: HÖGLUND and PERSSON 1971, Fig. 1, p 76) 24 h before the $pCO_2$ was sharply raised to about 20 mm Hg. This was achieved by adding hydrochloric acid to the aerated tap water (see Table 1) to a pH of 6.8—6.9. After 20 min of exposure to the raised $pCO_2$ the fish was taken out and stunned by a blow on the head, and aluminium clamps cooled in liquid nitrogen (WOLLENBERGER *et al.* 1960) were pressed on the back just in front of the dorsal fin, whereby a piece of frozen muscle tissue 2 mm thick was obtained. The whole procedure, from the time of removal of the fish from the water, took about 10 seconds.

### Analyses

Skin and visible bone were removed from the muscle sample which was then pulverized in liquid nitrogen as described by LOWRY *et al.* (1964). Lactate was determined in neutralized perchloric acid extract according to HOHORST *et al.* (1959). Lactic dehydrogenase and $NAD^+$ were obtained from Sigma Chemical Co.

### Statistics

Student's t-test was used in the statistical analyses, $p < 0.01$ being considered significant.

## III. RESULTS

The lactate estimates arrived at in the present study are presented in Table 2 along with earlier

[1] Towards a valid technique of sampling fish muscle to determine redox substrates.

Table 1. *Chemical characteristics of the aerated tap water in the laboratory in Uppsala* (Nov. 1974).*

| Temp. °C | pH | Tot. $CO_2$ [1] mM | $pCO_2$ mm Hg | $pO_2$ [2] as percentage of air saturation |
|---|---|---|---|---|
| 14 | 7.9 | 4.5 | 2—3 | 85—90 |

[1] Alkalinity determination according to BERGER; *in* KARLGREN (1962).
[2] Oxygen determination — ordinary WINKLER; *in* KARLGREN (1962).

Table 2. *Lactate content in body muscle (mmol/kg wet weight) and blood (mmol/l) of salmon parr.*

| | Control group | | Test group | |
|---|---|---|---|---|
| | Fish acclimatized to aerated Uppsala water (see Table 1) | | Fish exposed to $pCO_2$ of 20 mm Hg | |
| | Muscle [1] | Blood [2] | 20 min Muscle | 0—60 min Blood [2] |
| Mean ± SD | 1.01 ± 0.53 | 1.08 ± 0.42 | 2.32 ± 0.65 | 1.71 ± 1.26 |
| n | 6 | 10 | 10 | 13 |

[1] Data from BÖRJESON and FELLENIUS (to be published).
[2] Data from HÖGLUND and BÖRJESON (1971).

data from HÖGLUND and BÖRJESON (1971) and BÖRJESON and FELLENIUS (in preparation). The values obtained by the latter authors may be regarded as good estimates of muscle lactate in rested salmon parr. The hyperactivity induced by raising the $pCO_2$ caused a notable increase in the muscle lactate from 1.01 to 2.32 mmol/kg wet wt ($p < 0.01$). However, there is no statistically significant difference between the lactate levels in the muscle and in the blood of the parr belonging to the test group of Table 2 ($0.20 < p < 0.10$). HÖGLUND and BÖRJESON (1971) found only a slight increase in the blood lactate of salmon parr exposed to a high $pCO_2$ as compared with the controls. The significance level pertaining to this difference was erroneously noted in that paper (*op. cit.*, Table 2, p. 70). It should have been $0.20 < p < 0.10$, which further supports the statement made by these authors on p. 71. "It is concluded that the transient acidosis observed is not caused by a lactate accumulation. Accordingly the acidosis just seems to depend upon an unbalanced hypercapnia caused by the $CO_2$ treatment." Thus, it is evident from the present study that the lactate content and pH of the blood alone do not give a complete picture of the acid-base status of the fish. The lactate content in the muscle would seem to be a more sensitive index of the metabolic acidosis due to lactate than the lactate level in the blood.

## IV. DISCUSSION

The present results seem to support the view expressed by LOVE (1970, *cf.* p. 44), among others, that lactic acid is released rather slowly from fish muscle into the blood. Violent thrashing about gives rise to an increase in muscle lactate. This increase must be considerable, however, be-

* It should be noted that during the last few years the alkalinity of the tap water in Uppsala has decreased.

fore significant lactate accumulation can be observed in the blood either immediately or later. WITTENBERGER (1968) found that muscular work leads to an intensification of glycolysis in the white muscle, and of oxygen consumption in the red muscle. BILINSKI and JONAS (1972) noted a much higher rate of lactate oxidation in red than in white muscle from rainbow trout. This indicates that the lactate produced in the white muscle may be oxidized in the red muscle and thus partly may not be released into the blood circulation.

## V. SUMMARY

The lactate content in the body muscle of salmon parr (*Salmo salar* L.) increases during the initial phase of hyperactivity and acidosis due to hypercapnia deriving from a sudden rise of the $pCO_2$ in the respiratory water. This is not accompanied by any statistically significant elevation of the blood lactate level. The biological significance of this event is briefly discussed.

## VI. ACKNOWLEDGMENTS

We are indebted to Professor GUNNAR SVÄRDSON. Financial support from the Fishery Board of Sweden is appreciated.

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## VIII. KEY WORDS

Lactate, muscle lactate, blood lactate, metabolic acidosis, acidosis, hypercapnia, *Salmo salar*.

# The Acidification of Swedish Lakes

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## I. INTRODUCTION

Sweden has about 90,000 lakes. These have a total area of about 40,000 km² and thus occupy 9 per cent of the country (450,000 km²). The four largest ones, Lakes Vänern, Vättern, Mälaren and Hjälmaren, take up a quarter of this area and a further 4,000 lakes are bigger than one km², but the majority (85,000) are smaller, accounting for 20 per cent of the total lake area.

The yearly precipitation over the country ranges from 400 mm in the eastern parts to 1,800 mm in the mountain area in the north-west. The mean precipitation is about 700 mm.

The run-off increases from about 125 mm in the south-east to over 1,500 mm in the mountain area. The average run-off is about 400 mm per year.

Owing to different proportions between size of drainage areas and lake volumes, the theoretical retention time for the lakes varies from several decades (Lake Vättern, 60 years) to less than two years or even one year for most lakes.

## II. BEDROCK

The bedrock in Sweden consists mainly of slowly weathering granites, gneisses and porphyries. In some parts of the country, however, limestone or lime-rich greenstones (diorites, gabbroes, hyperites, diabases and amphibolites) are found.

The occurrence of lime in rocks and soils is shown in Fig. 1 (from MAGNUSSON *et al.* 1957).

Limestone and calcareous rocks are found in the following areas: Around Lakes Storsjön and Siljan; to the east of Lake Vänern and Lake Vättern; to the west of Lake Hjälmaren; in the southernmost part of Sweden; and on the islands Gotland and Öland.

From these areas and from the lime rocks in

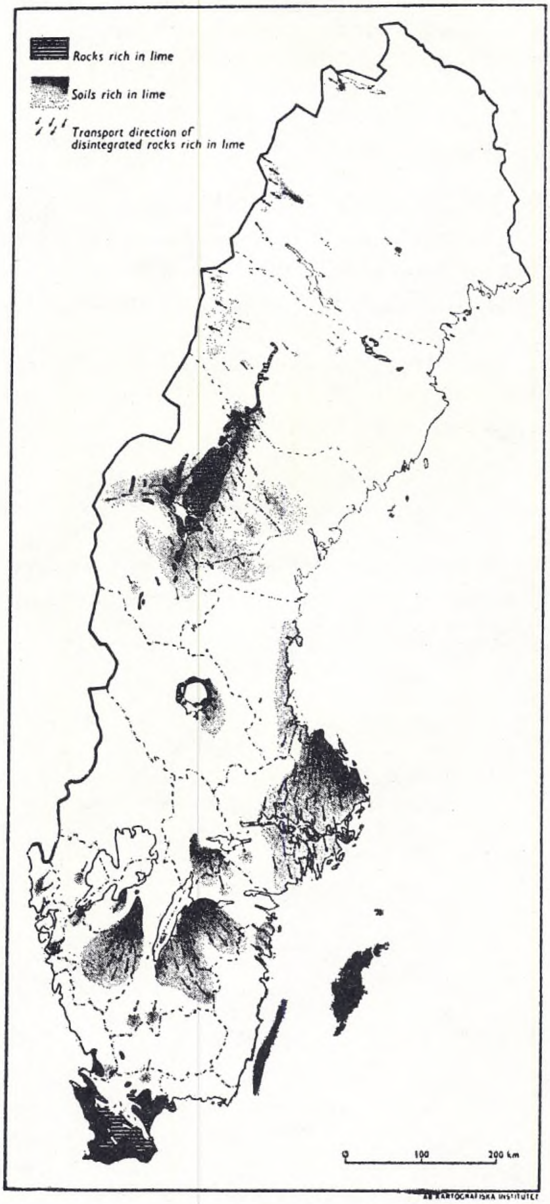



Fig. 1. The occurrence of lime in Sweden (from MAGNUSSON, LUNDQVIST and GRANLUND 1957).

the Gulf of Bothnia, lime was spread — mainly south-eastwards — by the inland ice during the last glacial period, making the soils in adjacent areas more calcareous than in other parts of the country.

On some places along the coast, shell banks give a high calcium content to the soil.

## III. LAKES

The bedrock and soil have a great influence on the composition of lake and ground water. In lime-rich areas, lakes will have a bicarbonate content, owing to the solution of the lime

$$CaCO_3 + H_2O + CO_2 \rightarrow Ca^{2+} + 2HCO_3^-,$$

that is often of the order of 0.5—2 mekv/litre, giving a pH of 7—8. Lakes on less weathering ground will have a natural alkalinity of 0.1—0.2 mekv/litre or even less and a pH of 6—7. The majority of Swedish lakes belong to this latter group, *i.e.* to the soft and low-buffered waters. In these the atmospheric contribution is responsible for the greater part of their salt content. In the mountain region several of these lakes have conductivities of about 10 μS, 20 C°, which is almost similar to that of distilled water.

Fig. 2 shows the alkalinity of Swedish lakes. The map is mainly based on the results from 1,250

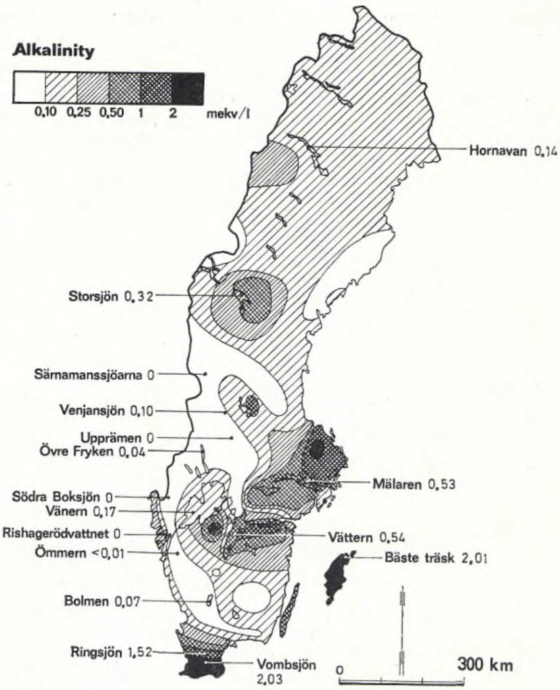



Fig. 2. Alkalinity in Swedish lakes.
The map is mainly based on the observations in 1,250 lakes in August 1972. Alkalinites above 0.5 mekv/l. are found in the lime-rich areas, *cf.* Fig. 1. Values below 0.1 mekv/l. are found in the western and south-western part, along the coast in the north and south-east of Stockholm (from JOHANSSON and KARLGREN 1974).

Table 1. *Some examples of alkalinity, pH and conductivity in lakes on weathering and less weathering ground. (Data from Mälaren and Vänern from* AHL *1973 a and b. Other data from the National Swedish Environment Protection Board, Research Laboratory, Drottningholm, 1972—74.)*

| Ground | Lake | Size km² | Date | Alkalinity mekv/l. | pH | μS 20°C |
|---|---|---|---|---|---|---|
| | Vombsjön | 12.4 | Aug 1972 | 2.03 | 8.9 | 371 |
| | Bäste träsk | 6.6 | Aug 1972 | 2.01 | 7.9 | 234 |
| weathering | Ringsjön | 41 | Aug 1972 | 1.52 | 8.8 | 238 |
| | Vättern | 1912 | Sep 1972 | 0.54 | 7.7 | 104 |
| | Mälaren | 1140 | 1964—1971 | 0.53 | 8.0 | 150 |
| | Storsjön | 456 | Aug 1972 | 0.32 | 7.3 | 36 |
| | Vänern | 5550 | 1973 | 0.175 | 7.1 | 80 |
| | Hornavan | 251 | Aug 1972 | 0.14 | 6.9 | 23 |
| less | Venjansjön | 34 | Aug 1972 | 0.10 | 6.9 | 21 |
| weathering | Bolmen | 184 | Aug 1972 | 0.07 | 6.6 | 64 |
| | Övre Fryken | 42 | Aug 1972 | 0.04 | 6.5 | 26 |
| | Ömmern | 10.3 | Aug 1974 | $<$0.01 | 5.5 | 64 |
| | Sö. Boksjön | 9.1 | Aug 1972 | 0 | 4.5 | 39 |

Table 2. *Contents of salts in precipitation 1955—59 and 1970—73. Station Plönninge in the south-western part (56° 42′ 12° 45′) and Forshult in Central Sweden 400 km to the north (60° 05′ 13° 47′). At station Plönninge there is a general increase of salts 1970—73 compared with 1955—59 (partly local contamination). At both stations, however, a rise in sulphur and nitrogen of 50—100 per cent is noted. The alkalinity of 0—0.02 mekv/l. during 1955—59 is replaced by an excess of hydrogen ions of 0.03 mekv/l. 1970—73 and a lowering of pH to 4.5. (Data from the International Meteorological Institute, University of Stockholm, 1974.)*

| | pH | H | Na | K | Ca | Mg | $NH_4$ | $SO_4$ | Cl | $NO_3$ | Alk |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | mekv/l. | | | | | |
| Plönninge | | | | | | | | | | | |
| 1955—59 | 5.0 | 0 | 0.097 | 0.007 | 0.042 | 0.032 | 0.044 | 0.074 | 0.104 | 0.025 | 0—0.02 |
| 1970—73 | 4.5 | 0.03 | 0.107 | 0.031 | 0.063 | 0.034 | 0.054 | 0.148 | 0.126 | 0.045 | 0 |
| Forshult | | | | | | | | | | | |
| 1955—59 | 5.4 | 0 | 0.017 | 0.004 | 0.039 | 0.010 | 0.012 | 0.045 | 0.012 | 0.012 | 0—0.02 |
| 1970—73 | 4.5 | 0.03 | 0.017 | 0.007 | 0.027 | 0.010 | 0.015 | 0.076 | 0.012 | 0.020 | 0 |

lakes analysed in August 1972 (from Johansson and Karlgren 1974). The good agreement between alkalinity and the occurrence of lime in Fig. 1 should be noted.

Examples from some lakes on weathering and less weathering ground are given in Table 1.

## IV. ACIDIFICATION

### *Bogs*

It has long been known that bog lakes can be very acid. Bog water usually has a very low salt content, which is entirely derived from the atmosphere. The vegetation around it, mostly peat mosses, have to depend on these salts for their nutrient requirements. The uptake of cations is larger than is that of anions, and is compensated for by a corresponding release of hydrogen ions, which in turn leads to an acidification of the bog water. Together with the dissolved acid decomposition products, humic acids, this process can bring about a very low pH, as low as pH 3 in small bog pools.

### *Coniferous forests*

A similar mechanism is considered to appear when spruce or pine is planted on former arable land. The uptake of cations and the release of hydrogen ions and acid humic substances may acidify the soil from a level even above pH 6 to values of only pH 4 within a few decades.

### *Oxidation*

Another type of acidification occurs when sulphide is oxidized in soil or sediment.

Hydrogen sulphide $H_2S + 2O_2 \rightarrow 2H^+ + SO_4^{2-}$

Iron sulphide $FeS + 2O_2 + 2H_2O \rightarrow 2H^+ + Fe(OH)_2 + SO_4^{2-}$.

The processes occur regularly and are considered to be very important for the weathering of the soil material. When soils that are usually submerged in water, come in contact with air during a long period of dry weather or low water, they will be oxidized and will acidify the next high water. Illustrative examples of this natural acidification have been found in lakes surrounded by sulphide soils. In such lakes a pH decrease to 3—3.5 has been found, as well as fish kills, clarification of the water owing to the precipitation of humic substances, and the adaptation of an extreme plankton (Högbom 1921, Vallin 1953).

The same type of acidification, in this case man-influenced, may also occur when bogs are ditched. Here, too, fish kills have been observed (Dahl 1923).

In nitrifying soils (above pH 5), ammonium will be oxidized to nitrate with a consequent acidification

$$NH_4^+ + 2O_2 \rightarrow 2H^+ + NO_3^- + H_2O$$

The process is well known to agriculturists and has become a problem in modern farming. The acidification from 100 kg ammonium-nitrogen requires a lime dose (CaO) of the order of 300 kg (Nömmik 1966).

*Man-made atmospheric acidification*

The acidification of soil and lakes due to atmospheric pollution by sulphur and nitrogen compounds has attracted increasing attention in Scandinavia during the last decade (ODÉN 1968, ANON. 1971). The problems are similar in Canada, where large acidifying emissions have influenced meagre soils and low-buffered lakes (BEAMISH and HARVEY 1972).

Today about 20 per cent of the total quantity of sulphur released into the atmosphere is emitted in Europe, from an area of only about 1 per cent of the total surface of the earth. In this region more than 75 per cent of the sulphur in the atmosphere has an anthropogenic origin (ANON. 1971). The emission has probably increased by about 3 per cent a year (MUNN and RODHE 1971).

In Sweden the composition in air and precipitation has been analysed monthly since the early 1950's by the International Meteorological Institute of the University of Stockholm.

The data show a sharp decrease in pH from around 5.5 in the 1950's to pH 4.3—4.5 up to Middle Sweden today, and an increase in sulphur and nitrogen compounds of 50—100 per cent (Table 2).

These changes are clearly connected with the continuous increase of acidic emissions and with the change-over from coal to oil combustion and from small to tall chimneys in Western Europe.

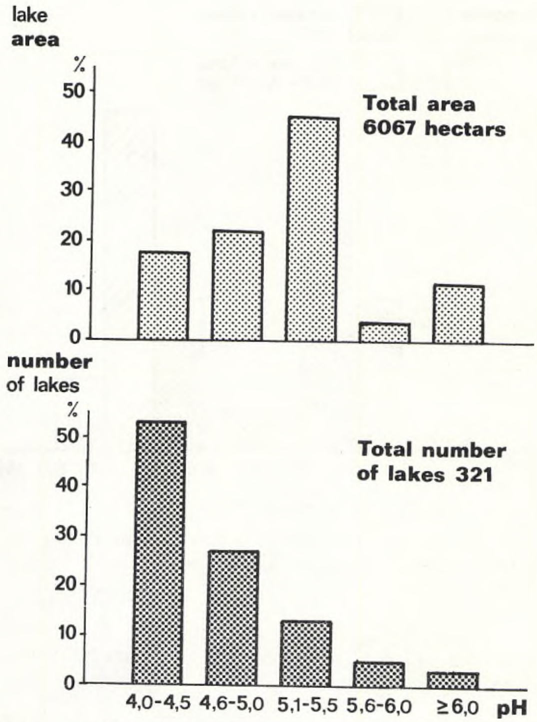



Fig. 3. pH in 321 lakes around Gothenburg during 1968—70. 85 per cent of the lake area had a pH of 5.5 or lower. 53 per cent of the total number of lakes even had a pH of 4.0—4.5 (from SCHMUUL 1972).

## V. EFFECTS IN LAKES

*pH and alkalinity*

Naturally there must have been a continuous increase in atmospheric pollution ever since the Industrial Revolution, with corresponding increased effects on waters. In Southern Norway, fish kills occurred as early as the 1920's in waters with a pH of around 5.0 and a connection between a mild, humid climate (southern winds?) and the acidity of the lakes was suspected (SUNDE 1926). This area is now the most highly acidified in Scandinavia.

In Sweden, the most sensitive lakes in the West Coast region, with "natural" pH values of about 5.5—6 (almost that of rainwater), lost their roach population in the 1930's possibly on account of the atmospheric acidification. "Clean rainwater" has a pH of about 5.6 and the roach will be affected already at values just below pH 5.5 (ALMER 1972).

During the last 20 years a large number of Swedish lakes have been acidified. Trends of the "Surface Water Network" in Sweden (ODÉN and AHL 1972) indicate that "if the present development continues, in less than 50 years about 50 per cent of our lakes and rivers may have pH values of 5.5 or even 5.0" (ANON. 1971).

This situation occurred long ago in the region around Gothenburg on the West Coast, which not only is extremely sensitive but also represents the most exposed area of Sweden. Of 321 lakes investigated during 1968—70, 93 per cent had pH 5.5 or lower and 53 per cent even had 4.0—4.5. Of the corresponding lake area, 85 per

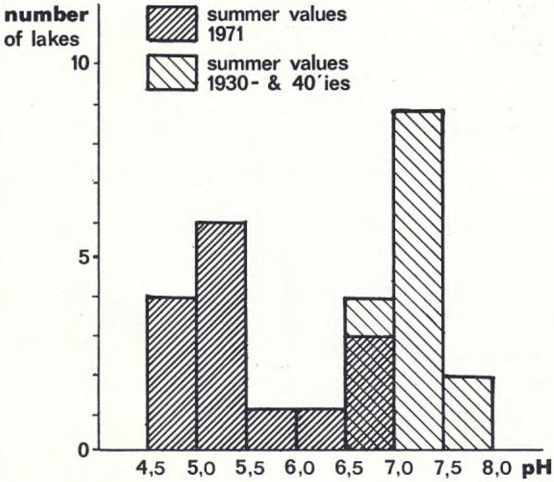



Fig. 4. pH change in West Coast lakes. Values from 1971 were often 1.5 pH units lower than during the 1930s and 40s in the same lakes.

cent had pH 5.5 or lower (Fig. 3, from SCHMUUL 1972).

A similar picture is found in the whole West Coast region. Extrapolated from the findings 1970—72 (Table 3, from ALMER *et al.* 1974), half of the number of lakes in the area, about 1,500 of a total of 3,000, would have a pH lower than 6, and some 800—900 would have pH values even lower than 5.0. In these lakes, the values had been lowered by up to 1.8 pH units from the 1930's and 40's to summer 1971 (Figs. 4 and 5).

The atmospheric pollution can be followed further north in almost the whole of Sweden, all lakes and rivers being affected, to a greater or lesser extent. Those with natural alkalinities below 0.1 mekv/litre are the most sensitive and they are the first where the pH will drop (Fig. 6). Most of them are located in the western part of the country (Fig. 2). They may have an area of several square kilometres. The total number of lakes now acidified to below pH 6 is probably around ten thousand and the number below pH 5.5 five thousand. Thousands will now have alkalinities of 0—0.05 mekv/litre and pH reduced by 0.5—1 unit.

The most acidified lakes are often located in the upper parts of a lake system, thus having small drainage areas and not being subjected to influence from agricultural land.

*Conductivity and ionic composition*

In Southern Sweden, since the 1930's and 40's, the conductivity in lakes has risen by 10—30 μS

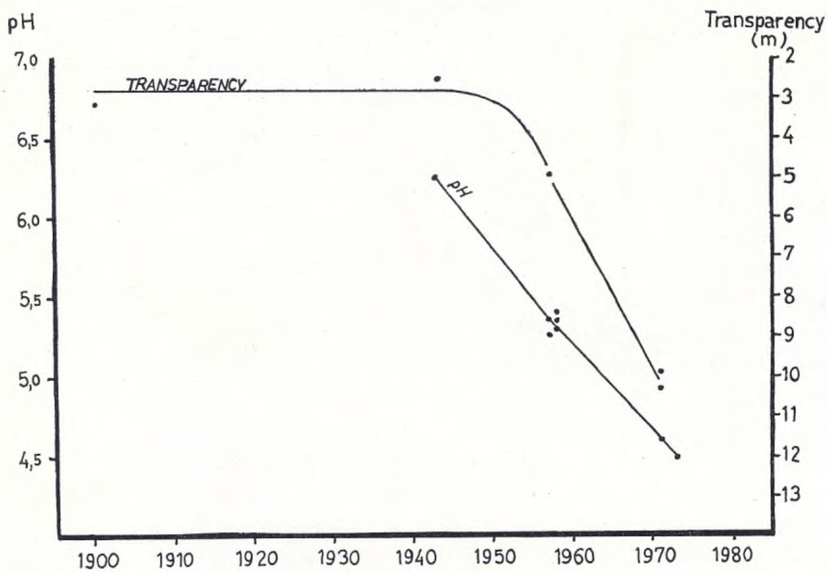



Fig. 5. Lake Stora Skarsjön. Size 0.6 km² (58° 13′ 11° 56′). From 1943 to 1973 the pH decreased from 6.25 to 4.5. The transparency increased more than 7 metres (from ALMER *et al.* 1974).

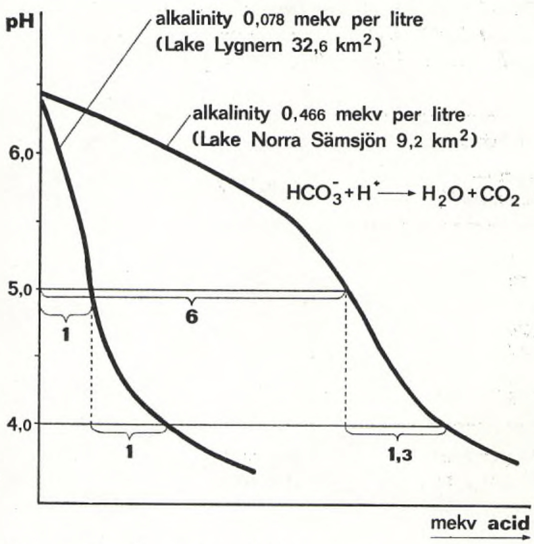



Fig. 6. The low-buffered lake, with an alkalinity of 0.078 mekv/l., needs only one sixth of the amount of acid added to the strongly-buffered lake to reach pH 5.0. Below this pH almost all bicarbonate is lost and pH will fall drastically in both lakes when more acid is added.

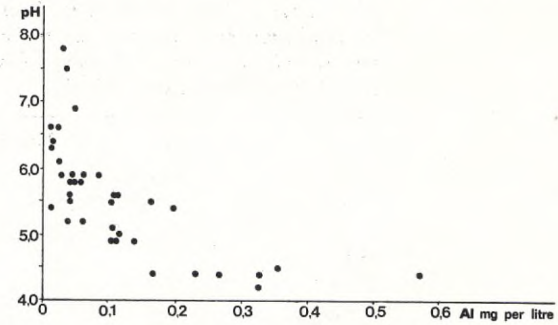



Fig. 7. Aluminium content in clear-water lakes. The solubility rises at low pH.

(20° C) and in Northern Sweden by 5 μS. The increase in sulphate is 0.1—0.3 mekv/litre and this accords well with the increased deposition. In many cases there is also noted an increase of cations, which is probably connected with the leaching activity of the acids.

In the so-called "Standard Composition" of lakes (Rodhe 1949), bicarbonate is the most abundant anion and the ratio of bicarbonate to sulphate is 4.7:1. The increase — at least a twofold one — in sulphate, and the decrease in bicarbonate have considerably reduced the validity of the "Standard Composition" as far as most Swedish lakes are concerned. The ratio of bicarbonate to sulphate is now usually less than 1:1.

*Transparency and nutrients*

Along with the acidification, the lakes will be clarified owing to precipitation of humic substances and a decrease in the plankton flora (Almer *et al.* 1974). In some cases the transparency has increased by ten metres during the last thirty years.

The content of phosphorus is very low, 2—10 μg/litre, possibly owing to the increasing precipitation of humus and a decreased decomposition of humus and detritus. The nitrogen content varies considerably according to the atmospheric fall-out; it ranges between 0.1 and 0.4 mg N/litre. A high proportion of this remains as nitrate even during the summer, owing to the low plankton activity.

*Solution of aluminium and manganese*

The content of aluminium rises in acidified lakes and is 0.2—0.6 mg Al/litre (0.01—0.07 mekv), whereas clear-water lakes with a normal pH have 0.05 mg/litre or less (Fig. 7). Below pH 5, aluminium will probably occur as a tri- or bivalent cation, but in less acid water it will exist in a monovalent or a non-ionic form (Pionke and Corey 1967, Malmer 1974). Manganese, too, goes into solution in acid water, and a high content, 0.3—0.4 mg/litre (Fig. 8), appears in the

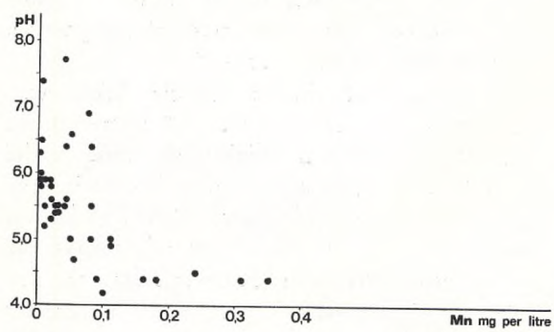



Fig. 8. Manganese in lakes. A considerable increase in solubility appears at about pH 4.5.

Table 3. *pH in lakes on the West Coast 1970—72, within 80 km from the sea. The total number of lakes in the region is about 3,000. Extrapolated from the lakes examined, some 1,500 would have a pH below 6 and 800—900 a pH below 5* (from ALMER *et al.* 1974).

| pH | Number of lakes | | |
|---|---|---|---|
| | November—December, 1970 | April—June, 1971 | August, 1972 |
| ⩽3.9 | 15 | 4 | 3 |
| 4.0—4.9 | 97 | 79 | 17 |
| 5.0—5.9 | 67 | 129 | 35 |
| 6.0—6.9 | 116 | 124 | 63 |
| ⩾7.0 | 19 | 47 | 43 |
| No. of lakes studied | 314 | 383 | 161 |
| <5.0 | 36 per cent | 22 per cent | 12 per cent |
| <6.0 | 57 per cent | 55 per cent | 34 per cent |

most acid lakes (corresponding to about 0.01 mekv/litre). No increased solubility of iron or silicon has been observed, so far as is known.

## *Composition of precipitation and lakes, three examples*

The water chemistry of most of the Swedish lakes on primary rocks is influenced to a marked extent by the atmospheric contributions. Three examples from different parts of Sweden will illustrate this.

### West Coast area

The precipitation around Gothenburg and the industrialized areas in this region is very acid, with a pH of about 4.2 (1973). The precipitation amounts to about 700 mm per year, and evapotranspiration will concentrate it about 1.6—1.8 times. The composition of the water of three forest lakes and some calculations of the possible contributions are made in Table 4.

The content of chloride in the lakes near the coast, 20 km from the sea, 100 metres above sea level, but below the highest shoreline of the sea, is 0.35 mekv/litre, or more than twice the amount that comes with precipitation (0.15 mekv). Even lakes above the highest shoreline have the same content of chloride. So in this area the dry deposition of sea salts will be equal or greater (about 1.0—1.5 times) than the amount from precipitation.

Of the sulphate content, 0.34 mekv/litre, some 0.16 mekv is derived from precipitation. To this must be added a small fraction, which is derived from sea spray (0.02 mekv/litre), while probably the greater part of the rest, 0.16 mekv/litre, is of anthropogenic origin airborne sulphur dioxide and sulphate. The total increase in sulphate, including the increase from precipitation, at least 0.1 mekv/litre, since a natural stage, is probably of the order of 0.2—0.3 mekv/litre.

The content of nitrate in precipitation is about twice as great as that in the precipitation of the 1950's, and ammonium has increased by around 25 per cent (Table 2). The concentrated deposition of ammonium and nitrate with precipitation (2.4 mg N/litre) is six times as great as that found in lakes (0.4 mg Total N/litre). The soil and forests evidently have a considerable ability to retain the nutrients needed.

There seems, however, to be a very considerable leaching of cations, calcium, magnesium, sodium, potassium and aluminium to the lakes (up to 0.25 mekv/litre) to compensate for the increased deposition of acid, sulphur and nitrogen. The hydrogen ion concentration in lakes at pH 4.9, for instance, is 0.09 mekv/litre less than what comes from precipitation, and to compensate for the sulphur a further 0.16 mekv of cations per litre is required (Table 4). Part of the amount of cations and sulphate may have leached out when

Table 4. *Contents of salts in three lakes in the West Coast area, (58° 12°) located about 20 km from the sea and within 15 km from the precipitation sampling station (Mjösund). Precipitation data from the Swedish Meteorological and Hydrological Institute, (SMHI) 1974. The values in precipitation of K, Ca, Mg, $NH_4$ and $NO_3$ are estimated from the size of the ions analysed (Na, H, Alk, $SO_4$, Cl) and with the aid of other stations in the region. The yearly precipitation is around 700 mm. The values of dry deposition and leaching are based on the assumption that all chloride derives from the sea and no leaching of it occurs. The content of calcium Ca, in these lakes is about half of the Ca+Mg content (around 0.15 mekv/l.).*

| | pH | Na | K | Ca | Mg | Al | Mn | H | Alk | $SO_4$ | Cl | $NH_4$ | $NO_3$ | $\Sigma+$ | $\Sigma-$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | mekv/l. | | | | | | | |
| precipitation March 1973 to June 1974 | 4.2 | 0.072 | ~0.003 | ~0.02 | ~0.02 | — | — | 0.063 | 0 | 0.093 | 0.086 | ~0.05 | ~0.05 | 0.228 | 0.229 |
| precipitation concentrated 1.7 times | 4.0 | 0.122 | ~0.005 | ~0.034 | ~0.034 | — | — | 0.107 | 0 | 0.158 | 0.146 | ~0.085 | ~0.085 | 0.387 | 0.389 |
| dry deposition from sea salts | — | 0.178 | ~0.004 | ~0.008 | ~0.041 | 0 | 0 | 0 | 0.001 | 0.021 | 0.208 | 0 | 0 | 0.231 | 0.230 |
| other airborne deposition and/or from leaching | — | 0.054 | ~0.009 | ~0.171 | | 0.013 | 0.004 | — | — | 0.157 | 0 | — | — | 0.251 | 0.157 |
| fixation from precip. | | | | | | | | 0.094 | 0.001 | — | 0 | ~0.085 | ~0.080 | ~0.179 | ~0.081 |
| contents in three lakes 1974 | 4.9 | 0.354 | 0.018 | 0.288 | | 0.013 | 0.004 | 0.013 | — | 0.336 | 0.354 | 0 | 0.005 | 0.690 | 0.695 |

Table 5. *Rishagerödvattnet near Stenungsund and Gothenburg in the West Coast area (58° 07′ 12° 00′). Mean values from 1947—52* (Lysén 1960) *and 1972—74.*

| | Ca+Mg | Alk | $SO_4$ | Cl | pH | µS/ 20° C | Colour mgPt/l. |
|---|---|---|---|---|---|---|---|
| | | mekv/l. | | | | | |
| 1947—52 | 0.17 | 0.03 | about 0.1 | 0.41 | 5.8 | 55 | 45 |
| 1972—74 | 0.33 | −0.01 | 0.33 | 0.40 | 4.9 | 89 | 15 |

ammonium and nitrate are fixed in the soil. The natural additions to these lakes are very small.

Since 1947—52 the conductivity in one of these lakes has risen from 55 to 89 µS, with a corresponding ionic increase of 0.16 mekv of calcium-magnesium per litre, and probably some 0.2—0.25 mekv of sulphate per litre (Table 5).

Lakes with less lime and a lower capability of cation leaching will, of course, be more acidified and consequently have pH 4—4.5.

The aluminium content in the lakes (pH 4.9) is around 0.17 mg/litre, corresponding to about 0.01 mekv as a bivalent ion ($Al(OH)^{2+}$) or 0.02 mekv as a trivalent ion ($Al^{3+}$). The manganese content is around 0.1 mg/litre (0.004 mekv $Mn^{2+}$/litre).

## Central Sweden

Lake Upprämen (60° 22′ 13° 53′) is situated 447 metres above sea level (and above the highest shoreline of the sea) and has a size of 3.8 km², corresponding to about 31 per cent of its drainage area. The yearly precipitation is about 800 mm and the run-off is of the order of 500 mm. The salts from precipitation will then be concentrated 1.5—1.7 times (Table 6).

When these concentrated values of the precipitation are compared with those of the lake (Table 6), one finds that if all chloride in the lake (0.03 mekv/litre) is derived from the atmosphere, which is plausible (Eriksson 1955, 1960), the dry deposition (0.01 mekv/litre), will be much less than in the West Coast area and will represent about 0.5—0.6 of the wet deposition.

Potassium is a nutrient retained by the vegetation and the lake has even less than what comes from precipitation. Still more evident is the retention of nitrogen. Only half of what is derived from the precipitation, 0.78 mg/litre, is to be found in the lake (0.4 mg Total N per litre).

Of the sulphate in the lake, 0.14 mekv/litre, 0.12 mekv is derived from precipitation and the small remainder, 0.01—0.02 mekv, may have come from dry deposition or from leaching. The lake content in a "natural" stage probably did not exceed 0.04 mekv/litre, and the increase since then should be at least three fold. Since 1955—59 there may have been a doubling (Table 2).

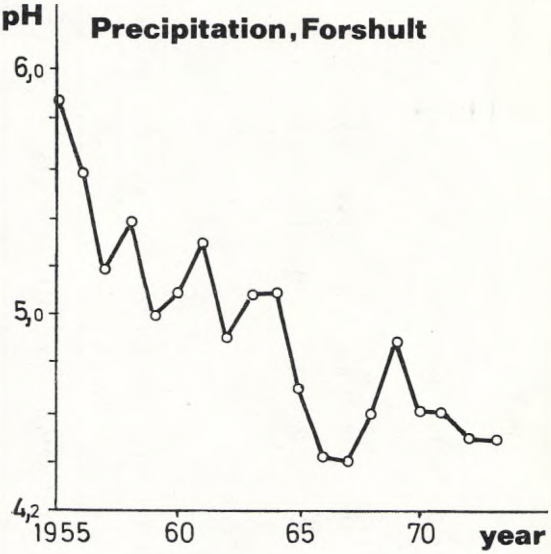
The content of calcium-magnesium in the lake is only 0.09 mekv/litre and at least two thirds of it is derived from the atmosphere.

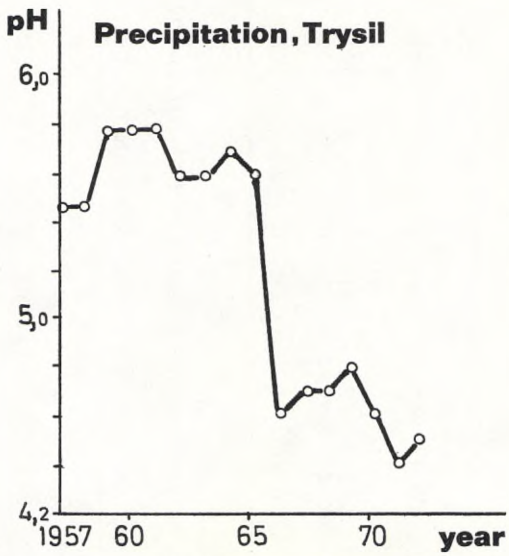
One third of the water supply to the lake is deposited on the water surface as unneutralized precipitation with a pH of 4.5, and the leaching to the lake of bicarbonate from the ground is far from sufficient to compensate for all the acid contributions. The pH of the lake is now 4.7, *i.e.* too acid for the stock of Arctic char, which has died out.

Lake Fjällrämen 20 km downstream has a pH of 5.4 (a decrease of 0.02 mekv/litre hydrogen ions) and 0.02 mekv of calcium-magnesium more per litre. The content of sodium and potassium is also higher.

In the clear-water lake, Upprämen, some 0.18 mg of aluminium is found, corresponding to 0.02 —0.01 mekv of $Al^{3+}$ and $Al(OH)^{2+}$, whereas Lake Fjällrämen, with a pH of 5.4 and rather brownish water, has 0.24 mg of aluminium, probably mostly bound to humic substances.

The precipitation values come from a station 50 km south of Lake Upprämen (Forshult, Hagfors), and Fig. 9 shows the trend of pH since




Figs. 9 above and 10 below. pH in precipitation at Forshult, Värmland (60° 05′ 13° 47′) and Trysil, Norway (61° 20′ 12° 15′). (Data from the International Meteorological Institute, University of Stockholm 1974.)

1955. The pH has dropped from 5.9 to 4.5 (International Meteorological Institute, University of Stockholm, 1974).

Mountain region

The lakes considered are the Särnamannasjöarna (61° 36′ 12° 45′) in the most southerly mountain area of Sweden. During the winter of 1972 the snow profile had a pH of 4.2 and contained twice as much sulphate and more than twice as much nitrate, ammonium and phosphorus as the lakes, where the pH was 4.9—5.0 (Table 7). The contents in the lakes rose, however, when the snow melted, and the pH dropped to 4.5. In July the pH had risen to 4.9 again.

In April 1964 a pH value of 5.4 was measured (Puke 1971, Andersson *et al.* 1971) and in April 1973 a value of 4.5 was recorded. The trend in precipitation has been the same (Fig. 10, Station Trysil, Norway).

At springtime, when the snow accumulated during the winter melts within a short time, there usually occurs a sharp drop of pH in low-buffered waters and this may cause kills of the fish fry, while pH during the rest of the year may be tolerable.

These three examples show the extreme dependence of the composition of lake waters on the precipitation. The anions, with the exception of bicarbonate (if there is any), may all derive from the atmosphere, and the same is true for nitrogen and phosphorus. An increased leaching of the cations occurs, however, probably to compensate for the increased acid deposition. But the leaching of cations and bicarbonate is often not sufficient, and this results in an acidification of the water.

*Heavy metals*

Parallel with this large-scale acidification, there is an increased deposition of heavy metals. The content in precipitation is several times higher than that found in lake water.

Table 8 shows the composition in precipitation during the winter 1972—73 at Drottningholm, 10 km from the centre of Stockholm. The content of cadmium (7 μg/l) was at least ten times greater than that found in lakes and the content of lead (64 μg/l) was from ten to fifty times greater. Though there is probably an influence from Stockholm, the values should nevertheless not be quite unrepresentative for certain areas of Southern Sweden. Snow investigations in Norway show

Table 6. *The composition of Lakes Upprämen and Fjällrämen in Dalecarlia and Värmland (60° 20′ 13° 50′) and of the precipitation at Forshult (60° 05′ 13° 47′). The values of dry deposition and leaching are based on the assumption that all chloride in the lakes is derived from the sea and no leaching of it occurs. Precipitation data from the International Meteorological Institute, University of Stockholm, 1974.*

| | pH | Na | K | Ca | Mg | Al | H | Alk | $SO_4$ | Cl | $NH_4$ | $NO_3$ | Σ+ | Σ− |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mekv/l | | | | | | | | | | | | |
| precipitation 1970—73 | 4.5 | 0.017 | 0.007 | 0.027 | 0.010 | — | 0.035 | — | 0.076 | 0.012 | 0.015 | 0.020 | 0.108 | 0.108 |
| precip. concentrated 1,6 times | 4.3 | 0.027 | 0.011 | 0.043 | 0.016 | — | 0.051 | — | 0.122 | 0.019 | 0.024 | 0.032 | 0.172 | 0.173 |
| dry deposition from sea salts | — | 0.009 | 0 | 0 | 0.002 | 0 | — | 0 | 0.001 | 0.011 | 0 | 0 | 0.011 | 0.012 |
| other airborne deposition and/ or from leaching | — | 0.004 | — | 0.029 | | 0.02 | — | — | 0.017 | 0 | — | — | 0.053 | 0.017 |
| fixation from precip. | — | — | — | — | | — | 0.031 | — | — | 0 | 0.023 | 0.018 | 0.054 | 0.018 |
| Lake Upprämen 1973—74 | 4.7 | 0.04 | <0.01 | 0.09 | | 0.02 | 0.02 | 0 | 0.14 | 0.03 | 0.001 | 0.014 | 0.18 | 0.18 |
| Fjällrämen 1973—74 | 5.4 | 0.06 | 0.01 | 0.11 | | — | — | 0.005 | 0.14 | 0.03 | 0.002 | 0.004 | 0.18 | 0.18 |

Table 7. *Contents of salts and nutrients in the mountain lake, Övre Särnamannasjön, (61° 36′ 12° 45′), during 1972 and in the snow in March 1972. The snow was very acid (pH 4.2) and had twice as much sulphate, and about three times as much nitrogen and phosphorus as the lake. After the spring flow, the values rose in the lake, and the pH fell to 4.5. In July the pH had risen to 4.9.*

| | pH | Na | K | Ca+Mg | H | Alk | $SO_4$ | Cl | $NH_4$–N | $NO_3$–N | Tot–N | $PO_4$–P | Tot–P | µS | FTU | $KMnO_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mekv/l. | | | | | | | µg/l. | | | | | 20° C | | mg/l |
| snow profile | | | | | | | | | | | | | | | | |
| 1972-03-21 | 4.2 | <0.01 | <0.01 | <0.02 | 0.063 | 0 | 0.18 | <0.01 | 217 | 610 | 1010 | 18 | 21 | 20 | 2.6 | 3 |
| Övre Särmannasjön | | | | | | | | | | | | | | | | |
| 1972-03-21 | 5.0 | <0.01 | <0.01 | <0.02 | 0.010 | 0 | 0.075 | <0.01 | 74 | 47 | 283 | 6.7 | 6.7 | 11.0 | 0.34 | 0 |
| 05-13 | 4.45 | <0.01 | <0.01 | <0.02 | 0.035 | 0 | 0.075 | <0.01 | 69 | 94 | 268 | 6.8 | 8.5 | 13.9 | 0.33 | 3.7 |
| 06-14 | 4.5 | <0.01 | <0.01 | 0.02 | 0.032 | 0 | 0.105 | 0.015 | 17 | 65 | 600 | 14 | 17 | 12 | — | 2.0 |
| 07-05 | 4.9 | <0.01 | <0.01 | 0.02 | 0.013 | 0 | 0.06 | 0.01 | 15 | 10 | 285 | 10 | 13.5 | 10 | 0.34 | 3.5 |

Table 8. *Composition of precipitation at Drottningholm November 29, 1972—February 28, 1973.*

| mm | pH | Na | K | Ca+Mg | Alk | $SO_4$ | Cl | µS | FTU | $KMnO_4$ | $NO_3$–N | Tot–N | $PO_4$–P | Tot–P | Fe | Mn | Cu | Zn | Ni | Co | Cr | Pb | Cd |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mekv/l. | | | | | | 20° C | | mg/l. | µg/l. | | | | | | | | | | | | |
| 84 | 4.1 | 0.03 | 0.01 | 0.03 | 0 | 0.19 | 0.04 | 51 | 4.7 | 17 | 873 | 2120 | 21 | 27 | 216 | 5 | 9 | 311 | 48 | <15 | <5 | 64 | 7 |

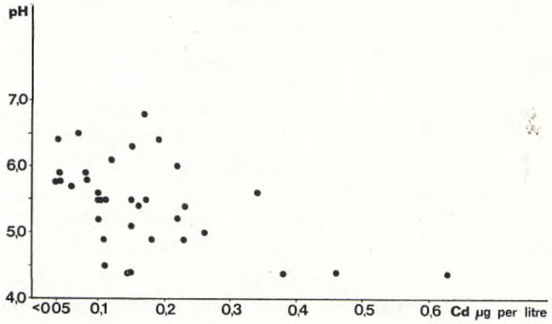



Fig. 11. Cadmium in lakes with differing pH. Acid lakes on the West Coast have high values.

similar results (Henriksen 1972, Elgmork *et al.* 1973).

Though the soil has an extraordinary ability to retain this content of heavy metals it is probable that when the snow melts some of the content is washed out to the lakes and in part sediments to the bottoms. The content of lead in otherwise unpolluted lakes in the West Coast region is about 150 mg/kg in the upper part of the sediment or at least 4 times as great as the "normal" value. Their waters have around 1—5 µg/litre. Lakes further north have less than this.

Zinc and cadmium show the same tendency. West Coast lakes have zinc values of 0.03—0.12 mg/litre and lakes further north have 0.01—0.03 mg/litre; the cadmium content is 0.08—0.63 µg/litre on the West Coast whereas further north it is < 0.05—0.23 µg/litre (only 35 lakes were investigated, in November 1973, up to 60° 20′ in Western Sweden).

There also seems to be a slight tendency towards a pH dependence (Fig. 11), as acid lakes are the most affected by the polluted precipitation; and owing to the acidity, more metals might be kept in solution.

Together with the acidity in soft waters, this mixed heavy-metal pollution will probably increase the poisonous effects on aquatic life.

## VI. CONCLUSION

The acidification affects thousands of Swedish low-buffered lakes. Many of these lakes are situated in virgin forests, in national parks and in mountain areas. According to the Government they were "not allowed to be polluted by any kind of matter". Some of them can be limed, but it is certain that the country as a whole is suffering through the loss of unpolluted water and much of the habitat for aquatic life.

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# Possible Odour Responses of Juvenile Arctic Char (*Salvelinus alpinus* (L.)) to Three Other Species of Subarctic Fish.

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## I. INTRODUCTION

This study deals with avoidance and attraction between fish species on account of hitherto undetermined chemical agents — presumably odours. The method employed is a modification of the fluviarium technique described earlier in detail by HÖGLUND (1961).

Juvenile specimens of arctic char (*Salvelinus alpinus* (L.)) have been tested upon presumed odour agents deriving, in the majority of the present experiments, from intact individuals of three other species of subarctic fish, but in a few experiments, also from injured and even dead specimens (experiments 7, 10 and 11 in Table 1). Two of the test objects, *viz.* whitefish (*Coregonus lavaretus* (L.)) and brown trout (*Salmo trutta* L.) are frequently found in competition with char. Vendace or cisco (*Coregonus albula* (L.)) on the other hand, are more seldom found in the same water, and when this species occurs it tends to form the prey of large-sized Arctic char.

For several years the research programme at the Freshwater Institute, Drottningholm, has included problems involving competition and other interactions between fish species. Traditional techniques for fishery biological investigations in the field have mainly been used in order to study competition for food, growth, fecundity, yield, and more recently *interactive segregation* as well (*i.a.* NILSSON 1965, 1967; NILSSON and PEJLER 1973; MOYLE, 1973). NILSSON and PEJLER (*op.cit.*) recently presented evidence for an interactive segregation between subarctic fish due to the exploitation of zooplankton.

It is obvious that behavioural phenomena have been important and sometimes decisive in the process of displacement or elimination of one species of fish upon the introduction or invasion of a new species. The possibilities of surveying physiological interrelations in interactive segregation have hitherto been limited, and this subject has been somewhat neglected. According to a review by NILSSON (1967), IVLEV (1961) and BREDER (1929) are among the few authors who have presented evidence clearly indicating that certain fish species avoid one another for reasons other than aggressiveness. Among such possible reasons NILSSON (*op. cit.*) mentions unfamiliar sizes, colours, movements, sounds and odours, all of which impressions, perceived by the interacting fish species, have a sound physiological basis.

WREDE (1932) and WHITE (1934 a, b) seem to be two of the first investigators to have pointed out chemical communication between fishes. Wrede (*op. cit.*) demonstrated intraspecific attraction and even individual recognition in the European minnow (*Phoxinus laevis* L.). Field observations in connection with the transplantation and reintroduction of young Atlantic salmon (*Salmo salar* L.) into certain streams in northeastern Canada led WHITE (1934 a, b) to the conclusion that chemoreception and recognition of water conditioned by populations of parr stages of this species constitute a guiding factor for ascending adults in selecting their streams for spawning.

In training experiments GÖZ (1941) showed that *Phoxinus laevis* were able to discriminate between odours from 15 species of fish and two species of frogs. The minnows also recognized individuals of their own species. Young jewelfish (*Hemichromis bimaculatus*) orientate to water from young of their own species and avoid chemical stimuli from other species (KÜHME 1963, 1964). The role of vision and chemoreception in parental preference in cichlids is also dealt with by MYRBERG (1966). Recently, it has been shown by REED (1969) that fright reactions are elicited among certain American prey fish in response to water conditioned by the presence of local (sympatric) predatory fish with undamaged skins.

OSHIMA *et al.* (1969) reported that chinook salmon (*Oncorhynchus tshawytscha*) showed olfactory bulbar responses to water conditioned by this species. Electroencephalographic evidence for imprinting and retention of olfactory cues in homing coho salmon (*Oncorhynchus kisutch*) has recently been presented by COOPER and HASLER (1974). For literature on the rather extensive sources of information regarding chemoreception and orientation of fish the reader is further referred to the reviews by HERTER (1953), UMEZU (1966), HARDEN-JONES (1968), KLEEREKOPER (1969, 1971), and HARA (1970, 1971). (See also the discussions below at p. 28—33.)

Thus there is increasing support for the view that chemical identification, largely due to olfaction, plays an important role in the behaviour and ecology of fishes. Local orientation of anadromous fishes is presumably influenced by pheromones (SOLOMON, 1973). NORDENG (1971) suggested that a migratory population of Arctic char (*Salvelinus alpinus*) may be attracted in their natural habitats by water conditioned by relatives, possibly even race-specifically. Later HÖGLUND and ÅSTRAND (1973) presented strong experimental evidence of intraspecific attraction among juvenile individuals of Arctic char. These preferential reactions were shown to be due to olfactory responses. To allow a better evaluation of the results arrived at in the present study regarding *interspecific preferential reactions* (avoidance or attraction) among juvenile Arctic char, these reactions have been compared with the *intraspecific attraction* demonstrated a year earlier by HÖGLUND and ÅSTRAND (1973) on the same population of char and with the same technique.

In Scandinavia the best-documented example of displacement of species is the introduction of whitefish (*Coregonus sp.*) into many trout-char lakes of northern Sweden. EKMAN (1910) listed nine northern Swedish lakes where whitefish had been introduced between the 1820s and the 1870s. In all these cases, as well as in those of more recent introductions, char has either been completely eliminated or has survived in small populations.

Thus in the present study an experimental approach has been used in an attempt to determine quantitatively whether *odour aversion (cf.* p. 33) exists. Two-summer-old individuals of Arctic char were given an opportunity to choose between a water current conditioned by the presence of another species and a current without odour, and their reactions were quantified. In Tables 2—5 the bulk of the primary observations from this study and from the earlier study by HÖGLUND and ÅSTRAND (*op. cit.*) have been treated statistically according to the nonparametric method of Mann and Whitney (SIEGEL, 1956). The material has also been treated by the method used by HÖGLUND (1961) and HÖGLUND and ÅSTRAND (1973), resulting in the diagram in Fig. 5. Finally, the significance of the results is discussed in relation to problems concerning interactive segregation among the fish species dealt with in this investigation.

## II. MATERIAL AND METHODS

### *Fish material*

All experiments were carried out at the Institute of Zoophysiology in Uppsala. Sixty-one juvenile char (*Salvelinus alpinus* (L.)), hatched in April 1972, were tested in 16 experiments as shown in Table 1, and 5 more were studied in a test for hand-dipping (p. 27). These fish originated from a population in Lake Hornavan, Swedish Lapland. They were hatched and reared by the breeding plant of the Fishery Board at Älvkarleby and were moved to our institute in November 1972. The tested specimens thus belonged to the same population as were used in the earlier study by HÖGLUND and ÅSTRAND (1973). They were kept indoors in the laboratory in a storage tank supplied with aerated Uppsala tap water in a continuous-round-flow. The fish were fed daily with EWOS pellets (standard fodder, size 3). Small specimens were selected for the tests, except for the experiment of November 8 (Table 1). The mean body weight and length ($\pm$ SD) were $18.5 \pm 7.5$ (range 9.4—43.7) g and $13.0 \pm 1.5$

Table 1. *Survey of experiments performed. See also Figs. 1 and 5.*

| Experiment no. | Date 1973 | Odour donor fish: No. of fish | Odour donor fish: Species and sex | Odour donor fish: Mean length cm | Odour donor fish: Mean weight g | Test fish: No. of fish | Test fish: Species | Test fish: Mean length cm | Test fish: Mean weight g | Experimental design: Spatial arrangement of current | Experimental design: Flow rate cm/sec | Experimental design: Water temp. °C | Experimental design: Light [1] in test yard | Results in: Fig. | Results in: Table |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Nov. 1 | 1 | Whitefish | 22.0 | — | 3 | Char | 12.9 | 17.7 | Two currents | 2.1 | 9.5 | Bright | | |
| 2 | 2 | 2 | Whitefish | 23.6 | — | 3 | Char | 12.6 | 16.4 | Two currents | 1.1 | 10.0 | Bright | | |
| 3 | 6 | 4 | Whitefish | 23.9 | — | 5 | Char | 12.2 | 14.2 | Even flow | 1.1 | 10.0 | Feeble | 2 | |
| 4 | 7 | 4 | Whitefish | 24.2 | — | 5 | Char | 12.9 | 17.3 | Even flow | 1.1 | 10.5 | Bright | and | 2 |
| 5 | 8 | 4 | Whitefish | 23.6 | — | 5 | Char | 17.6 | 40.1 | Even flow | 1.1 | 10.0 | Bright | 5 | |
| 6 | 9 | 4 | Whitefish | 23.9 | — | 5 | Char | 14.0 | 21.5 | Even flow | 0.6 | 10.0 | Bright | | |
| 7 | 13 | 4 | Dead whitefish [2] | — | — | 5 | Char | 13.7 | 20.7 | Even flow | 1.1 | 9.5 | Feeble | | |
| 8 | Nov. 19 | 4 | Cisco | 23.4 | — | 5 | Char | 14.1 | 22.0 | Even flow | 1.1 | 9.5 | Feeble | | |
| 9 | 20 | 4 | Cisco | 24.1 | — | 5 | Char | 12.2 | 13.6 | Even flow | 1.1 | — | Feeble | 3 | |
| 10 | 21 | 4 | Cisco [2] | 23.8 | — | 3 | Char | 13.5 | 19.5 | Two currents | 1.6 | 9.0 | Feeble | and | 3 |
| 11 | 22 | 4 | Cisco [2] | 23.1 | — | 3 | Char | 13.1 | 18.7 | Two currents | 1.1 | — | Feeble | 5 | |
| 12 | Nov. 28 | 5 | Brown trout | 13.4 | 11.2 | 3 | Char | 12.0 | 13.4 | Two currents | 1.6 | 8.5 | Feeble | | |
| 13 | Dec. 3 | 8 | Brown trout | 10.8 | 11.7 | 3 | Char | 13.4 | 19.0 | Two currents | 1.6 | 8.0 | Feeble | 4 | |
| 14 | 4 | 8 | Brown trout | 10.8 | 11.7 | 4 | Char | 10.9 | 10.7 | Two currents | 1.6 | 7.5 | Feeble | and | 4 |
| 15 | 5 | 8 | Brown trout | 10.8 | 11.7 | 4 | Char | 10.9 | 10.7 | Two currents | 1.6 | — | Bright | 5 | |
| 16 | 6 | 8 | Brown trout | 10.8 | 11.7 | 4 | Char | 12.2 | 13.8 | Two currents | 1.6 | 7.5 | Bright | | |

[1] "Bright" means 610 lux and "Feeble" 4.5 lux adjacent to the fish (*cf.* the text on p. 25).
[2] With the exception of these experiments, living intact specimens were used as odour donor fish; in experiment 7, dead fish were used and, in experiments 10 and 11, injured specimens.

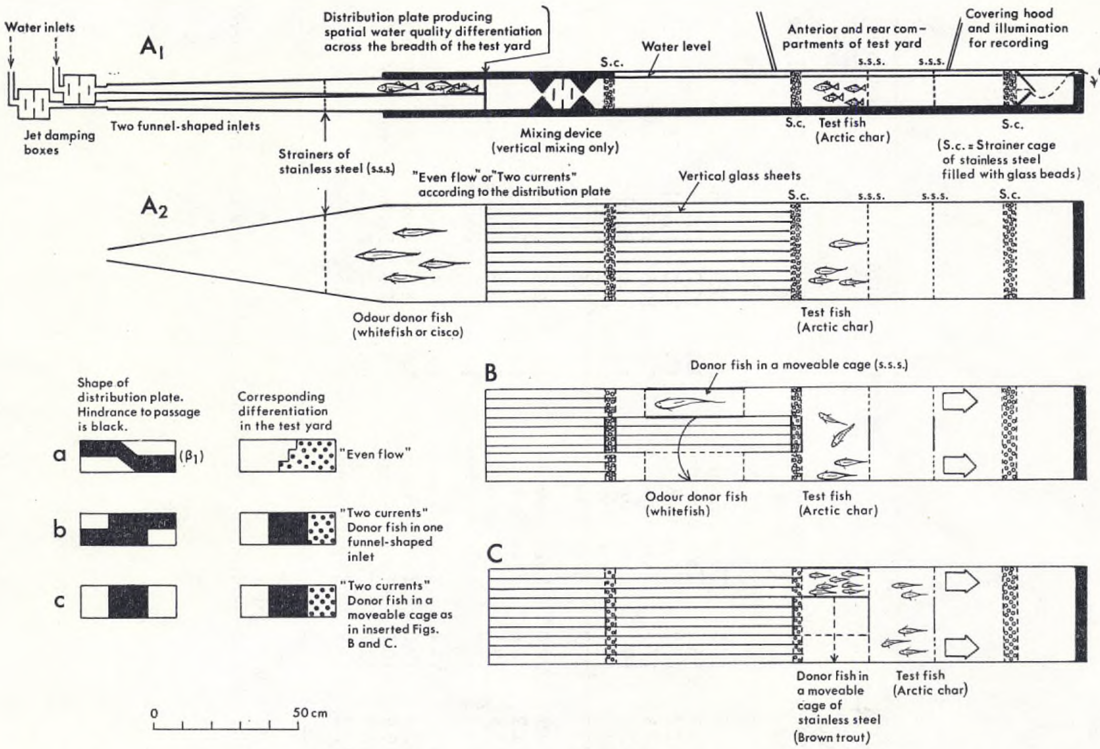



Fig. 1. Sketch of the test apparatus and the arrangements for the main types of experiments. $A_1$ and $A_2$ show lateral and horizontal views of the fluviarium, indicating the positions of donor and test fishes. In "even flow" experiments (see Table 1) the distribution plate ($\beta_1$ according to HÖGLUND, 1961) was used. In "two current" tests (see Table 1) the plates b or c were used, depending on whether the donor fish were kept in front of or behind the distribution plate. Unless otherwise stated, the apparatus is built of polyacrylic plastic material. For further details see HÖGLUND (1961, Figs. 1 and 2) and HÖGLUND and ÅSTRAND (1973, Figs. 1 and 2). The experiments were performed in a dark room; the only illumination source was two symmetrically placed tubular electric lamps giving an indirect light from the sheltering hood above the test yard, which is not fully drawn in the figure. The dots in the transverse sections indicate assumed odour from the donor fish.

In B and C the donor fish are kept in one current on the K-side corresponding to sections 8—10. During the next test period the donor fish are kept on the other side corresponding to sections 1—3, here called the A-side (below in the diagrams of Figs. 2—4), alternating this way from one 90 min period to the other.

(range 10.6—17.8) cm. As will be seen in Table 1, the same specimens were used in the experiment of December 4 and 5. At the time of writing (March 1974) this population of char has been kept isolated from other species in our storage tanks in good health for more than 15 months.

The whitefish were caught during their spawning run from Lake Siljan to the River Öster-Dalälven in the province of Dalarna, central Sweden. They belonged to the species *Coregonus lavaretus* (SVÄRDSON, 1957, 1961, and Professor G. SVÄRDSON, personal communication). Their total length ranged from 22.0 to 27.0 cm. The cisco belonged to the population of Lake Mälaren (*cf.*, *e.g.*, NORTHCOTE and RUNDBERG, 1970) and ranged in length from 21.5 to 27.0 cm. The brown trout were obtained from the same hatchery as the arctic char; their total length ranged from 6.7 to 20.0 cm.

Before being used in an experiment, the fish were kept in the test yard of the fluviarium at a low flow rate for at least 12 hours.

*Water quality*

The water supplying the storage aquarium and the test apparatus was aerated Uppsala tap water. The chemical composition of this originally subsoil water from an esker nearby has been reported by HÖGLUND and ÅSTRAND (1973, Table 1). It is rich in $HCO_3^-$ (300—320 mg/l), $Ca^{2+}$ and $Mg^{2+}$ (95—119 mg/l in the proportion of about 15 % Mg and 85 % Ca). The oxygen content was kept at a level just below saturation, and the pH varied between 7.4 and 7.8. (See also HÖGLUND (1961) and HÖGLUND and BÖRJESON (1971).) Oher experimental conditions are given in Table 1.

*Experimental designs*

Essentially, the experimental procedures developed by HÖGLUND (1961) were followed supplemented with some modifications introduced by HÖGLUND and ÅSTRAND (1973). In the tests performed with cisco and whitefish, however, the earlier arrangement for keeping the "donor fish" in the fluviarium could not be used owing to the comparatively large size of the fish. Instead these fish were kept in one of the inlet funnels (Fig. 1). Other arrangements for keeping the donor and test fishes, as well as for the distribution of current and presumed fish odour from the donors, are also shown in Fig. 1 and in Table 1.

The juvenile Arctic char were allowed to swim about in the confined space of the test yard. There they were given a choice between pure water and water conditioned by the presence of another species. The experimental events were arranged symmetrically and all disturbances of the test fish were avoided. The test yard was evenly illuminated by two symmetrically placed tubular bulblamps (40 W each) screened by milkglass sheaths. The light intensity adjacent to the test fish was 610 lux ("bright" in Table 1), and 4.5 lux ("feeble" in Table 1). In the latter case a BRAUN electronic flash aggregate was used for the recordings.

As shown in the graphs of Figs. 2—4, the experiments lasted for eight hours. The distribution of the two water qualities was reversed at two-hour intervals. In this way the distribution of pure and conditioned water was changed from one side to the other. The momentary positions of the test fish were recorded every 30 sec by means of a 16 mm film camera. The recording started 30 min after the reversal of the experimental conditions. The side walls of the fluviarium are called A and K, as shown in Fig. 1. Accordingly consecutive test periods lasting for 90 min each are called Aa, Ka, Ab and Kb; the capital letters designate conditioned water on the A or K side, respectively, and the lower-case letters the ordinal number. The first 30 minutes, when no records were obtained, have been designated Ca, Cb, etc., in the graphs. During that period the new reversed distribution of pure and conditioned water was established within 10 minutes. During about the next 20 minutes the test fish became accustomed to the new spatial arrangement of presumed fish odour in the test yard.

Two spatial arrangements regarding the distribution of current were used. In Table 1 these are designated "Two currents" and "Even flow". (1) "Two currents" means that two equal currents were established along each side with a stillwater belt between, the latter covering the imaginary sections 4—7 of the test yard. (2) "Even flow" means that as far as possible an even flow over the whole breadth of the open trough was maintained. In (1) only one current contained the presumed active stimulants deriving from the donors placed in a cage upstream in front of the test yard. By moving the cage from one current to the other, alternative test conditions — A or K — were established (*cf.* Fig. 1). Using an even flow (2), the corresponding alternation from A to K was accomplished by turning the distribution plate, called a in Fig. 1, upside down.

*Definition of reaction values and statistical treatment*

The film recording was analysed in order to find out wether the char preferred to stay more often in pure water or in conditioned water. The number of momentary visits in each section as

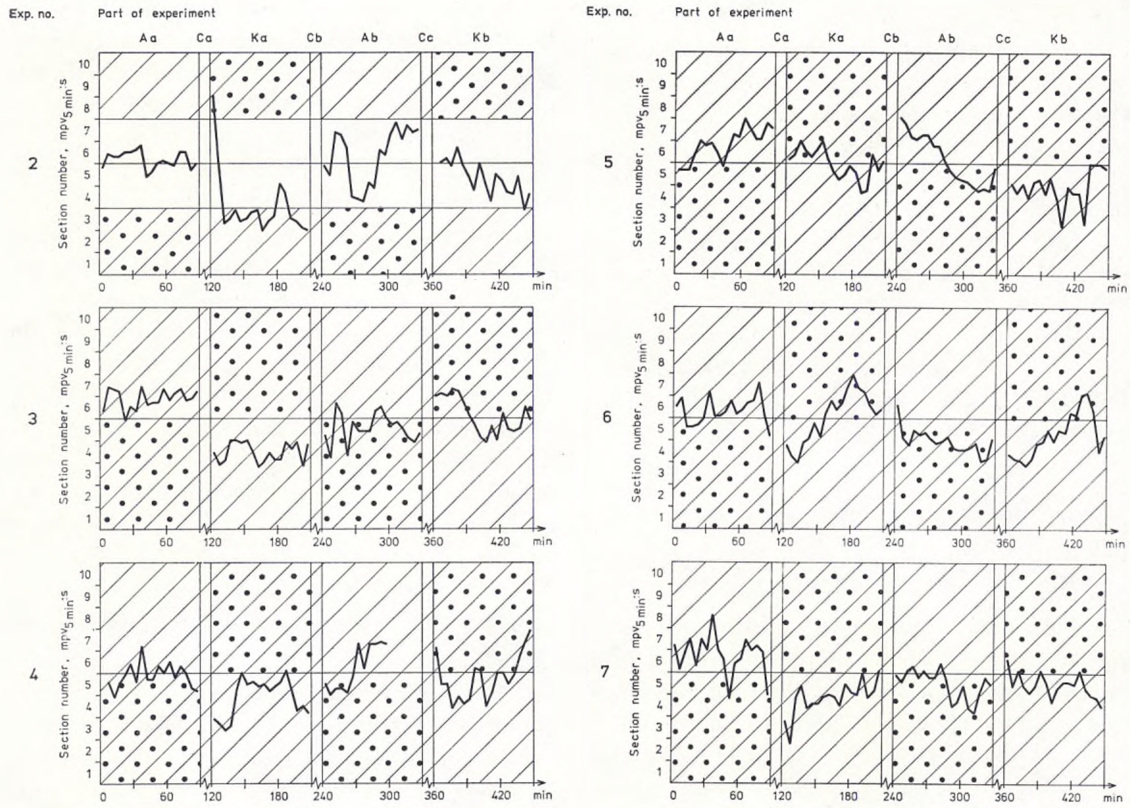



Fig. 2. Preferences among juvenile Arctic char (*Salvelinus alpinus* L.) for pure water and water conditioned by the presence of whitefish (*Coregonus lavaretus*). Cross-hatched areas in the graph of experiment 2 symbolize the distribution of flowing water in the test yard. In experiments 3—7 an even flow was used. Dots indicate assumed whitefish odour. For further details regarding the designs of experiments, see Table 1, Fig. 1 and p. 25.

recorded on each frame of the film was multiplied by the corresponding section number (1—10). The total sum of these products from ten frames was divided by the total number of observations. This represented a mean position value for five minutes ($mpv_{5\ min}$ according to HÖGLUND, 1961, pp. 46—48). Thus in the present tests, owing to the number of fish tested, each $mpv_{5\ min}$ represented 30—50 momentary positions. The $mpv_{5\ min}$ values are plotted in Figs. 2—4, which show the mean preferences among the fish during the course of different parts of the experiments. By definition, a mean position value of 5.5 thus indicates a reaction of indifference. The difference between this value and the actual mean position value for a certain period of time gives a reaction value, rv, with an index indicating the length of a period with identical experimental conditions. Positive values indicate *attraction* and negative values *avoidance*, in accordance with the definitions suggested by HÖGLUND (1961, pp. 48—49).

The change of reaction within an experiment was evaluated by means of the two-tailed MANN-WHITNEY U-test (SIEGEL, 1956). Thus, in this context a reaction value was defined as the difference between the medians of the generally occurring 18 $mpv_{5\ min}$ values from consecutive test periods. The medians (Mdn) and the semi-interquartile range (Q) recorded are given in Tables 2—5 with the pertinent statistics from the U-test.

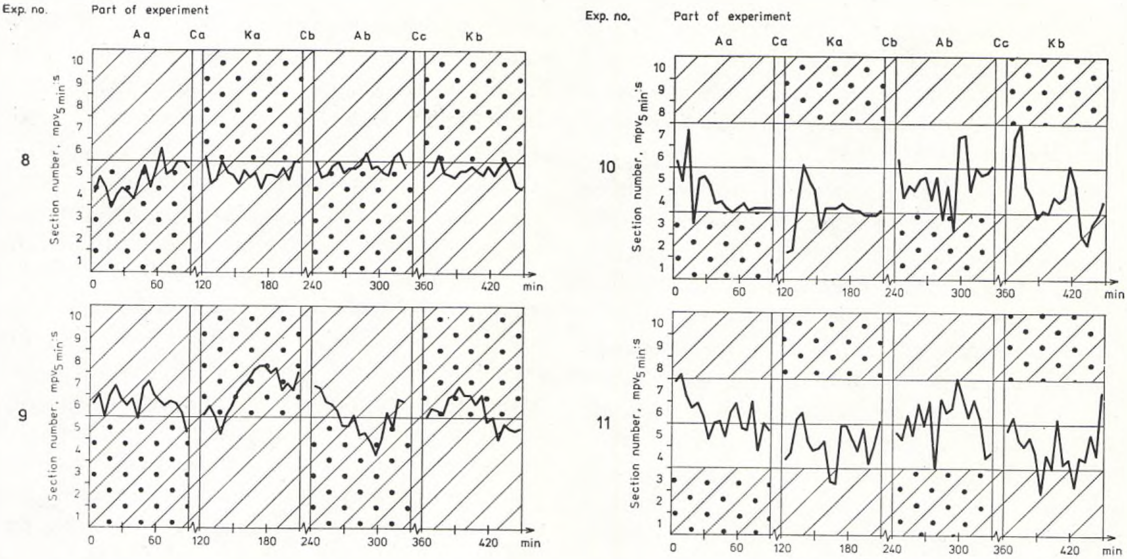


Fig. 3. Preferences among juvenile Arctic char (*Salvelinus alpinus* (L.)) for pure water and water conditioned by the presence of cisco (*Coregonus albula* L.). For details see Table 1 and p. 25.

### *Measures to eliminate possible irrelevant factors*

All five species of Pacific salmon belonging to the genus *Oncorhynchus* exhibit alarm reactions and detect repellent odours from human hand rinses and mammalian skins (BRETT and MACKINNON, 1954; ALDERDICE *et al.*, 1954; IDLER *et al.*, 1961, and others). Electroencephalic responses from the olfactory bulb of the rainbow trout (*Salmo gairdneri*) to handdipped water and $10^{-6}$ M L-serine have also been recorded by HARA (1971). Thus a control test was performed to find out whether the juvenile char in our experiments responded if the experimenter dipped his hands in the fluviarium water for some time before a test. In this control test the resulting preference reaction was quite indifferent. In the tests presented in this paper, however, the fish were transferred from the storage aquarium into the experimental tank with the aid of a net, thus avoiding all interference from this type of irrelevant factors.

## III. RESULTS

### *Presentation*

All results are presented in Figs. 2—5 and Tables 2—4. For comparison, in Table 5 the primary observations presented earlier by HÖGLUND and ÅSTRAND (1973) have been subjected to the same statistical treatment as the observations in Tables 2—4.

In experiment 1 (see Table 1), the char exhibited a fairly stable reaction throughout the test period. The results from this test are presented only in Fig. 5. For all other experiments (2—16), the intensity of reaction during the course of the test can be followed in full graphic representations (see Figs. 2—4). In these diagrams the $mpv_{5\,min}$ values are plotted against time, and changes of the experimental conditions between different test periods are indicated by symbols, which are explained in the figure texts.

The medians of the 18 $mpv_{5\,min}$ values from each 90 min test period were calculated and are given in Tables 2—5, which also present statistics from the MANN-WHITNEY U-test. By comparing the reactions from consecutive test periods with reversed areal distributions of conditioned and pure water in the test yard, the significance of avoidance or attraction was estimated, and this is given in these Tables.

Finally, the total reaction of an experiment was calculated according to the original method introduced by HÖGLUND (1961). These reaction values ($rv_{6\ hours}$ values) were based on the four $rv_{90\ min}$ values included in each experiment. In Fig. 5 the mean, range and standard deviations are given. As a complement, the observation material presented by HÖGLUND and ÅSTRAND (1973) regarding intraspecific olfactorily-stimulated reactions among juvenile arctic char was treated in the same way and is also presented in Fig. 5. In this figure a standard deviation extending on both sides of the zero line has been considered to be indifference reaction. Thus it is concluded that avoidance was exhibited in experiments 2, 3, 4, 5, 7, 11, 13 and 19. With this definition, it also appears from Fig. 5 that clear attraction was shown in experiments 17 and 18.

The avoidance or attraction found with the non-parametric statistical treatment employed (see Tables 2—5) should be regarded as reaction patterns within experiments. These estimates are presented mainly as a complement to the overall reactions in Fig. 5.

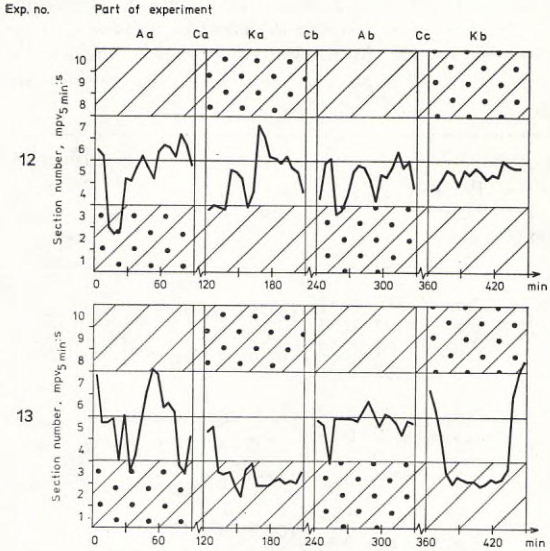
### *Comments on the experimental results*

#### Reactions of juvenile Arctic char to whitefish

The preferences among juvenile arctic char for pure water or for water conditioned by the presence of whitefish are presented in Figs. 2 and 5 and in Table 2. As shown in Fig. 5, experiment 1 gave an indifference reaction, which is probably explained by the facts that (1) only a single whitefish was used as donor and (2) the water velocity of the two currents in this experiment was comparatively high, *viz.* 2.1 cm/sec. The amount of presumed odour or other stimulant from this one individual passing the test fishes per unit of time would thus seem to have been too low to give a significant reaction with the present technique. However, indifference or no reaction at all was also recorded in experiment 6, where the flow rate was as low as 0.6 cm/sec over the whole breadth of the flume and a group of four "odour donor" whitefish were placed in one of the funnel-shaped inlets of the fluviarium. One possible explanation for this result is that the current was too low to activate the char to display searching and explorative behaviour. Consequently the probability of detecting the presumed whitefish odour in the spatially differentiated water mass might be diminished in this test. Another possible explanation for the absence of preference in this test is that the presumably existing biologically active agents may disintegrate fairly quickly, so that the ethological impact of these substances fades on the way from the donor fish to the test fish. In this case the time for reaching the test-yard area amounted to as much as about four minutes over a distance of about 1.4 m. It is obvious from Fig. 2 and the statistics in Table 2, as well as from Fig. 5, that in the remaining five experiments there were clear avoidance reactions. Thus, it appears from this study that under certain circumstances juvenile char actually detect and tend to avoid whitefish, probably through olfactory stimulation. The action of the biologically active compounds presumably responsible for these effects seems to be restricted as to time and space. With the exception of experiment 7, intact and actively swimming specimens of *Coregonus lavaretus* were used as "odour donors" in this group of tests. The avoidance reaction demonstrated in experiment 7 emanated from water conditioned by injured or dead whitefish and may be similar in nature to the fright or alarm reaction (Schreckreaktion) described by several authors (*cf., e.g.,* PFEIFFER 1967; HARA 1971, p. 107 f.), though according to HARA (1971, p. 108) the fright reaction may occur only in the *Ostariophysi.* In all other respects, the test conditions in experiments 2, 3, 4, 5 and 7 (see Table 1) were identical, except for the fact that two light intensities were used.

#### Reactions of juvenile Arctic char to cisco

When juvenile arctic char were tested against water conditioned by the presence of four intact



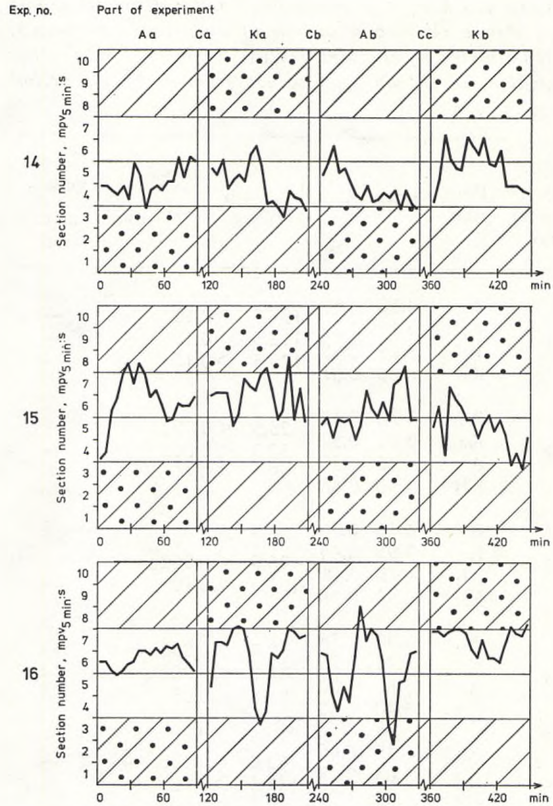




Fig. 4. Preferences among juvenile Arctic char (*Salvelinus alpinus* (L.)) for pure water and water conditioned by the presence of brown trout (*Salmo trutta* L.). Symbols as in Fig. 2. For further details see Table 1 and p. 25.

cisco, no reaction whatever — *i.e.* indifference — was obtained (see experiments 8 and 9 in Figs. 3 and 5, and Table 3). In experiment 10, slightly injured cisco were used instead to condition the water and, in experiment 11, specimens that were somewhat more injured but still alive were used. It appears from Fig. 5 and Table 3 that an avoidance reaction was particularly evident in the latter case (*cf.* the comments on experiment 7).

### Reactions of juvenile Arctic char to brown trout

In the experiments with brown trout as donor (nos. 12—16, Figs. 4, 5 and Table 4), on the whole no reactions were found except in experiment 13. According to Table 4, however, a slight attraction was shown in certain parts of experiments 14—16. The clear avoidance found in experiment 13 is difficult to explain, but may have been due to occasional aggression among the trout.

It has been shown that in situations of distress or unpleasant emotion increased amounts of catecholamines are excreted in the urine in man (LEVI, 1965). The release of agents in response to antagonistic or agonistic behaviour among the donor fish may be detected by the char and result in the avoidance reactions found in the present tests. But even if this does occur, it would not seem to be sufficient to explain the more or less consistent avoidance to whitefish demonstrated in experiments 2—5 and 7.

### Intraspecific reactions of intact and olfactorily-extirpated juvenile Arctic char

PFEIFFER (1974) very recently has reviewed the literature on pheromones and chemical communication especially concerning "alarm reactions" among fish.

Table 2. *Changes of reactions (avoidance or attraction) of Arctic char to whitefish in different experiments, as estimated by the* Mann-Whitney *U-test. Mdn = the median of 18* $mpr_{5\,min}$ *values from each test period (see p. 27).*

| Experiment no. | Period | Mdn | Q | U-value | P | Significance levels: avoidance | Significance levels: attraction |
|---|---|---|---|---|---|---|---|
| 1 | Aa | 4.90 | 0.55 | 157.5 | >0.10 | — | — |
| | Ka | 5.15 | 1.20 | 112.5 | >0.10 | — | — |
| | Ab | 5.65 | 0.90 | 129.5 | >0.10 | — | — |
| | Kb | 6.05 | 0.57 | | | | |
| 2 | Aa | 5.70 | 0.32 | 25.5 | <0.002 | +++ | — |
| | Ka | 3.02 | 0.52 | 32.5 | <0.002 | +++ | — |
| | Ab | 6.15 | 1.20 | 99.5 | <0.10 | — | — |
| | Kb | 4.90 | 0.66 | | | | |
| 3 | Aa | 6.35 | 0.36 | 0.0 | <0.002 | +++ | — |
| | Ka | 3.95 | 0.44 | 26.5 | <0.002 | +++ | — |
| | Ab | 4.95 | 0.48 | 102.5 | <0.10 | — | — |
| | Kb | 5.50 | 0.78 | | | | |
| 4 | Aa | 5.30 | 0.38 | 50.5 | <0.002 | +++ | — |
| | Ka | 4.72 | 0.75 | 45.0 | <0.002 | +++ | — |
| | Ab | 5.50 | 1.00 | 83.0 | <0.05 | + | — |
| | Kb | 5.20 | 0.80 | | | | |
| 5 | Aa | 6.45 | 0.65 | 62.0 | <0.002 | +++ | — |
| | Ka | 5.65 | 0.48 | 147.5 | >0.10 | — | — |
| | Ab | 5.45 | 1.08 | 63.5 | <0.002 | +++ | — |
| | Kb | 4.55 | 0.50 | | | | |
| 6 | Aa | 5.75 | 0.48 | 149.5 | >0.10 | — | — |
| | Ka | 5.80 | 1.00 | 71.0 | <0.02 | — | ++ |
| | Ab | 4.45 | 0.30 | 141.0 | >0.10 | — | — |
| | Kb | 4.60 | 0.98 | | | | |
| 7 | Aa | 6.45 | 0.50 | 19.5 | <0.002 | +++ | — |
| | Ka | 4.43 | 0.38 | 84.5 | <0.02 | ++ | — |
| | Ab | 5.25 | 0.55 | 137.0 | >0.10 | — | — |
| | Kb | 4.92 | 0.40 | | | | |

Table 3. *Changes of reaction (avoidance or attraction) of Arctic char to cisco in different experiments, as estimated by the* Mann-Whitney *U-test. Mdn = the median of 18* $mpv_{5\,min}$ *from each test period (see p. 27).*

| Experiment no. | Period | Mdn | Q | U-value | P | Significance levels: avoidance | Significance levels: attraction |
|---|---|---|---|---|---|---|---|
| 8 | Aa | 4.75 | 0.58 | 121.5 | >0.10 | — | — |
| | Ka | 4.92 | 0.33 | 79.5 | <0.02 | ++ | — |
| | Ab | 5.24 | 0.25 | 98.5 | >0.10 | — | — |
| | Kb | 5.07 | 0.20 | | | | |
| 9 | Aa | 6.20 | 0.32 | 101.0 | <0.10 | — | — |
| | Ka | 6.80 | 0.88 | 58.0 | <0.002 | — | +++ |
| | Ab | 5.60 | 0.72 | 122.5 | >0.10 | — | — |
| | Kb | 5.70 | 0.68 | | | | |
| 10 | Aa | 3.80 | 0.64 | 100.5 | <0.10 | — | — |
| | Ka | 3.65 | 0.34 | 59.0 | <0.002 | +++ | — |
| | Ab | 5.05 | 0.60 | 88.5 | 0.02 | ++ | — |
| | Kb | 4.02 | 0.68 | | | | |
| 11 | Aa | 5.70 | 0.62 | 52.0 | <0.002 | +++ | — |
| | Ka | 4.65 | 0.68 | 69.5 | <0.02 | ++ | — |
| | Ab | 5.85 | 0.82 | 64.5 | <0.002 | +++ | — |
| | Kb | 4.15 | 0.65 | | | | |

According to Newcombe and Hartman (1973), as judged from their experiments in a two-choice maze, ripe rainbow trout (*Salmo gairdneri*) of both sexes show intraspecific attraction, probably due to olfactory information. These authors also attend to some earlier observations on intraspecific chemical signals in the reproductive behaviour of fish, *e.g. Poecilia reticulata* (Gandolfi, 1969), *Colisa labia*, and *C. labiosa* (Rossi, 1969), and in the blind goby, *Typhogobius californiensis* (MacGinite, 1939).

Døving *et al.* (1974) have given electrophysiological evidence for differential responses from the olfactory bulb cells in *Salvelinus alpinus* to odorants released with skin mucus from different populations of this species. The possible role of such agents acting as pheromones and assisting in near-migrations to homestream spawning sites was also discussed. It is interesting in the present context, as pointed out earlier by Døving *et al.* (1973), that heating and storage of water containing the odorants, only slightly enhanced their stimulating potence.

Clear intraspecific attraction among juvenile Arctic char (*Salvelinus alpinus*) due to olfaction presumably caused by pheromones (Karlson and Lüscher 1959) was also demonstrated in the fluviarium experiments by Höglund and Åstrand (1973). Further treatment of the data presented in that paper (*op. cit.*) indicates that olfactorily-extirpated char in experiment 19 (Fig. 5 and Table 5) showed clear avoidance reactions. To judge from Table 5 avoidance was also shown

Table 4. *Changes of reaction (avoidance or attraction) of Arctic char to brown trout in different experiments as estimated by the* Mann-Whitney *U-test. Mdn = the median of 18 mpv$_{5\ min}$ values from each test period (see p. 27).*

| Experiment no. | Period | Mdn | Q | U-value | P | Significance levels: avoidance | Significance levels: attraction |
|---|---|---|---|---|---|---|---|
| 12 | Aa | 5.50 | 0.70 | 125.0 | >0.10 | — | — |
| | Ka | 5.00 | 1.10 | 157.0 | >0.10 | — | — |
| | Ab | 4.95 | 0.75 | 150.5 | >0.10 | — | — |
| | Kb | 4.94 | 0.24 | | | | |
| 13 | Aa | 5.35 | 1.38 | 22.0 | <0.002 | +++ | — |
| | Ka | 2.65 | 0.31 | 4.5 | <0.002 | +++ | — |
| | Ab | 5.35 | 0.20 | 89.5 | <0.05 | + | — |
| | Kb | 2.75 | 1.81 | | | | |
| 14 | Aa | 4.43 | 0.46 | 146.5 | >0.10 | — | — |
| | Ka | 4.45 | 0.80 | 149.0 | >0.10 | — | — |
| | Ab | 4.15 | 0.60 | 69.0 | <0.02 | — | ++ |
| | Kb | 5.25 | 0.86 | | | | |
| 15 | Aa | 6.20 | 0.80 | 141.5 | >0.10 | — | — |
| | Ka | 6.62 | 0.78 | 94.0 | <0.05 | — | + |
| | Ab | 5.43 | 0.60 | 100.5 | <0.10 | — | — |
| | Kb | 5.15 | 0.88 | | | | |
| 16 | Aa | 6.10 | 0.40 | 99.0 | 0.05 | — | + |
| | Ka | 6.85 | 0.95 | 109.5 | >0.10 | — | — |
| | Ab | 6.15 | 1.18 | 67.0 | <0.02 | — | +++ |
| | Kb | 7.25 | 0.52 | | | | |

Table 5. *Changes of intraspecific reaction (avoidance or attraction) between intact or olfactory-extirpated Arctic char and intact char. Estimates are made by the* Mann-Whitney *U-test on primary observations presented by* Höglund *and* Åstrand *(1973). Mdn = the median of 18 mpv$_{5\ min}$ values from each test period (see p. 27).*

| Experiment no. | Period | Mdn | Q | U-value | Significance levels: avoidance | Significance levels: attraction |
|---|---|---|---|---|---|---|
| Intact test fish | | | | | | |
| 17 | Aa | 4.85 | 0.82 | 41.0 | — | +++ |
| | Ka | 7.24 | 1.28 | 30.5 | — | +++ |
| | Ab | 4.25 | 1.30 | 4.0 | — | +++ |
| | Kb | 8.15 | 1.12 | | | |
| 18 | Aa | 4.68 | 0.75 | 1.0 | — | +++ |
| | Ka | 7.85 | 0.82 | 0.0 | — | +++ |
| | Ab | 4.30 | 0.30 | 8.5 | — | +++ |
| | Kb | 6.55 | 0.72 | | | |
| Extirpated test fish | | | | | | |
| 19 | Aa | 5.85 | 0.15 | 0.5 | +++ | — |
| | Ka | 4.60 | 0.30 | 5.0 | +++ | — |
| | Ab | 5.65 | 0.16 | 93.5 | + | — |
| | Kb | 5.40 | 0.22 | | | |
| 20 | Aa | 6.92 | 0.60 | 128.0 | — | — |
| | Ka | 6.63 | 0.38 | 46.5 | +++ | — |
| | Ab | 7.25 | 0.15 | 86.0 | ++ | — |
| | Kb | 6.85 | 0.72 | | | |

in the latter parts of experiment 20. The reason for this is, of course, difficult to discuss, as the causative stimulant(s) are not known at the present stage of investigation. It is possible that some stimulant(s) may be perceived by gustatory receptors, or by free nerve endings in the skin, the so-called common chemical sense.

According to Hara (1971, p. 89) reactions following stimulation of the latter organ are usually negative or defensive reactions. Mechanoreception may also contribute to the negative response, as according to Hemmings (1966 a, b) the attractions for a schooling fish (*Rutilus rutilus* L.) are vision and olfaction and the repulsive modality is the lateral sense. Chemoreceptive stimulation and physiological depressants among aquatic animals possibly due to unknown metabolites, have also been discussed by Berrie and Visser (1963) and Yu (1968).

Of special interest are the studies on chemical communication of fishes by Todd *et al.* (1967) (see also Todd, 1971). According to the latter investigators the exquisitely sensitive organs of smell in the bullhead catfish (*Ictalurus natalis*) are mainly used for chemical communication in their social contacts. The detection of distant food, on the other hand, is exerted by the taste buds on the body of this species.

However, the significance of different senses in chemical communication and in the behaviour of fish evidently varies widely from one species to the other.

## IV. FINAL DISCUSSION

In this study an experimental approach has been tried in an attempt to provide quantitative evidence of possible preferential reactions (attraction

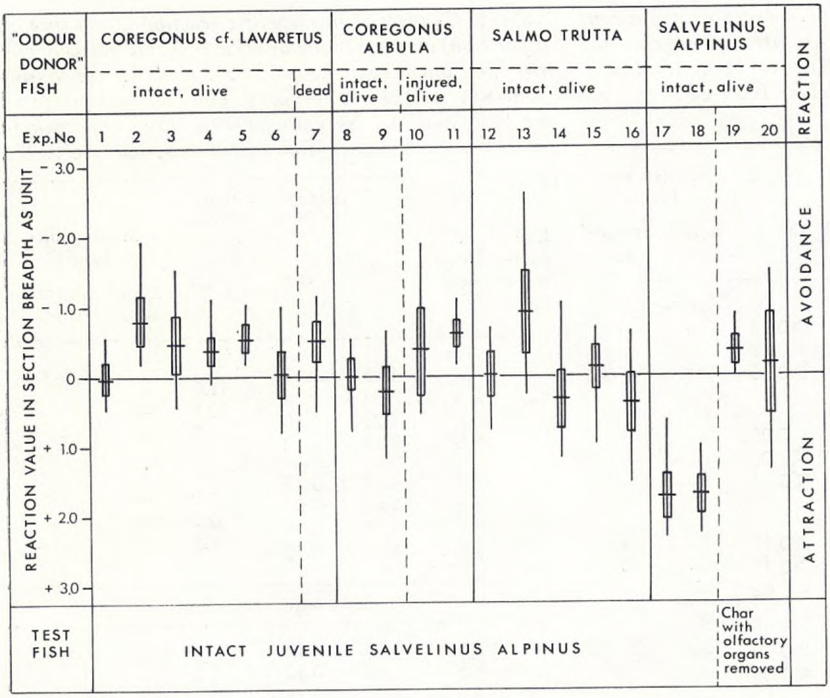



Fig. 5. Diagram showing all preference reactions arrived at in the experiments of the present study (experiments 1—16) as well as of the study by Höglund and Åstrand (1973) (experiments 17—20). Horizontal streaks refer to reaction values, $rv_{6\ hr}$ values (for definition see p. 28). Vertical thin lines represent the range of the reaction values ($rv_{90\ min}$ values) obtained from the four test periods of each experiment, and vertical bars the standard deviation (n=4). A standard deviation covering the median zero line has been regarded as indicating an indifferent reaction.

or avoidance) to presumed odour responses between subarctic fish species. Of especial interest are avoidance reactions which may contribute to the elimination of one species from its natural habitat when faced with another species, or a segregation between species into different ecological niches.

As pointed out above, the present results suggest that such a process may play a role in the case of juvenile Arctic char in relation to whitefish (*Coregonus lavaretus* (L.)). In relation to cisco (*Coregonus albula* L.) and brown trout (*Salmo trutta* L.) the reaction of char was less clear-cut, which supports the idea that complex behaviour patterns are involved. A comparison between the results from earlier investigations on the reaction of char to specimens of the same species (Höglund and Åstrand, 1973), which are included in Fig. 5, experiments 17—18, has strengthened the view that the avoidance reaction displayed by char in experiments 1—7 are in fact real. The experiments with olfactorily-extirpated fish (Fig. 5, Nos. 19—20) also suggest that other agents than odours may be involved (*cf.* the discussion above).

The existence of alarm substances has been shown by several authors ever since the classic experiments of von Frish (1938, 1941 a, b); see also Pfeiffer (1960; 1962; 1963; 1964; 1967) and Hara (1971). Fright reactions to individuals of the same species only occur when the fish are injured, as the cells producing alarm substances of that kind are not open to the surface of the skin (Pfeiffer, 1960). It has also been proved, however, that non-injured predatory fish can produce a "predator odour" that causes alarm reactions in fish of other species (Reed, 1969). The alarm reactions of salmonids to mammalian skin, mentioned above (p. 28) are also of interest in this context, but hand-dipping before starting the recording was found to give no reaction in the present tests (p. 27). In this investigation it has been shown that one species (whitefish), which is not known to be predatory on the other species (Arctic char), caused reactions similar to the above-mentioned alarm response. We therefore suggest that avoidance on account of interspecific odour responses similar to those demonstrated in the present study should not be overlooked as a possible element in the process of non-predatory competitive elimination or interactive segregation. We propose the term "odour aversion" for this phenomenon, which to judge from the present results seems to be a factor of ethological and ecological importance in interaction between fish species. As mentioned in the Introduction, the char used in these experiments normally lives sympatrically with whitefish and theoretically the avoidance reaction should therefore serve to segregate the species. It remains to find out wether similar reactions also occur in char populations living in allopatry with whitefish. For that account it is planned to repeat the present tests with a population of Arctic char from Lake Sädvajaure situated at a higher level in the same mountain region as Lake Hornavan. This future test object has probably never been in contact with any species of whitefish (*Coregonus spp.*).

## V. ACKNOWLEDGMENTS

Our thanks are due to Professor GUNNAR SVÄRDSON at the Institute of Freshwater Research, Drottningholm, for valuable help on this project. We are grateful to Professor KERSTIN LINDAHL-KIESSLING for providing laboratory facilities at the Institute of Zoophysiology, University of Uppsala. We are also indebted to Professor JAN-ERIK KIHLSTRÖM, Uppsala, who suggested the use of the MANN-WHITNEY U-test for statistical evaluation of the results. The research was financially supported by grants from the Swedish Board of Fisheries and from the foundations of EVA and OSCAR AHRÉN and of O. E. NYCANDER.

## VI. SUMMARY

Reactions of juvenile Arctic char (*Salvenius alpinus* (L.)) to chemical stimulation, presumably odour(s) released into the water by the presence of any of three other subarctic fish species, were studied quantitatively by means of the fluviarium technique. In five out of seven tests second-summer char showed clear avoidance to intact as well as to dead whitefish (*Coregonus lavaretus* (L.)). In the other two tests the reactions were indifferent. Two tests using intact vendace or cisco (*Coregonus albula* (L.)) as "odour donor" also gave no significant reaction, but avoidance was noted in two tests with injured cisco. Brown trout (*Salmo trutta* L.) gave rise to significant avoidance in one test, but to slight attraction in the other four tests performed. The present results have been compared with earlier findings regarding intraspecific attraction among juvenile Arctic char. The interspecific avoidance behaviour of juvenile char versus whitefish observed in the present tests has been discussed as a possible guiding factor in interactive segregation. The term "odour aversion" is suggested as a designation for the phenomenon of restriction in behaviour and occurrence of one species on account of such olfactory responses in the field situation.

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# Population Biology of the Cestode *Caryophyllaeus laticeps* (Pallas) in Bream, *Abramis brama* (L.), and the Feeding of Fish on Oligochaetes

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## I. INTRODUCTION

The traditional way of obtaining detailed information on the food preferences of various fish is by careful analyses of the stomach contents.

Quite often, however, the representativity of such analyses can be questioned, especially in the case of over-night nettings of fish when the water temperature, and hence the rate of digestion, is reasonably high. In such nettings the fish may fasten in the nets late in the afternoon, but the stomach contents will not be preserved until the following morning.

In view of the fact that some food items like oligochaete worms lacking chitinous, hard head capsules, thick shells etc. may be fully digested within an hour after consumption (*cf.* GALINSKY and NIKITIN, 1972) it is easily understood that such soft-bodied food items are nearly always underestimated in any stomach analyses, however detailed. Nets are rarely examined at hourly intervals, which would be required, and the bristles and crotchets of oligochaetes are only exceptionally searched for in such analyses.

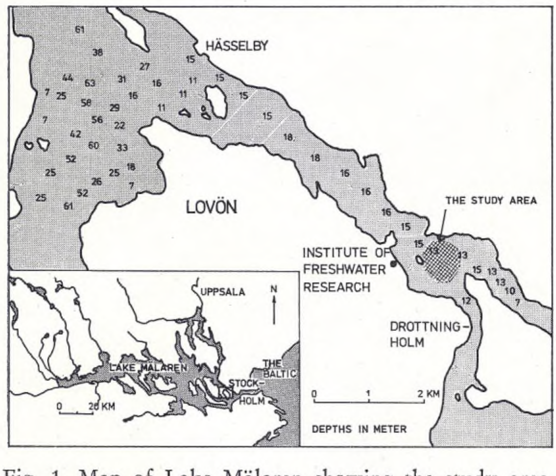


Fig. 1. Map of Lake Mälaren showing the study area at Drottningholm. Some depths (in m) are indicated on the map.

The order Oligochaeta is generally one of the major entities — beside chironomid larvae — in freshwater soft-bottom communities. Oligochaetes, however, are seldom if at all mentioned in studies on the food of fish.

Several recent works have focused attention upon this obvious discrepancy (PODDUBNAYA 1962, NIKOLSKI 1963, GRIGYALIS 1966, KAJAK and WISNIEWSKI 1966, KENNEDY 1969 a, 1969 b, AL-DOORI 1971, BRINKHURST and JAMIESON 1971, GALINSKY and NIKITIN 1972, YAROCHENKO *et al.* 1972, AAREFJORD *et al.* 1973, and others). POPCHENKO, 1971, emphasizes the importance of oligochaetes in the diet of coarse fish but also of salmonids. On the whole, East-European fishery biologists seem to be traditionally more observant than others upon the role of oligochaetes in the diet of fish.

A careful examination of the intestinal parasite fauna with regard to caryophyllid cestodes (KENNEDY 1969 a, working with dace) is among the most sophisticated methods of getting information about the intensity of feeding upon oligochaetes by fish, especially coarse fish. The mere presence of small specimens of *Caryophyllaeus laticeps* (PALLAS) in the anterior part of the guts of various coarse fish is in itself an indication of a fairly recent consumption of tubificid oligochaetes.

In the present work light is cast upon the life cycle of *C. laticeps* in its final host — bream, *Abramis brama* (L.) — in eastern Lake Mälaren. The parasite is usually associated with representatives of the family Cyprinidae, but also other fish may become infested more or less regularly,

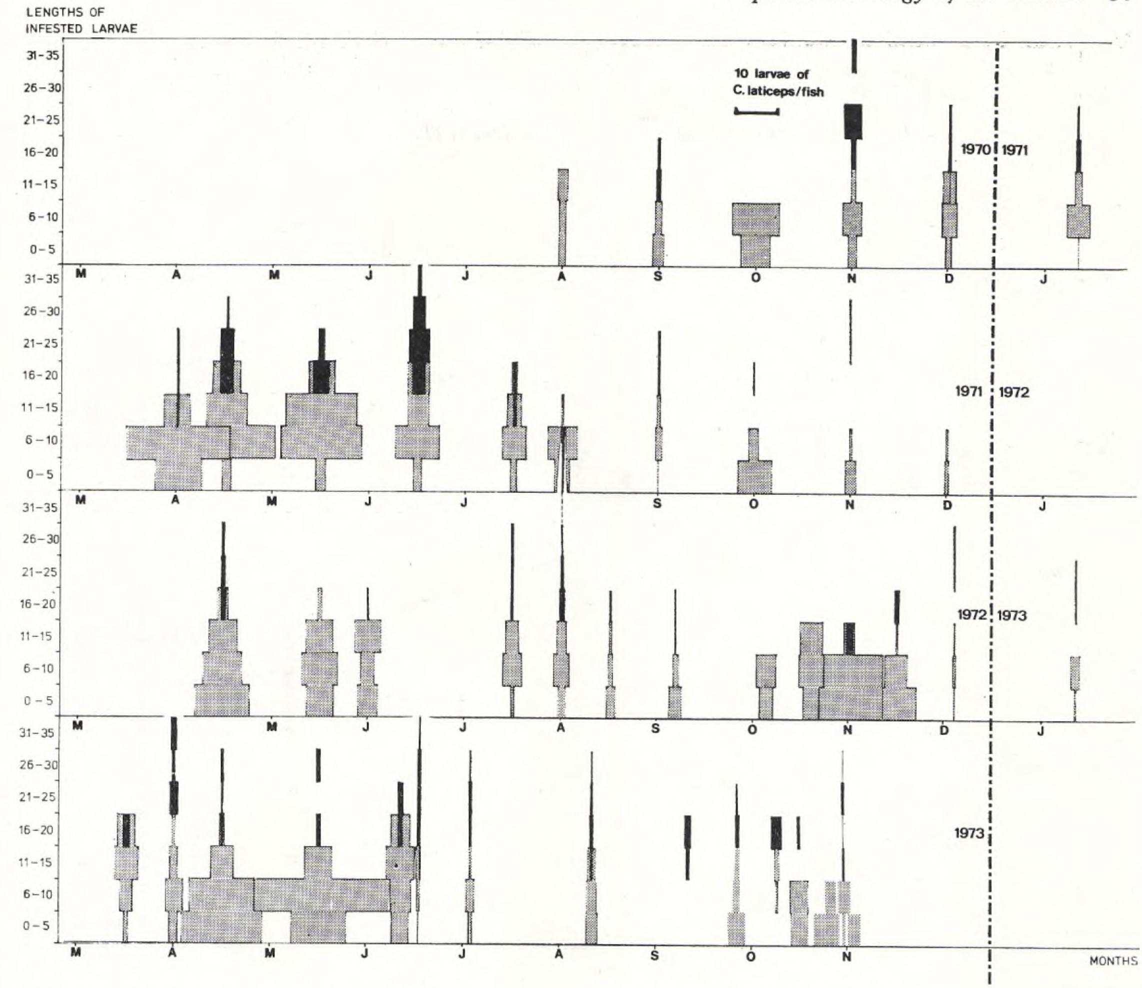


Fig. 2. The pattern of changes in length and state of maturity of *Caryophyllaeus laticeps* in bream at different times of the year. The data are expressed as actual numbers of mature (unshaded) and gravid worms with developed vitellaries (shaded) found each month.

even rainbow trout (Brandes 1958) and possibly *Gobius minutus* (Markowski 1935) in brackish water. Kulakowskaja (1973) presents a list of 12 fish species frequently infested by the parasite.

For further information on the general biology of *C. laticeps,* as well as other caryophyllids, the reader is referred to the comprehensive and important work by Mackiewics (1972).

## II. MATERIAL AND METHODS

*Caryophyllaeus laticeps* is a fairly common intestinal parasite in bream in Lake Mälaren. Blue bream, *Abramis ballerus* (L.), is also regularly infested, while silver bream, *Blicca bjoerkna* (L.), and tench, *Tinca tinca* (L.), are only infested at times.

The present study started on the initiative of Dr. C. R. Kennedy, University of Exeter, England, in late July 1970 and has continued since then. The intention was to capture about 20 bream monthly by means of gill nets in an area not far from the Institute of Freshwater Research in eastern Lake Mälaren (Fig. 1). Despite intensive fishing (11 gill nets each time) it proved very difficult to catch more than about 10 fish per

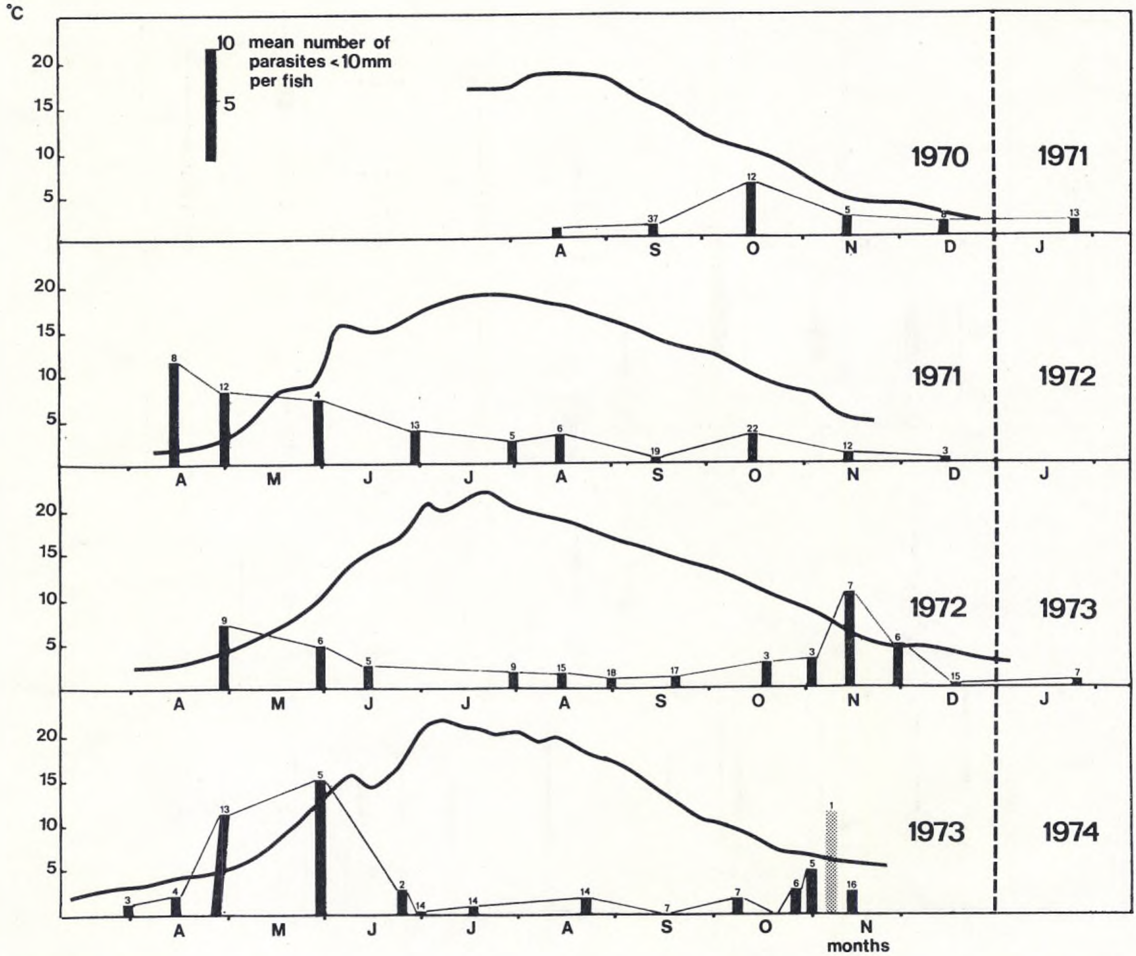


Fig. 3. The mean number of *C. laticeps* shorter than 10 mm per bream at different times of the year (August 1970—November 1973). Surface water temperatures (in degrees C) covering the period of investigation are also given in the figure. The actual number of examined fish at each sampling event is indicated on top of each staple. (Note that one staple in November 1973 is very uncertain.)

month. Until the end of 1973 in all 346 bream were caught in 46 trials over 33 ice-free months. As a rule the period of permanent ice-cover lasts from the middle of December to the middle of April in this part of Lake Mälaren (see Fig. 6).

In comparison with captured bream from adjacent waters the average bream inside the study area was markedly smaller, *i.e.* about 25 cm long and 7—8 years of age. For this reason and others there is little doubt that most captured fish belong to a fairly homogeneous population.

All captured fish were weighed and measured and detemined to sex.

The fullness degree of the intestines was estimated. The intestines were examined under a low-powered microscope for the presence of macroscopic remnants of oligochaetes. It has not been considered necessary to apply dyeing procedures as suggested by Galinsky and Nikitin (1972).

Samples of the stomach contents and smears of the gut mucosa were routinely examined under a high-powered microscope (400—600×) for the presence of bristles and crotchets of oligochaetes.

The entire stomach contents of some fish were regularly analysed.

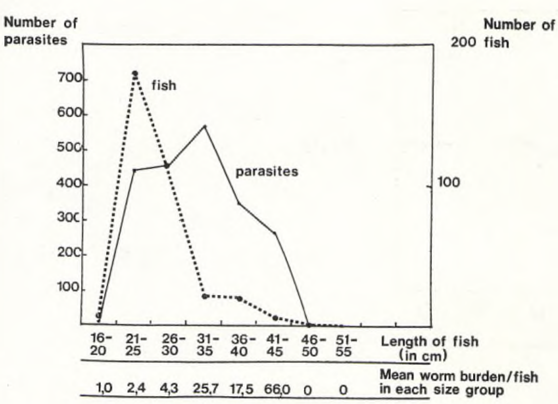


Fig. 4. The mean worm burden (*C. laticeps*) per bream in each size group.

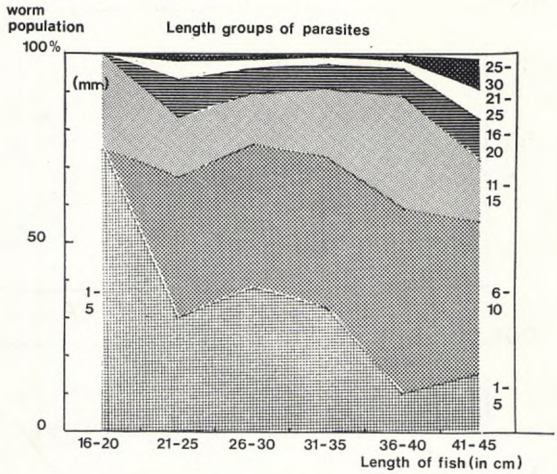


Fig. 5. Length groups of *C. laticeps* found in each size group of bream — from January 1971 till the end of 1973.

All intestinal parasites obtained were allowed to relax in cold tap water until they ceased to respond to tactile stimuli (*cf.* KENNEDY 1969 a), which facilitates later measurements, and were thereafter preserved in formalin. All caryophyllids were measured to the nearest millimetres and gravid worms with developed vitellaries were specially recorded.

Measurements of surface water temperatures in the study area, covering the whole period of investigation, were made every day during the ice-free season (Fig. 3).

## III. RESULTS

### *Population biology of Caryophyllaeus laticeps in bream*

The annual incidence cycle of *C. laticeps* in its final host, bream, was on the whole identical throughout the four years studied (Fig. 2). Two quite distinct peaks of infection — one in the spring and one in the autumn — were generally distinguished (*cf.* 1972 in particular), while the level of infection in late summer was invariably very low.

Mass infection often took place from March to the middle of May and from late October to mid November (Fig. 3). In autumn the raised incidence of infection could be associated with a concurrently lowered surface water temperature to about 8° C (Fig. 3). The spring maximum on the other hand started when the water temperature was still below 5° C. On the whole, infections were most numerous in the coldest half of the year. The peak periods are generally difficult to trace, the autumn peak in particular being very narrow. In the autumns of 1970 and 1971 the real peak values were probably not obtained. The frequency of sampling was probably too low in those periods.

Very few full-grown parasites were generally recorded in August and September. The majority of gravid worms had obviously left their hosts by June and the months following immediately thereafter which is in good agreement with similar investigations in other countries (see discussion below).

In spite of the fact that the water temperature in Lake Mälaren in the winter is normally very low, and the lake is frozen over at least 3—4 months a year, newly infested bream are caught all the year round. At the time of consumption by the fish the larval parasites are obviously of very different age. According to KENNEDY (1969b) they may vary in length at least between 2.5 and 10 mm — often even up to 15 mm (*cf.* Fig. 3 and discussion below).

The mean worm burden per fish is strongly correlated with the length of fish (Fig. 4). Large

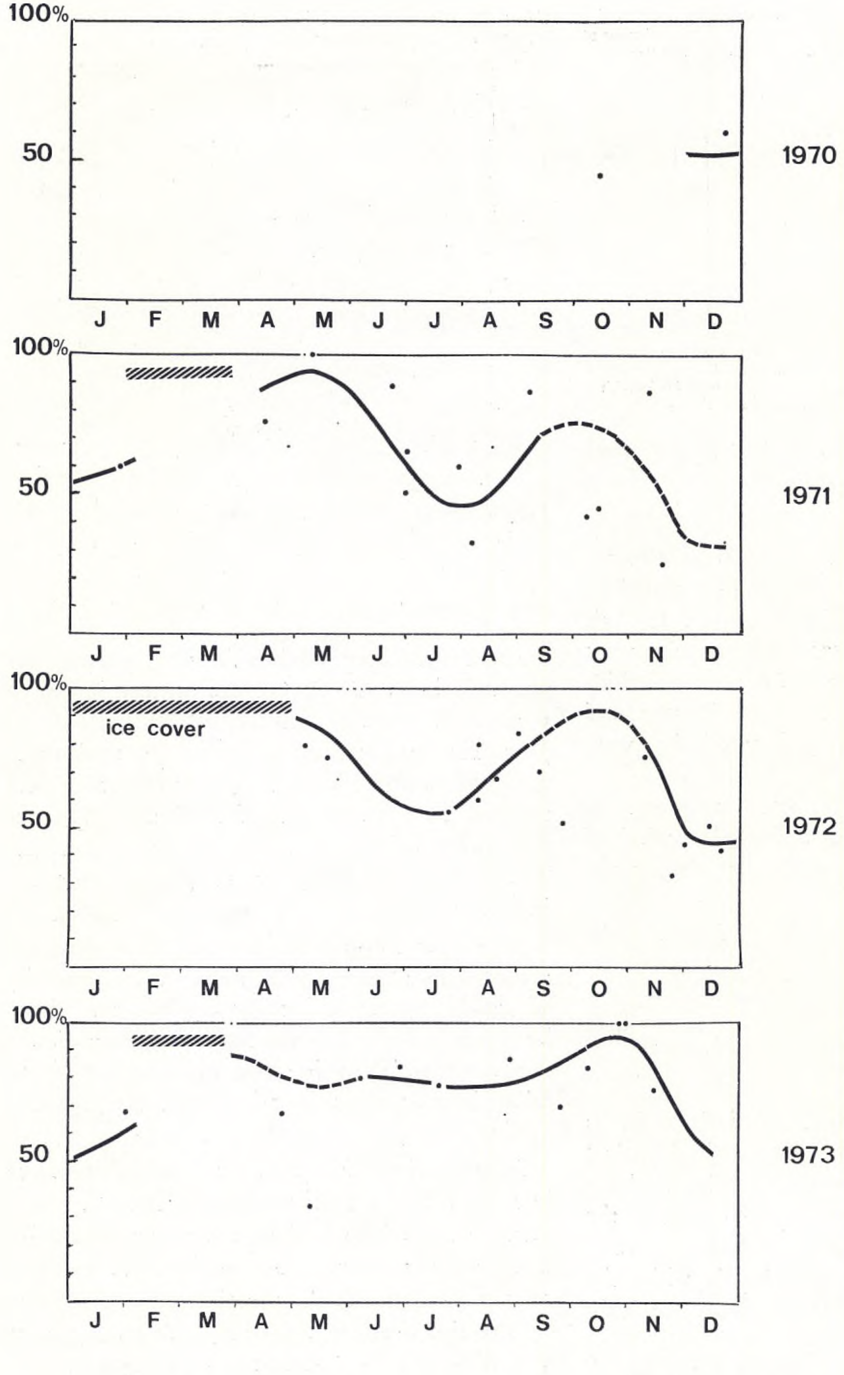


Fig. 6. The yearly variation in the degree of infection (in %) of bream caught at Drottningholm. Large dots indicate a minimum of 10 bream examined at each sampling event. Small dots represent a minimum of 3 fish. The periods of permanent ice-cover are indicated in the figure (shaded).

fish, in particular, may carry great numbers of parasites in different stages of development (see below and ANDERSSON 1974).

Most fish caught within the area are between 20 and 30 cm long (Fig. 4). The yearly worm burden for these size groups is generally between 2 and 4, which means that during the peak periods the mean worm burden is probably between 10 and 20 for the same size groups. Fish between 30 and 45 cm carry far more parasites at any time of the year (Fig. 5) and in comparison with smaller fish they also carry a greater proportion of nearly full-grown cestodes. Bream between 15 and 20 cm have only been found carrying small cestodes, about 75 % of the latter are shorter than 5 mm (and nearly 25 % are between 11 and 15 mm). In comparison with the smaller fish those between 36 and 45 cm carry, on average, only about 15 % worms shorter than 5 mm, while 45 % are between 11 and 30 mm.

Due to the relative scarcity of fish longer than 30 cm in the catches the calculated worm burdens per fish in these length groups are a bit uncertain. The general trend, however, persists; the larger the fish the heavier the worm burden. This is also in full agreement with BACKIEL and ZAWISZA (1968) basing their conclusions on masses of compiled data on bream.

The mean infection degree of bream caught in the vicinity of the laboratory has rarely been found to be below 50 % (about 40 % once in December 1971). Over a period of about three and a half years (Fig. 6) the average value is more likely to be above than below 70 %. Values of about 90 % or more are common during the peaks of infection in the spring and in the autumn. The cycles of 1971 and 1972 very much resembled each other and the autumn peak of infection of 1973 was almost identical, too. Remarkably enough, in the summer of 1973 the infection built up a plateau between May and September at no less than 80 %.

The author has repeatedly obtained evidence that *C. laticeps* spontaneously moves backwards in the intestines of captive bream even if, as in the present case, the newly caught fish are immediately put into a tank with circulating water from their natural habitat.

Bream kept in a tank, however big, over a night or two have repeatedly turned out to be more or less devoid of *C. laticeps* when examined later. In some cases the parasites could be found in the rear part of the intestinal canal. KENNEDY (unpublished) has even observed worms actually leaving the fish.

The parasite is otherwise rarely found in the rear part of the guts under natural conditions but commoly in the first loop of the intestinal canal. MACKIEWICS (1972) presented similar observations concerning the same fish and parasite, "an important consideration when utilizing preserved fish for incidence or distributional studies".

### *Distribution and hosts of larval Caryophyllaeus laticeps*

Normally, each tubificid host can only carry one developing parasite (*cf.* KENNEDY 1969 a). To complete the cycle parasitized tubificids are bound to be eaten by fish — in this case a bream. (The abbreviated and very special cycle of the genus *Archigetes* will not be discussed here; *cf.* NYBELIN 1962 and MACKIEWICS 1972).

Oligochaete material obtained since 1965 from all over Lake Mälaren has been analysed and all identifiable procercoids in tubificids have been carefully recorded. The mean degree of infection has only occasionally been found to exceed 0.15 %. As a rule it is far below that value (*cf.* Lake Hjälmaren below). Infected tubificids have been identified from sampling sites throughout the whole lake, but local aggregations of infected specimens have never been noted. In most cases no parasitized tubificids could be found at all which may imply that the true values are more probably below 0.1 % than above. The value given above — 0.15 % — has been repeatedly obtained from Görväln, a bay of Lake Mälaren about 10 miles to the north of Drottningholm. A value of even 1 % was once estimated from a small series of samples in that basin (T. WIEDERHOLM, personal information; *cf.* below).

Since a large lake in this respect is an open ecosystem, it is always difficult to check whether the fish actually feed where the bottom samples come from or whether infested tubificids are locally more frequent elsewhere, in areas where the fish may move. This problem was thoroughly discussed in ANDERSSON (1974).

The bream near the Institute at Drottningholm are known to migrate short distances from shallow reaches to deep water (15—18 m) when water temperature falls rapidly in late autumn. Other migratory movements are very poorly known, but naturally cannot be excluded. As mentioned above, there are some indications that the exchange with neighbouring basins of the lake is of rather limited importance.

The question of host specificity of *Caryophyllaeus laticeps* and other caryophyllids does not seem to be exhaustively treated in literature. In the compilation of data in MACKIEWICS (1972) only four tubificid species from fresh water are stated to serve as intermediate hosts. Most references do not separate different tubificid species at all, which is probably due to insufficient taxonomical knowledge rather than a lack of interest in which species are involved in the cycle.

Several tubificid species belonging to the genus *Potamothrix* were the only hosts for *C. laticeps* in different parts of Lake Mälaren, *i.e.* the species *P. hammoniensis, P. heuscheri, P. vejdovskyi,* and *P. bedoti* (*cf.* TIMM 1972). They seem to be infected to roughly the same extent. Accordingly, on average some 75—80 % of all tubificids in the lake are presumptive hosts for *C. laticeps.* There are no indications, however, that fish can systematically pick one tubificid species out of the whole population.

In view of the fact that no infested tubificids have been found inside the area so far (not very surprising with regard to the limited material available), although present in most other parts of Lake Mälaren, it could be of interest to discuss what species of tubificids commonly occurring in the area — in addition to those belonging to the genus *Potamothrix* — may be susceptible to infection.

*Psammoryctides barbatus,* which is mostly confined to the littoral and sub-littoral at Drottningholm, does not seem to be an intermediate host at all in the lake. However, it was the only host identified in the nearby Lake Hjälmaren, the average degree of infection being estimated at 0.1 % (MILBRINK 1973 a). The species made up only some 5—6 % of all tubificids in that lake, while *P. hammoniensis* made up 80—90 %.

Other species of interest in this context — all very abundant — are *Tubifex tubifex,* which is the only host, or the main host, in numbers of Swedish lakes, *Limnodrilus hoffmeisteri,* of which one infested specimen has possibly been found in the lake, and *Limnodrilus claparedeanus,* which, for instance, was the intermediate host for *C. laticeps* in the Lake of Geneva (JUGET 1958).

*Food of bream as revealed by stomach analysis*

The very high level of infection throughout the year is naturally in itself an indication that the fish are feeding extensively on oligochaetes. In order to test the correlation between the frequency of remnants of oligochaetes in the gut contents and the burdens of parasites in the intestinal canals, bream of different size from different times of the year were carefully examined. (The stomachs of 333 bream were examined under low magnification only). In Table 1 the gut contents are given of 13 bream, 2 silver bream, 1 rudd (*Scardinius erythrophtalmus*), 1 blue bream, and 1 tench. The various fish were taken out at random. Blue bream have been found to be as much infested by *C. laticeps* as bream in Lake Mälaren (MILBRINK, unpublished). Silver bream are seasonally and regionally infested (mostly in late summer) and so are tench. In the lake rudd have never been observed carrying caryophyllids.

Of these fish 8 bream contained *C. laticeps,* but none of the other fish. The frequencies of different entities are given in the table in a relative scale comprising only three degrees. The first bream in the table caught in September 1970 was accordingly about 30 cm long. No identifiable remnants of oligochaetes could be observed in a low-powered microscope (about 10—50×), which is generally the instrument used in standard stomach analyses.

A magnification degree of about 400×, however, revealed that the bream mentioned first had lots of bristles and crotchets of tubificid and naidid oligochaetes in its food bolus. Smears from the gut mucosa analysed the same way gave a similar result. Still, the bream carried only one parasite.

In fact, all bream analysed for the purpose had remnants of oligochaetes in the food bolus, but here there was no correlation at all between the momentary intake of oligochaetes and the parasite burden. Accordingly, one bream caught in late October 1970 had only traces of oligochaetes

Table 1. *The stomach contents of 11 bream, 2 silver bream, 1 blue bream and 1 rudd selected at random.*

| | bream, large (30 cm) 8/9 1970 | bream, large (30 cm) 8/9 1970 | bream, large (43.5 cm) 15/4 1971 | bream, medium-large (29 cm), 28/1 1971 | bream, medium 8/9 1970 | bream, medium 16/11 1973 | bream, medium (23.5 cm) 28/1 1971 | bream, medium 8/9 1970 | bream, medium 20/10 1970 | bream, large (30 cm) 13/10 1970 | bream, medium (27 cm) 13/10 1970 | silver bream, medium 8/9 1970 | silver bream, medium (20 cm) 8/9 1970 | silver bream, medium (23.5 cm) 14/10 1971 | rudd, medium 8/9 1970 | tench, large (30 cm) 9/9 1970 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Caryophyllaeus laticeps* | 1 | — | 152 | 15 | — | — | 1 | 30 | 1 | 185 | 2 | — | — | — | — | — |
| identifyable remnants of oligochaetes | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| microscopic details of oligochaetes such as bristles and crotchets | ××× | ×× | ××× | ×× | × | × | ×× | ×× | ××× | ×(×) | × | × | × | — | — | — |
| chironomid larvae, tanypodines | × | ×× | ××× | ××(×) | — | ×× | ××(×) | — | ×× | ××× | ××× | — | × | — | — | × |
| — others | — | — | ×× | ×× | — | × | × | × | × | ×× | — | ×× | ×× | × | — | × |
| Trichoptera | — | — | — | × | — | — | — | — | — | — | × | × | — | — | — | — |
| Ceratopogonidae | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | × |
| Aphididae | — | — | — | — | — | — | — | × | — | — | — | — | — | — | — | — |
| Copepoda, *Cyclops* spp. | — | — | — | × | — | — | ×× | — | × | — | — | — | × | — | — | — |
| Cladocera, *Bosmina* spp. | — | ×× | — | — | — | — | — | × | × | × | — | ××× | ×× | — | × | — |
| — others | ×× | ×× | — | — | — | — | ×× | ××× | ×× | ××× | × | ×× | ××× | — | — | — |
| Ostracoda | ×× | ×× | × | — | — | — | — | ××× | ×× | ×× | ××× | — | ×× | — | — | × |
| *Asellus aquaticus* | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | × |
| rests of amphipods | — | — | × | — | — | — | — | — | — | — | — | — | — | — | — | ×× |
| Mollusca, *Pisidium* spp. | — | — | — | — | — | — | — | — | — | — | × | — | — | — | — | — |
| — others such as Planorbidae and *Valvata* sp. | — | — | — | — | — | — | — | — | — | — | × | × | — | — | — | ×× |
| rests of molluscs | — | — | — | ××(×) | — | — | — | — | — | — | ×× | — | — | — | ××× | × |
| fins and scales of fish | — | — | — | ×× | — | — | × | — | — | — | — | — | — | — | — | × |
| fish roe | — | — | — | × | — | — | — | — | — | × | — | — | — | — | — | — |
| Rotatoria | — | — | — | — | — | — | — | — | — | — | — | — | × | — | — | — |
| not identifyable animal residues | × | ×× | ××× | — | — | × | ××× | ××× | ×× | ××× | — | ××× | ×× | — | — | × |
| material from rooted vegetation | — | × | × | ×× | — | — | — | — | — | — | × | ×× | — | — | ××× | ×× |
| periphyton | — | — | × | — | — | × | × | — | × | — | — | × | × | — | ××× | ××× |
| seeds | — | — | — | × | — | — | — | — | — | — | — | × | — | — | — | — |

in the gut mucosa, but the worm burden (comprising all sizes) was still 185 (Table 1).

The stomach analyses revealed that the bream had a most varied intake of different food objects. Chironomid larvae formed a significant part of the food intake, but cladocerans, copepods and ostracods were always well represented. Food items of plant origin were also common.

Fish scales and fins in the guts are naturally difficult to explain. Bream are not known to prey very much on other fish although single fish may feed on poorer ones (PODDUBNAYA 1959, *In* BACKIEL and ZAWISZA 1968).

In view of the fact that all bream examined had remnants of oligochaetes in their guts, there is little reason to believe that bream switch from a tubificid diet in early spring to, for instance, a crustacean diet in summer. On the contrary, almost the same bottom animals dominated the diet at different times of the year.

Attempts have been made to link the presence of bristles and crotchets in the gut mucosa of fish to a non-specific ingestion of bottom material including remnants of dead tubificids (KLUST 1935).

Bristles and crotchets of tubificids built of mucopolysaccarids are not very stable in the bottom ooze and are quickly decomposed. Accordingly in contrast to chitinous cuticular fragments of arthropods these structures are known to be of little or no value in palaeozoology. On the above grounds the non-specific ingestion theory is easily rejected.

### *Feeding habits of bream as indicated by parasitic infestation*

The presence of small specimens of *C. laticeps* in bream indicates that the fish have recently fed upon infected tubificids and so their presence in bream in all months of the year (Fig. 2) indicates clearly that bream feed on tubificids all through the year.

The incidence cycle of *C. laticeps* is naturally dependent amongst other factors upon the feeding intensity of the fish at different seasons. A reduced incidence of infection in bream from December to February is probably a direct consequence of the reduced feeding activity of the fish in that part of the year. No stomachs of bream, however, were then totally empty, due to fact that bream do not stop feeding completely and the rate of digestion is generally very much retarded in winter. The spring peak of infection coincides well with the resumption of feeding in spring, while the water temperature is still very low (*cf.* Fig. 3 and discussion below).

The scarcity of parasites in the warmest months is probably due to the temperature control system proposed by KENNEDY for dace (KENNEDY 1969 b; *cf.* discussion below). The feeding activity of bream, however, is certainly kept at a high level also in summer and this high level generally persists till the end of November. The autumn peak may again be explained with a reduced physiological resistance by the host to infections, while at the same time feeding has not started yet to decrease.

The present study indicated that older bream were more heavily infested with *C. laticeps* than younger bream (Fig. 4), which maybe merely reflects a slightly different way or intensity of feeding on the benthos (*cf.* WALKEY 1967). In several other fish species the parasite burdens generally decrease with age, which may be an indication of an acquired immunity response (*cf.* MACKIEWICS 1972). BORGSTRÖM and HALVORSEN (1968) found slightly different burdens of *Caryophyllides fennica* in males and females of roach, which in accordance with NIKOLSKI (1963), for instance, may merely reflect a differential feeding behaviour between the sexes during the spawning season. Although not especially looked for here, no such differences between the sexes have been observed.

It has been clearly demonstrated that not every parasite ingested adheres to the gut mucosa. Physiological reactions from the fish, especially during the warm season make the cestode larvae pass through the entire digestive canal — possibly due to immune reactions (*cf.* discussion below) — without adhering to the walls of the intestines. Furthermore, a high proportion of the cestodes are probably too damaged by the pharyngeal teeth of the fish to be able to make an infection successful (KENNEDY 1969 a). In addition to the above there is a continuous gain and loss of parasites (KENNEDY 1969 a), and only a fraction of the specimens are probably capable of staying in the intestines of the fish for a longer period at any time of the year.

With the above limitations in mind it seems quite obvious that the fish must consume large numbers of tubificids over a short period.

The average number of *C. laticeps* per bream in a summer month (June) in Lake Hjälmaren was 5 (MILBRINK 1973 a) and the same number was obtained for both bream and blue bream in the basin of Ekoln in northern Lake Mälaren (September—November; MILBRINK, unpublished). Calculations based on the whole Drottningholm material (from July 1970 to January 1974) gave 6.6 to be the average worm burden per bream throughout the year (*cf.* above).

As was said above about 75—80 % of all tubificids in Lake Mälaren, *i.e.* those belonging to the genus *Potamothrix,* are presumptive hosts of *C. laticeps.* Accordingly, the average fish above with 6.6 parasites must have eaten at least 6.6 specimens of *Potamothrix* spp. However, with a maintained infection degree of tubificids (see above), let us assume 0.1 %, the acquisition of this number could have involved the ingestion of 6,600 specimens of *Potamothrix* spp. within a fairly short period. Since there is no evidence that any species of fish feeds selectively on species of tubificids the fish could actually have consumed about 8,500 tubificids.

Fish with worm burdens of between 150 and 200 have been caught within the area (see Table 1). Worm burdens of about 50 are rather common. With a maintained assumed level of infection of 0.1 % the fish must have recently consumed between 190,000 and 250,000 tubificids to secure the maximum worm burdens mentioned first.

Even if the level of infection of tubificids was ten times higher (1.0 %), the fish would still have to consume 19,000—25,000 worms, thereby giving a possible minimum value of consumption.

If local accumulations of infested worms are unlikely a level below 0.1 % seems quite reasonable and a range up to 1.0 % should be safely within the margins.

Naturally these figures are very approximate. However, since the infection involved at least about 50 % of the fish at any time of the year (*cf.* Fig. 6), it is obvious that tubificids must form a significant part in the diet of most bream (*cf.* KENNEDY 1969 a).

### *Other intestinal parasites in the bream population*

Bream caught in the vicinity of Drottningholm are quite often heavily infested with another cestode, *Ligula intestinalis* (L.), the average incidence being close to 15 % in the study area (1970: no records available, 1971: 17.9 %, 1972: 13.3 % and 1973: 16.8 %; a preliminary value for 1974 would be slightly above 12 %).

The presence of *Ligula* in bream rarely seems to be associated with heavy infections of *C. laticeps.* On the whole the incidence of infection of *C. laticeps* seems to be a bit lower than the average when both species are present in the fish, maybe suggesting an interspecific antagonism between those cestodes (*cf.* MACKIEWICS 1972). This could also be due to individual food preferences amongst fish, such that individuals feeding on plankton prefer not to feed on benthos and *vise versa.* Naturally, the presence of *Ligula* could also bring about a change in the physiological condition of the host and thereby a change in feeding habits or feeding intensity.

In Fig. 7 the mean number of *C. laticeps* per bream is plotted versus the mean number per *Ligula*-infested bream. Each point in the diagrams stands for one sampling event with at least a total of 6 bream each time. Open circles represent samplings with at least 3 *Ligula*-infested fish each time and a total of 10—20 fish, while half-filled circles represent the same except that only 2 *Ligula*-infested fish were recorded each time. Fig. 7 a covers from January 1971 till the end of 1973 and Fig. 7 b from January 1974 till the end of November the same year. A brief glance at the diagrams will give an impression that most points (quotients) are lying above unity. All of the open circles are above unity and none of the quotients they represent are below 2. Furthermore very high quotients have been regularly obtained in late April, in May and the first half of June (Fig. 7 a). The above would imply that fish with *Ligula* are at the same time less liable to infection by *C. laticeps* than the average fish

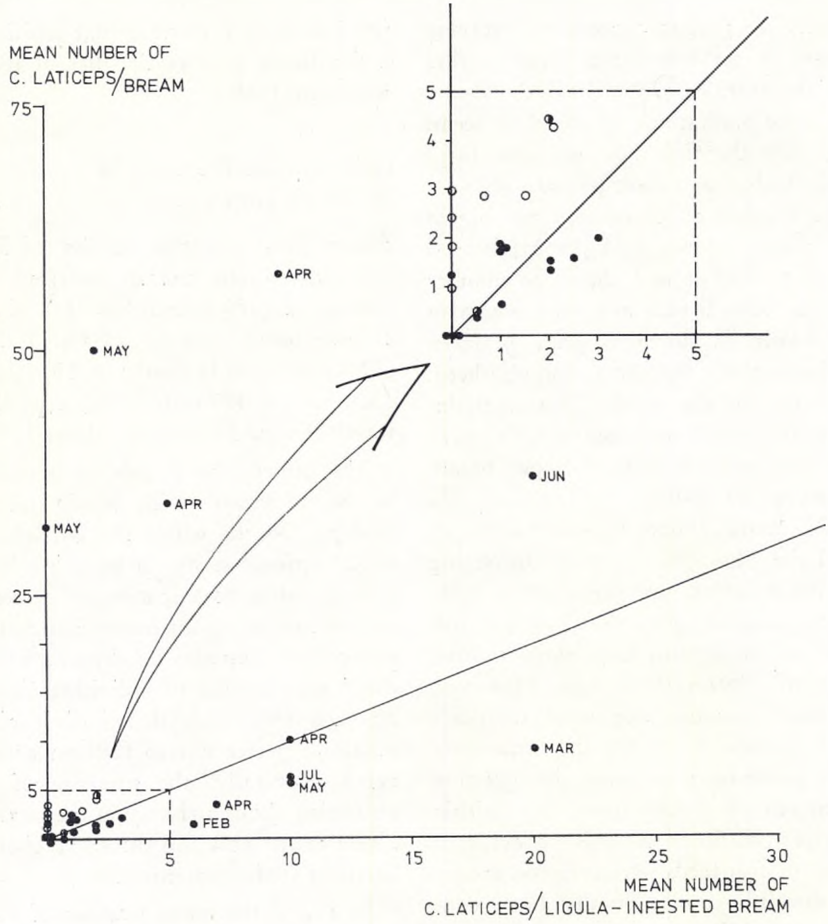


Fig. 7 a.

and heavy infection by the latter are not likely to occur even during the peak periods.

During the peaks of infection in late October and early November, in particular, *C. laticeps* co-exists with *Neoechinorhynchus rutili* (MÜLLER) in the study area.

## IV. DISCUSSION

The sequence of events that constitute the development of *C. laticeps* in Lake Mälaren are arranged in an annual cycle. KENNEDY (1969 a, 1969 b) has convincingly demonstrated what factors are most important for the timing of that cycle in the final host — dace — and in the intermediate tubificid host, *Psammoryctides barbatus*, as well. KENNEDY suggested that the time of maturation of the parasite was of particular importance. The production of eggs are closely correlated with the time of fish spawning. The hormone balance of the host is supposed to provide the stimulus to this production. The maturation cycle of the parasite and that of the host are obviously closely related.

The cycle of *C. laticeps* in Lake Mälaren could readily be explained by the Kennedy model — a temperature control system (see below).

Most eggs of the parasite are probably shed into the lake from May to the beginning of July. Bream tend to spawn at the end of June and most of the adult cestodes leave their hosts at that time (*cf.* Fig. 2). KULAKOVSKAJA (1962) has demonstrated that the eggs may remain infective

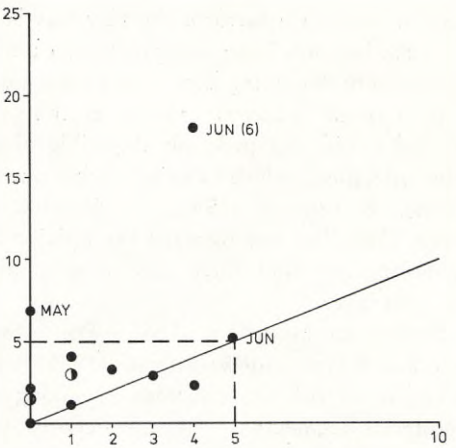


Fig. 7 b.

Fig. 7. The mean number of *C. laticeps* per bream plotted versus the mean number per *Ligula*-infested bream. Samplings resulting in mean numbers exceeding 5 *C. laticeps* per bream are here indicated with FEB, which stands for February, MAR for March etc. (see text).

Fig. 7 a covers from January 1971 till the end of 1973 and Fig. 7 b from January 1974 till the end of November the same year. Each open circle represents a minimum of 3 *Ligula*-infested bream and a total number of at least 10 bream. (For further explanations, see text.)

to tubificids for about three months, which would suffice to explain the cycle, since the bulk of young tubificids of *Potamothrix* (see above), which seems to be the only intermediate host genus in the lake, generally emerge in August—September. *Potamothrix hammoniensis,* although it may reproduce over the greater part of the year (*cf.* Brinkhurst 1966), generally breeds in June—July in Lakes Hjälmaren (Milbrink 1973 a) and Mälaren (Milbrink, unpublished). It is well-known that young tubificids are more readily infected than older ones. Young worms of *P. hammoniensis* may thus be susceptible to infection all the year round.

Most of the cestode larvae in their intermediate hosts are probably not infective to fish until the middle of October and onwards. Those cestodes not immediately eaten by the fish will remain in the tubificids in a "cestode larvae pool" at least till the start of the next summer, if not consumed before then, at the time most tubificids breed and die. It is not known what happens to the larvae of *C. laticeps* if their intermediate hosts will survive for another year — or perhaps for another couple of years or even more, Jonason's (1972) slightly mystifying results taken into account.

Kulakovskaya (1962) found that the majority of these cestodes have a total life span of more than one year (Sekutowics (1934) suggested, a life span of about two years). Since *P. hammoniensis,* has a cycle of development longer than one year in Lake Mälaren (Milbrink, unpublished) this same situation pertains here also.

The newly infested caryophyllid larvae continue their growth in the final host and rapidly pass from one size group to another. A great proportion of the larvae, however, are not capable of staying in the fish long enough to complete the cycle (*cf.* above). Although there are always larvae capable of reaching sexual maturity at any time of the year (*cf.* Fig. 2), most of them probably produce eggs in May and June.

*C. laticeps* generally spends 3 months in bream in Lake Mälaren, or possibly even up to 6 months in the winter, considering that living processes are much retarded in the coldest months of the year (*cf.* Fig. 2). Anderson (1974) similarly found 3 months for bream, while Polish data would suggest periods about twice as long (Mackiewićs 1972). In the USSR *Caryophyllaeus* lives for 1—1.5 (2) months in carp according to Bauer (1959) and Kulakowskaya (1964).

The timing of the cycle in Lake Mälaren is in good agreement with those described by Kulakowskaya (1964), Kennedy (1969 b, from the River Avon) and by Anderson (1974, see below), although the whole cycle in Lake Mälaren is slightly retarded and in that respect may agree better with Halvorsen (1972) from the River Glomma in Norway. Local conditions, however, may be responsible for the spectacular change of the normal one-peak cycle into, as here, a definite two-peak cycle.

The characteristic shape of the cycle of *C. laticeps* in Lake Mälaren may also be explained in another way also involving a discontinuous availability of masses of infective cestode larvae. In support for this opinion is the fact that the majority of the newly infested cestodes are shorter than 5 mm both in the spring and in the autumn.

Accordingly those larvae forming the spring peak of infection are not necessarily recruited from the "cestode larvae pool" (see above), in which they may remain infective up to lengths of about 15 mm.

The incidence cycle of *C. laticeps* in the fish is naturally dependent upon the availability of infective larvae. Other factors are the specific feeding habits of the fish and the physiological resistance of the fish to infections (KENNEDY 1969 b).

Even if there is a cyclic availability of infective parasites with a pronounced minimum in late summer, this would probably not bee enough to explain the small numbers of parasites in the host at the same time.

The stomach analyses of the food of bream gave here little support for a radical dietary change with season. (This is in accordance with CRAGG-HINE, 1964 *In* KENNEDY 1969 a.) Small seasonal variations in the feeding habits of bream, are likely to occur, but such seasonal changes are not of the magnitude required to explain variations in the recruitment of *C. laticeps.*

Bream obviously continue to feed even in the coldest months in Lake Mälaren, though on a much reduced scale. BAUER (1959) cites one example of mass infections of bream in the winter from Lake Kals in Latvia. The fish continued to feed in the winter due to a relatively high water temperature (influence from hot springs).

If fingerlings of carp hibernate at relatively high temperatures (3—4°C) infection by *Caryophyllaeus* may occur throughout the winter (DOGIEL 1962). *C. laticeps* may, in the same host species, be present all the year round in one locality (KENNEDY 1970, referring to unpublished PhD.-theses), but with a well-defined seasonal cycle in another locality. ANDERSON (1974) found infected bream all the year round in a gravel pit lake in Essex, England.

The parasite was present in carp in Poland only during April through August (WUNDER 1939), in bream only in the spring (DUBININA 1949, *In* MACKIEWICS 1972) or in the spring and early summer (HALVORSEN 1972), from April to October (A. N. DERZHAVIN, *In* DOGIEL 1962), and in dace (the River Avon) only from December through July (KENNEDY 1969 b).

Another important factor is the very way bream feed on the bottom. There are plenty of examples from literature describing how bream take mouthfuls of bottom material, spit everything out again and eventually pick out digestible objects, thereby ingesting whole worms (with possible parasites). KARZINKIN (1952, *In* BACKIEL and ZAWISZA 1968) has put forward the opinion that large bream can find their food even under a 15 cm mud layer.

According to BORUTSKY (1960), PODDUBNAYA (1962), and KAJAK and WISNIEWSKI (1966) bream, carp and other fish often succeed in seizing only the posterior segments — *i.e.* the exposed parts — of tubificids. A rapid regeneration of the posterior segments of the worm, however, is supposed to help the maintenance of the population at the same level.

GRIGYALIS (1966) and NOSKOVA (1967) have demonstrated that most of the protein reserves are concentrated in the middle and posterior segments of oligochaetes. In that case the fish could actually consume great quantities of oligochaetes (posterior segments) without getting parasitized to any great extent, since the well-developed procercoids of *C. laticeps* are nearly always found in the anterior part of the worm.

The low frequency of infestation in the summer as well as the apparently continuous loss of cestodes when the water temperature rises could all be explained with changes in the physiological resistance of the fish to infection — a temperature control system (KENNEDY 1969 b). The ability of dace to respond to infection was least in the coldest months. However, with the spring rise in temperature and with the increased worm burden the "response increased in strength, thus simultaneously preventing the establishment of new infections and eliminating existing ones".

Temperature alters the physiological condition of the host, which makes it difficult for the worms to overcome or neutralize the peristaltic press tending to sweep them down the intenstine (KENNEDY 1969 b).

KENNEDY and WALKER (1969) found no evidence for an immune response of dace to infection. They did not, however, eliminate the possibility that antibodies were involved in this self-cure reaction.

Lakes in heavily industrialized and densely settled areas are often under severe pressure of eutrophication primarily due to accelerated inputs of inorganic salts. This is unfortunately the classical scheme of evolution of lakes in the civilized world and has been frequently documented by, for instance, HASLER (1947), THOMAS (1968, 1973) etc.

In Switzerland, in particular, where it was first documented, as well as in the rest of Europe, eutrophication was accompanied by the "cyprinid problem", which has been reviewed by KRIEGSMANN (1955), ROTH (1969), and others. Catches of trout, char and coregonines gradually decrease, while first perch and later cyprinids increase tremendously in abundance and gradually invade even the pelagic parts of the lakes.

Amongst cyprinids, as a rule, roach, in first hand, and bream are by far the most favoured fish and normally respond quite vigorously to a raised nutrient standard. Due to the gradual extention of areas of rooted aquatic vegetation in time with the nutrient enrichment of the lakes, suitable spawning areas for various cyprinids have largely expanded.

An increase of roach is often accompanied by an increase of bream. In Swedish waters they do not seem to compete very much. Bream and ruffe, *Gymnocephalus cernua* (L.), however, are known to compete sometimes very hard for the same food items (NIKOLSKI 1963, and MILBRINK unpublished). As a "sucker" bream is mostly bound to the bottom fauna, even if euzooplankton at times may be an important source of nourishment.

Amongst the benthos, chironomid larvae and oligochaetes are generally the most important food items for bream — even if consumption of oligochaetes is not sufficiently verified in the literature (*cf.* above).

Oligochaetes — mainly tubificids — as well as several filtering chironomid larvae are indirectly dependent upon the sedimentation of masses of dead phytoplankton that flourish in a eutrophied environment. The sedimenting algae in turn favour the various bacterial strains that tubificids in all probability feed on (BRINKHURST and JAMIESON 1971). As a rule oligochaetes and filtering chironomid larvae increase tremendously in such situations. Accordingly, both oligochaetes and bream are known to be much favoured by the enrichment of inland waters but this connection is rarely mentioned in the literature.

Bream is naturally not the only fish extensively feeding upon oligochaetes. Ruffe was mentioned above, tench, silver bream, and blue bream are others. Bottom-feeding coregonids and eel (Dr N.CAMPELL, personal information) may make extensive use of this protein reserve, at least temporarily. SÖDERSTRÖM and FREIDENFELT (unpublished material) were fully aware of the role of oligochaetes in the diet of bottom-feeding coregonids in Lake Vänern and Dr P. AASS found that tubificids were the main food items for the same fish in one locality in Lake Mjösa, Norway (MILBRINK 1973 b). By means of very careful analyses of stomachs of roach from two bodies of water in Scotland ALDOORI (1971) found that oligochaetes occurred in the food to a much greater extent than previously recorded. There are numerous Easteuropean examples on the role of oligochaetes in the diet of various fish, even salmonids (*cf.* POPCHENKO 1971 and BACKIEL and ZAWISZA 1968, 3: 36). POPCHENKO has convincingly demonstrated that not even fish but also various invertebrates prey quite vigorously upon oligochaetes.

In the present study the contents of the intestines of about 350 bream have been surveyed under a low-powered microscope. These surveys gave no indications at all that the fish had fed upon oligochaetes. Yet the parasitological results revealed that at least 50 % of the same fish had consumed tubificids not long before. Similar results on dace were presented by KENNEDY (1969 b).

Erroneous conclusions in earlier literature concerning the presumed limited role of oligochaetes in the diet of various fish could definitely be attributed to results from insufficient stomach analyses. The parasitological approach presented here is a very useful complement to ordinary stomach analyses, although it is mainly qualitative and indicative. Unfortunately it is only available for a limited number of fish species.

## V. SUMMARY

The population biology of *Caryophyllaeus laticeps* in bream was studied at Drottningholm

in Lake Mälaren over a period of three and a half years. The cycle of infection was found to have two definite peaks, one in the spring and one in the autumn. As far as the author knows, two peaks have never before been described for *C. laticeps,* only one single peak in the spring being earlier observed. Furthermore, newly infested specimens of *C. laticeps* could be found in these bream at any time of the year. Under normal conditions records of infection by the parasite in the autumn and in the winter are otherwise very few in the literature.

The literature gives ample evidence that the possibility of detecting ingested oligochaete worms in any fish is extremely limited.

A parasitological approach to the problem (KENNEDY, 1969 a, 1969 b) has given very promising indications on the real consumption of oligochaetes by dace in England. Since July 1970 bream have been caught regularly at Drottningholm for the same purpose. At the end of 1973 in all about 350 fish had been examined with regard to the cestode fauna in the intestines. Possible macroscopic details of oligochaetes visible under low magnification were recorded at the same time. Some stomachs were also analysed in detail under high magnification.

Not one single bream was ever caught containing remnants of oligochaetes visible in low magnification only. However, the cestode fauna and smears from the gut mucosa revealed that 70 % of the fish, on average, had consumed oligochaetes not long before. During the peak periods of occurrence of *C. laticeps* in the spring and in the autumn even 90—100 % of the fish had recently consumed oligochaetes.

By knowing the approximate degree of infection of the intermediate hosts in the lake — tubificids of the genus *Potamothrix* — it has been possible to estimate, although still in rather approximate figures, the "real" consumption of oligochaetes by bream. Provided the infection degree of tubificids is about 0.1 %, which is the usual figure obtained in the lake, a fish with 50 specimens of *C. laticeps* must have consumed a minimum of about 85,000 tubificids within a couple of months. If the real infection degree was even 1.0 % the fish must still have consumed no less than about 8,500 tubificids. Even if this is merely an arithmetical example there should be little doubt that tubificid oligochaetes must form a significant part in the diet of most bream. Keeping in mind the enormous quantities of oligochaetes present in any eutrophied waters it is remarkable that the connection between a protein-rich bottom fauna and a simultaneous occurrence of masses of bream and other bottom-feeding fish has been so little noticed.

## VI. ACKNOWLEDGMENTS

The author would like to thank Dr. C. R. KENNEDY, University of Exeter, England, who encouraged me to do this parasitological work and who kindly read the manuscript. Thanks are also due to Mr. GÖSTA MOLIN, who made most of the fishings for me.

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# Some Effects of Acidification on Roe of Roach, *Rutilus rutilus L.*, and Perch, *Perca fluviatilis L.* – With Special Reference to the Åvaå Lake System in Eastern Sweden

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## I. INTRODUCTION

The problem of the acidification of inland waters has long been of deep concern to fishery biologists in the Scandinavian countries. Among the first striking indications of increasing acidification are radical changes in the natural fish fauna, *e.g.* a gradual reduction in the number of fish species, a rapid decline of recruitment into the younger stages of certain species, a simultaneous increase in size of the surviving adults of those species and sudden fish kills.

The fish that represent the last steps in the food chain in the lakes are naturally much affected by all the changes in the earlier steps of this chain. Most adult fish, however, can obviously stand moderate acidification even if this implies a switch to food items otherwise not chosen (*cf.* GRAHN *et al.* 1974).

In this context there is little doubt that the most critical periods in the development of the fish are the stages that proceed from the spawning event until the hatching of the eggs, or even longer (EIFAC, Report 1969; JOHANSSON *et al.* 1971, BEAMISH *et al.* 1975).

In Scandinavia the first effects of acidification were noticed in Southern Norway already in 1915—20. Whole populations of trout could be totally wiped out from lakes and rivers more or less suddenly. This area is now the most acidified part of Scandinavia.

At the start of the 1960's the acidification of lakes and rivers near the Swedish West Coast had reached an alarmingly high level, causing profound changes in the natural fish populations of these lakes, a dramatic shift in the composition of phyto- and zooplankton slightly below pH 6.0 and a general and gradual increase in transparency in the most exposed waters. The majority of these waters like those in Southern Norway had a very low ionic concentration and low alkalinity (HULTBERG and STENSON 1970, ALMER 1972, ALMER *et al.* 1974 and GRAHN *et al.* 1974).

Several studies made by the different County Administrations and Fisheries Authorities in the western part of Sweden document the gradual extension of lakes under obvious acidification (ALMER 1972, GRAHN *et al.* 1974).

In 1972 ALMER (unpublished) found that several well-known "char lakes" in Southern Sweden (with low buffering capacities) had become more acid and the char had eventually been wiped out. However, not only the "char lakes" on the western coast, but also several in the inner, central parts if South Sweden and even some in the middle part of Sweden (Lakes Rösjöarna), in the mountain areas close to the Norwegian border, were affected by acid fallout (ANDERSSON *et al.* 1971; *cf.* DICKSON, 1975).

The eastern parts of South Sweden, however, have not been much involved in discussions concerning acidification. The reason for this is probably that lakes on the eastern coast are generally stronger buffered than those on the western coast, alkalinity is higher due to calcareous soils and nutrient enrichment from human settlements and farmland areas, the ionic contents are higher etc. (*cf.* DICKSON, 1975).

## II. MATERIAL AND METHODS

The present work is focused on the Åvaå lake system in South-Eastern Sweden, not far from Stockholm (Fig. 1), famous for its impressive

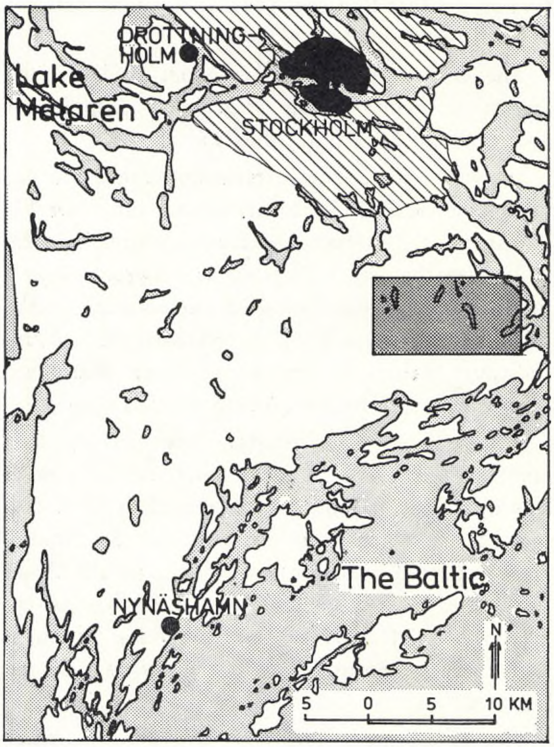
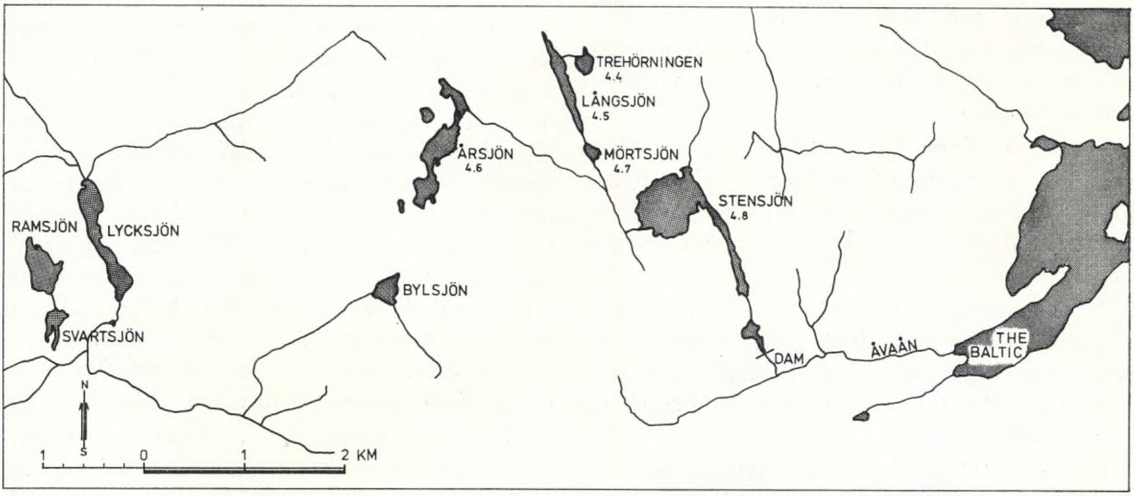



Fig. 1. Map of the Åvaå area in the vicinity of Stockholm (Fig. 1 a). Extremely low pH-values obtained in late March 1974 are given for the largest lakes of the Åvaå Lake system (Fig. 1 b). Some minor tributaries to the River Åvaån are also indicated in the figure.




population of large sea-run trout. At present this area appears to be under the influence of acid fallout, whatever the sources may be.

The sea-running trout population can only use the lower reaches of the River Åvaån for spawning since there is an old dam about 500 metres to the south of Lake Stensjön, serving as a migration obstacle (*cf.* Fig. 1).

In 1972 the County Administration and the Fisheries Authorities of Stockholm drew attention to the fact that the Åvaå area was threatened by acidification.

The lake system comprises about 10 lakes, the largest of them — Lake Stensjön — occupies an area of about 0.5 km² (*cf.* Fig. 1).

In two of these lakes, Årsjön and Trehörningen, colourimetrical pH-measurements and "secchi-transparancy" estimations were made already in the 1940's (see below). Comparisons between colourimetrical methods and modern electrometric methods have given a good agreement, the colour-indicator values seldom being more than two tenths of a unit higher (ALMER *et al.* 1974).

Standardized test fishings (with survey nets comprising vertical net sections of different mesh size combined at random) performed by M. LARSSON, the County Administration of Stockholm, were repeated twice in most lakes in the Åvaå area, the first in April 1974 and the last in October 1974 (Table 1). The data obtained have been generously put at the disposal of the authors.

Roe of roach, in late May and perch in early June, were placed in Lakes Stensjön and Trehörningen in two submerged baskets, each containing 6 chambers, each chamber in its turn containing at least 300 eggs. The baskets allowed free water passage through the chambers for proper oxygenation of the roe.

Roach roe from Lake Mälaren (though fertilized in water from Lake Stensjön) was tested in Lake Stensjön together with similar roe from the last lake, each batch tested simultaneously in 3 chambers.

In the tests of roach roe in Lake Trehörningen roe (fertilized in water from Lake Trehörningen) was taken from the nearby Lake Stensjön and from Lake Mälaren, since there are no roach in Lake Trehörningen.

One batch of roe from Lake Mälaren was allowed to develop "*in situ*" in the above manner at Drottningholm.

The same device was later used for perch roe, with the only difference that no roe was obtained from Lake Stensjön for this purpose (the time of spawning turned out to be one to two weeks earlier here than in Lake Mälaren). Accordingly perch roe from Lake Mälaren was placed both in Lake Stensjön and in Lake Trehörningen and was tested simultaneously in 3 chambers in each lake.

The field material, the actual pH-values and water temperatures were checked at least twice a week. Unfertilized or dead eggs were counted and carefully removed. The different baskets were placed in shallow water and were shadowed from possibly deleterious effects of direct sunlight. Both roach and perch generally spawn in shallow water. Accordingly the estimations of pH and water temperature were made on surface water.

Both for roe of roach and perch a fertilization degree of about 80—95 % is generally expected. (However, one batch of perch roe tested "*in vitro*" was only fertilized to about 55 %.)

For further information on the methods used in the laboratory tests of roe of roach and perch, the reader is referred to JOHANSSON and MILBRINK (1975) and to similar tests of pike roe to JOHANSSON and RUNN (in manuscript).

## III. RESULTS

The primary purpose of the present study was to get more information on the lower limits of tolerance to acidification of the most common freshwater fish of Sweden, *i.e.* perch, roach and pike, both in the field and in laboratory tests. Secondly it was considered of great interest to test whether roe from one particular acidified lake displayed greater viability through acclimatization than roe brought from more alkaline waters and thirdly to correlate the lower limits of tolerance obtained for roach and perch to the natural occurrence of these fish specifically in the Åvaå lake system (*cf.* discussion below).

The present authors know of very few studies on the effects of acidification on various fish made simultaneously (or nearly so) both "*in situ*" and "*in vitro*".

The outcome of the "*in situ*"-experiments in Lakes Stensjön, Trehörningen and Mälaren revealed very strikingly that the percentage of hatching both for roe of roach and perch was highly dependent on the pH-level (Fig. 2). As said

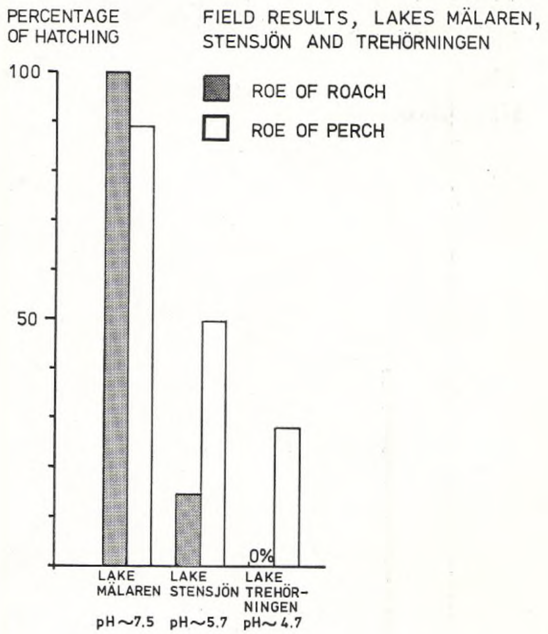



Fig. 2. The hatching percentage of roe of roach and perch in waters of different pH-level.

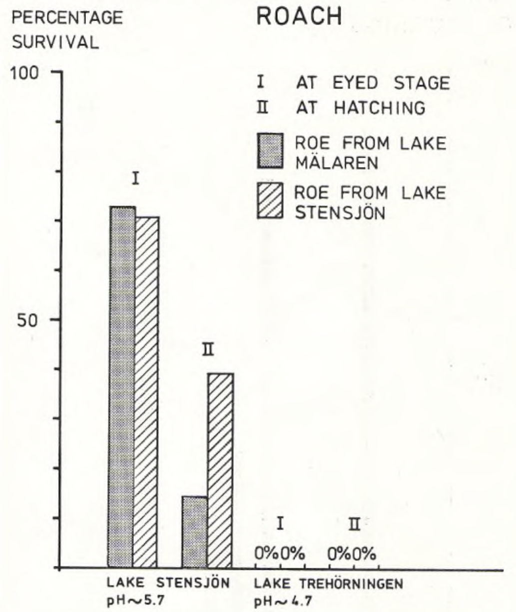



Fig. 3. Results of acclimatization-tests in Lakes Stensjön and Trehörningen with roach roe from Lakes Mälaren and Stensjön.

above the roe of roach and perch caught at Drottningholm, was divided in three batches and tested in Lakes Mälaren, Stensjön and Trehörningen. All the eggs of roach hatched at Drottningholm (pH ~7.5), about 14 % hatched in Lake Stensjön (pH~5.7), but no eggs at all hatched in Lake Trehörningen (pH~4.7). For perch the corresponding percentages were 89, 50 and 28, respectively (Fig. 2). Accordingly, about half of the perch survived till the early fry stage at a pH of about 5.7, while roach only reached a hatching success of about 14 %. Nearly 1/3 of the perch could survive till the same stage at a pH below 5, while roach did not survive at all. The results would indicate a difference in tolerance against acid water, which fits rather well into the present situation in these lakes (see below).

The perch fry that hatched in Lake Trehörningen showed no symptoms of being malformed. However, nothing could be said of the prognosis for these fry. All the perch caught in the lake were very large, which means that natural reproduction may have ceased completely (see below). Preliminary data from "*in vitro*"-experiments with roe of sea-trout from the River Dalälven, on the Swedish East Coast, would suggest that low pH-values may seriously affect the swimming capability of the fry below pH 5.0 (Johansson and Milbrink, preliminary data).

For the purpose of studying the possible effects of acclimatization roach roe from Lake Mälaren and from Lake Stensjön were simultaneously tested in Lakes Stensjön and Trehörningen (Fig. 3). It was not possible to discern any differences at all at the eyed stage of the roe in Lake Stensjön (73 % and 71 %, respectively reached this stage). However, with reservation for the limited number of times each situation was repeated, the roach roe from Lake Mälaren hatched to only about 14 % in Lake Stensjön, while indigeneous roe hatched to about 39 %. The roe did not hatch at all in Lake Trehörningen, nor did it reach the eyed stage. With regard to the great differences in specific conductivity between Lake Mälaren, about 23—29 mS/cm (25°C), and Lakes Stensjön and Trehörningen, about 4.6—5.8 mS/cm (25°C), the above differences in hatching success seem

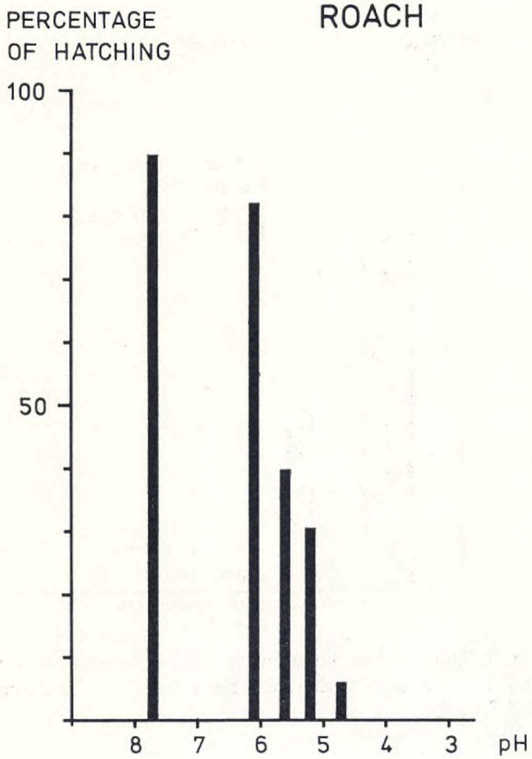



Fig. 4. The hatching percentage of roach roe from Lake Mälaren kept at different pH-levels — here given as mean values — in the laboratory.

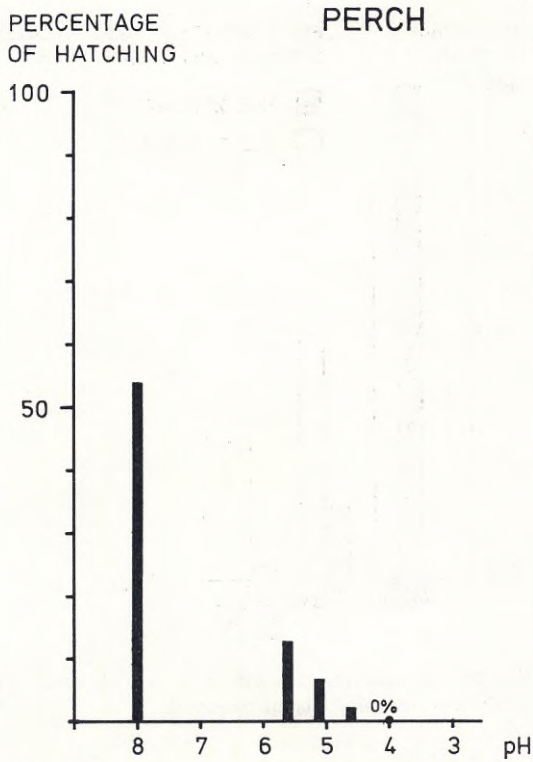



Fig. 5. The hatching percentage of perch roe from Lake Mälaren kept at different pH-levels — here given as mean values — in the laboratory.

natural. However, further study of acclimatization effects should be made in the Åvaå lakes to verify these findings.

Parallel acidification tests were also made "*in vitro*" with roe of roach and perch caught at Drottningholm. The outcome of these experiments is discussed in some detail in JOHANSSON and MILBRINK (1975) and will only be briefly commented here for comparison. The same is relevant for the "*in vitro*"-tests of pike (JOHANSSON and RUNN, in manuscript).

Batches of roach roe kept at five different pH-intervals (Fig. 4) gave few deviations from the results obtained "*in situ*" in the Åvaå lake system. One possible difference was the circa 5 % survival of the roe kept at pH 4.7—4.8, while no eggs survived in Lake Trehörningen (*cf.* Fig. 2).

A sudden drop in the viability of the roe seemed to take place slightly above pH 5.5, which is well in accordance with the results obtained in the field (*cf.* Fig. 2), and corresponds well to the actual situation in the most acid lakes of the Åvaå lake system (*cf.* Table 1).

The laboratory test with perch roe, however, gave rather inconsistent results, probably much depending upon a low degree of fertile eggs. On the whole, a very small proportion of the eggs hatched at any pH-interval (Fig. 5).

Similar tests of pike roe in a series of pH-intervals between 7.5 and 4.0 (JOHANSSON and RUNN, in manuscript) gave a very modest decline in hatching success — only about 30 % between the extremes (Fig. 6). However, all the fry below pH 4.5 were seriously malformed. They could not possibly be given any changes of survival. It is most interesting to note that about 10 % of the fry were malformed already at pH 5.1.

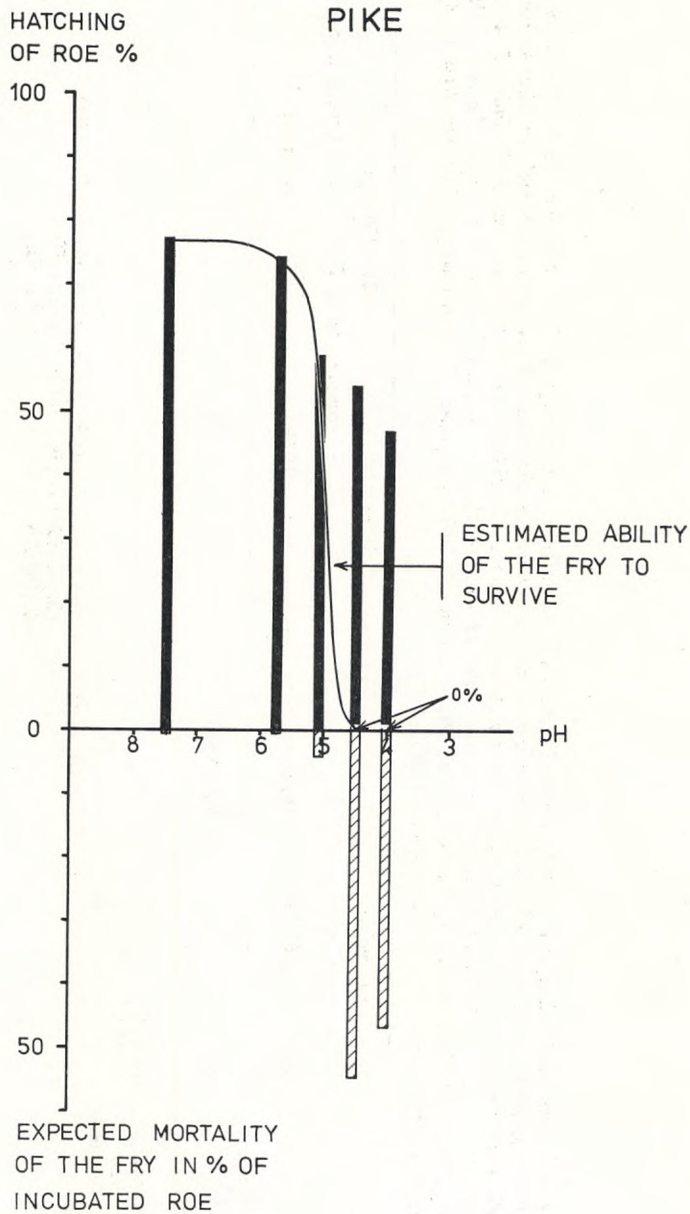



Fig. 6. The hatching percentage of pike roe from Lake Mälaren kept at different pH-levels — here given as mean values — in the laboratory. The expected mortality of the fry — due to severe malformation — is indicated in per cent of the incubated roe. The curve represents the estimated ability of the fry to survive.

## IV. DISCUSSION

One of the main reasons for choosing the Åvaå lake system for these field experiments was because the natural fish fauna in some of the lakes had undergone a well-documented change during recent decades as also had some chemical-physical properties of the water such as the pH and the transparency. These changes in the fish fauna brought in mind similar changes in acidified lakes on the Swedish West Coast (ALMER *et al.* 1974).

Lakes Trehörningen and Årsjön (Fig. 1) are particularly interesting in this context because

Table 1. *A compilation of older and recent pH-values, "secchi-transparency"-data and results from test fishings in Lakes Stensjön, Trehörningen and Årsjön in the Åvaå area at different seasons of the year. The early spring values were obtained at the time the ice started to melt in late March. Figures in the table with no indices were obtained by* M. Larsson, *the County Administration of Stockholm in 1974. Figures with the index I were obtained by the present authors in 1974, with the index II by* H.-G. Andersson, *the Fisheries Authorities of Stockholm in 1972, with the index III by* R. W. Kolbe *in 1948* (Kolbe 1950), *with the index IV by* C. Puke *from 1945 and onwards, with the index V by* Anonymus *1926* (*In* Alm 1928) *and finally with the index VI by* A. Höckenström *1926* (Höckenström 1927).

| Lakes | Season | pH-values | | "Secchi-transparency", in m | | Test fishings | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | composition of fish | | mean weight, in g | |
| | | 1974 | previous data | 1974 | previous data | 1974 | previous data | roach | perch |
| Stensjön | *early spring* (late March—early April) | 4.8 | | 5.7 (April) | | | | | |
| | *spawning time*, roach | 5.6—6.0 I | | | | | | | |
| | *spawning time*, perch (late May—early June) | 5.6—5.8 I | | | | | | | |
| | *summer* (late July) | 6.1 | | 5.7 (August) | 4.9—5.1 (Sept. 1926) VI | roach, perch pike, ruffe cisco | roach, perch pike, ruffe rudd, cisco (1927) V | 50 | 100 |
| | *autumn* (late October—November) | 5.8 | | | ~6 (1926) V | | | | |
| Trehörningen | *early spring* | 4.4 | | 2.4—2.9 | | | | | |
| | *spawning time*, roach | 5.0 I | | | | | | | |
| | *spawning time*, perch | 4.7—5.0 I | | | | | | | |
| | *summer* | 5.3 | 5.36—5.38 (1948) III | 3.0—3.6 | 1.2 (1948) III | perch, pike (extremely few) | | — | 590 (5 specimens) |
| | *autumn* | 4.2 | | | | | | | |
| Årsjön | *early spring* | 4.8 | 6.0 (1945) IV | 7.6 (1972) II 5.6 (1974) | 4.1 (1948) III | | | | |
| | *summer* | 5.5 | 5.9 (1945) IV | 6—7 | 4.3 (1945) IV | perch, pike, (very few), ruffe | roach, perch, pike, eel (1945) IV | | |
| | *autumn* | 5.1 | 6.0 (1946) IV | 6.2 (1971) | 2.9 (1946) IV | | | | |

pH- and transparency-measurements had been made in the 1940's. R. W. KOLBE studied the composition of phytoplankton — alongside with some elementary chemical-physical background data — in Lake Trehörningen in 1948 (KOLBE 1950). Lake Årsjön was one of the lakes in C. PUKE's limnological survey of lakes in the Stockholm area from 1945 and onwards (mimeographed material), and there are also some 50-year old data, mainly on the fish fauna, from Lake Stensjön (ALM 1928).

Some of the previous data from these lakes have been compiled in Table 1 together with results obtained in the last couple of years. Most of the data from 1974 were obtained by the County Administration of Stockholm (see text to Table 1).

According to available data from the 1940's the pH-value of Lake Årsjön was steadily about 6.0 from the end of March until November (C. PUKE, unpubl.). From Lake Trehörningen a summer series from 1948 estimated the pH-range to be 5.36—5.38 (KOLBE 1950). The corresponding "secchi-transparency" values were in the order of 3 to 4 metres in Lake Årsjön and about 1 metre in Lake Trehörningen.

At present (1974), Lake Årsjön is considerably more acid than about 30 years ago, the pH varying between 5 and 5.5 units and the "secchi-transparency" generally between 6 and 7 metres (Table 1). Lake Trehörningen is now one of the most acid lakes in the system, the pH varying between 4 and 5 units and the transparency has increased to about 3 metres.

There is little doubt that the pH-level now is considerably lower than in the 1940's. A general increase in transparency is another well-known effect of acidification when the composition of phytoplankton has drastically changed and a great proportion of the humates has precipitated (*cf.* ALMER *et al.* 1974, and DICKSON 1975).

The authors are not aware of early pH-data from Lake Stensjön. However, in 1926, the "secchi-transparency" was estimated at about 6 metres (ANONYMUS 1926, *In* ALM 1928), or 4.9—5.1 metres in September (HÖCKENSTRÖM 1927), which are about the depths obtained today. Minimum values of about 3.5 metres during periods of intensive development of algae, obtained in August 1926 (ANONYMUS, *In* ALM 1928), are comparable to minimum values varying between 3.2 and 3.6 metres obtained under similar conditions in the summer of 1972 (the County Administration, unpubl. material). The lake is the strongest buffered of the lakes in the system. The pH-level varied between 5.5 and 6.0 in 1974. At present, there are few indications of acidification in the lake.

The pH-values obtained in the early spring of 1974 were very low in the whole lake system (*cf.* Fig. 1 b), none of the lakes having a pH above 5.0. In early June, however, at the time of spawning for roach and perch the pH had reached 5.6—6.0 units in Lake Stensjön, which would suffice for normal reproduction (*cf.* above). On the other hand the pH in Lake Trehörningen at the time of spawning for these fish never exceeded 5.0, which would at least be critical for roe of roach. There is no roach left in the lake (*cf.* below).

The natural composition of fish species is under change — *i.e.* a reduction in the number of species — even in the moderately acid lakes of the system. (M. LARSSON, the County Administration of Stockholm, unpubl. material. The full material will be published in another context.) Brown trout and cisco (both species introduced) are still present in the lakes lying lowest in the system, above the dam (Fig. 1). Test-fishing in Lake Stensjön in the spring and autumn of 1974 revealed a diversified fish fauna (Table 1), which had not markedly changed since the 1920's (ALM 1928). The average weights of roach and perch appeared quite normal. Similar fishing in Lake Trehörningen in the spring gave only 5 perch, the mean weight being close to 600 g, and one small pike. Correspondingly high mean weights of perch have been obtained from other lakes in the system. In Lakes Långsjön and Mörtsjön (the "roach lake") roach were very few but with high mean weights. Recruitment for the species seemed to have ceased more or less. One particularly interesting piece of information in this context is that roach was apparently present in Lake Årsjön in 1945 (C. PUKE, personal information). Now, there are no roach left in the lake.

The surveys of lake systems under acidification in Western Sweden mentioned above have naturally provided guide-lines as to where on the lower part of the pH-scale certain fish species tend to disappear. Laboratory tests in Norway have confirmed field observations and have given detailed information on the pH-tolerance of salmon, sea-trout and brown trout.

Such limits, however, are very dependent upon the local conditions in the water, upon the dynamic relationships between different fish species etc. Fish may propagate even in the case of a low hatching success in one particular water but may be on the verge of extinction, despite a nearly maximal hatching success, in another water. For these reasons the lower limits of tolerance may mean different things in different waters. On the other hand such limits may be of considerable value for predictions on the fish fauna.

A scheme of the lower pH-limits of tolerance for the most common freshwater fish on the Swedish West Coast was compiled on empirical grounds by fishery consultant U. LUNDIN. Brown trout generally tended to disappear at pH 5.0, perch at pH 4.0 and pike at pH 4.3 (unpubl. material). On the basis of data from the south of Sweden and from Germany Dr B. BERZINS found the lower limits of tolerance for populations of brown trout, roach, perch and pike to be pH 5.0, 5.2, 4.2 and 5.0, respectively (unpubl. material).

The results presented in this paper mainly refer to the hatching success of roe of roach and perch, irrespective of the ability of the fry to survive. For pike, however, this ability (*i.e.* lack of ability) has been included. The following lower limits of tolerance may be suggested: for roach about pH 5.5, for perch slightly above pH 4.7 and for pike pH 5.0. These limits for roach and perch, would be slightly higher if the survival of the fry is included. Below these limits the propagation of populations of fish will be very uncertain; possible effects of local acclimatization, however, are not taken into account, see below.

Naturally there are several other factors than the mere concentrations of the $H^+$-ions, which affect the fish populations at low pH-values. Some of these factors are obviously linked more or less to the variations of pH, such as the concentrations of free $CO_2$ in the water, the degree of mobilization of some toxic ions and substances etc.

Adult fish may display avoidance reactions against water of low pH (HÖGLUND 1961). One obvious consequence of that would be that fish populations may propagate in waters that are otherwise too acid simply by reproducing in more alkaline tributaries.

Populations of fish may also become acclimatized to acid water (*cf.* above), a fact which makes it particularly hazardous to establish the lower pH-limits of tolerance for various species of fish. ANDERSSON (1972), for instance, found self-reproducing populations of brown trout in Central South Sweden at pH 4.7—4.9. All the above factors discussed in a series of EIFAC Reports must be taken into account, but so far they have not been studied to any great extent.

As mentioned earlier the fish of primary concern in the Åvaå area is the sea-running trout. The question is how resistant this stock of sea-trout is to acidification. The River Åvaån, in which the trout spawn, drains Lake Stensjön. This lake may be as acid as pH 5 in the winter and early spring (*cf.* above), when the fry of trout appear. This level could be critical for salmonid fish (*cf.* above). However, the river receives some additional enriched water via small tributaries coming from farmland areas between the dam and the river-mouth. The pH-level is probably still high enough in the river, 5.5—6.0 (the Country Administration of Stockholm, unpubl. material) not to threaten the propagation of the population of sea-trout.

The erection of a station net covering the whole of Europe for registration of the chemical composition of the air and precipitation (*In* ODÉN 1968) and another river-registering station net covering Sweden, Norway and Finland (ODÉN and AHL 1970) now makes it possible to trace the general pathways (trajectories) of air pollutants in Northern Europe and to predict the general development of different water systems with regard to acidification.

Accordingly it would be possible to calculate how many years it will take for water systems, for instance the Åvaå Lake system, to reach certain critical pH-levels.

The geographical position of the Åvaå area, close to a very densely populated and heavily industrialized area (Fig. 1) to the north-east and other possible sources of emission in other directions, naturally makes it very exposed to acid fallout. The lake system is, no doubt, in the middle of dynamic process with a successive decrease of the pH-level. The general trends of development seem obvious, even if earlier pH- and transparency-data from the area are not so many. The pH-level in the source lakes has decreased in the order of 0.5 pH-units and the "secchi-transparency" has approximately doubled since the 1940's.

On the basis of what we know of biological limits of tolerance in general and of the present lake system in particular we now have a key for evaluating both the temporal and the areal situation with regard to the proximity to the sources of acidifying emissions.

## V. SUMMARY

The lower limits of tolerance to acidification were studied for two of the most frequent freshwater fish in any Scandinavian waters, roach and perch. It has been convincingly demonstrated in the literature that the early development of most fish is particularly sensitive in this context. Accordingly, the impact of acidification was simultaneously tested on roe of roach and perch in the laboratory and in the field.

The lower limit of tolerance for roach turned out to be slightly above pH 5.5 both *in situ* and *in vitro*. Similarly the lower limit for perch was close to pH 4.7. For comparison, the same lower limit for pike — pH 5.0 — obtained in a similar way has also been included in the text (JOHANSSON and RUNN, in manuscript). The values obtained for these three fish are in good agreement with empirical results obtained in waters all over Scandinavia.

Fish may get acclimatized to acid conditions when exposed for a long time. In order to test the possible effects of acclimatization roach roe was brought from Lake Mälaren to lakes in the Åvaå Lake system to the south-east of Stockholm for comparison with indigeneous roe. With reservation for a rather limited material the indigeneous roe gave a better hatching success and thus revealed a lower limit of tolerance to acidification.

Over the last decade special attention has been paid to the acidification of waters in Western Scandinavia. We now know that even weakly buffered lakes in the eastern part of Sweden are threatened. The Åvaå Lake system, chosen for the purpose of this study, is under the obvious impact of acidification. The fish fauna is under species reduction. Roach gradually disappear from the source lakes of the system and the remaining perch, if any, increase in size due to little competition in the search for food. According to available evidence the transparency has doubled since the 1940's and pH has decreased in the order of half a unit in the source lakes of the system.

## VI. ACKNOWLEDGMENTS

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# Behaviour of Fish Influenced by Hotwater Effluents as Observed by Ultrasonic Tracking

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## I. INTRODUCTION

The effects of above-normal temperatures on fish behaviour are extremely difficult to evaluate because they are interrelated with other environmental factors. Thermal effects thus influence the physiology and metabolism at all stages of the life history of the fish including the environment in which it lives. There are no simple rules for assessing these effects and *in situ* studies are relatively few (*cf.* KRENKEL and PARKER 1969) where fish behaviour is concerned. Both from the sports and commercial fisheries as well as the scientific point of view it is essential to know how fish react to induced flows of heated water, *e.g.* from thermal power stations. The techniques available to study orientation of unconfined fish in field conditions include fisheries statistics, various sampling techniques, biochemical parameters and others (reviewed by STASKO 1971). These methods are however indirect and provide little information on the behaviour of the individual fish. For a more detailed tracking of fish underwater telemetry so far provides the most efficient system. Ultrasonic telemetry is fairly recent. It all started in late 1956 when a silver salmon was equipped with a transmitter, released in a Seattle lake and followed a little over an hour by two biologists in an open boat (JOHNSON 1971).

The technical evolution in this field in the last decade has immensely widened the range of potential study areas including the monitoring of many internal functions like heart and tail beat. Other sensors are capable of monitoring swimming depth, ambient light conditions, temperature and velocity (STANDORA 1972). Another aspect of these technical innovations pertains to the size of the transmitters, which was the limiting factor of early investigations. Today, use of small internally carried ultrasonic transmitters make possible tracking of fish down to a size of approx. 25 cm total length.

The applied studies have usually been on open-water orientation (*e.g.* HASLER *et al.* 1969, GREER WALKER *et al.* 1971, MADISON *et al.* 1972) but the phenomenon of homing has also been investigated (*e.g.* MCCLEAVE and HORRALL 1970, PODDUBNYI 1971, MCCLEAVE and LABAR 1972). Other applications have had a more practical goal, for instance the study by LEGGETT and JONES (1971) on net avoidance behaviour in American shad. These authors reported how shad moved to within 1—2 m of the net before sensing its presence and since net avoidances were observed also when light conditions were inadequate for visual detection it was concluded that other senses may function when sight is impaired.

The objective of the present study was to test the feasibility of using ultrasonic telemetry for studying the behaviour of selected species of fish confronted by the hotwater plumes of thermal power stations. It was also hoped that these studies would add to the knowledge of recreational and commercial fishermen how and when the fishing should be operated, and possibly also help evaluate effects on the fish fauna when chosing future sites for thermal power stations. To the knowledge of the present author this paper is the first presentation of such an application of the underwater telemetry technology.

## II. MATERIALS AND METHODS

Eight silver eels (*Anguilla anguilla* (L.)), 2 yellow eels (*A. anguilla*), 5 brown trout (*Salmo trutta* L.)

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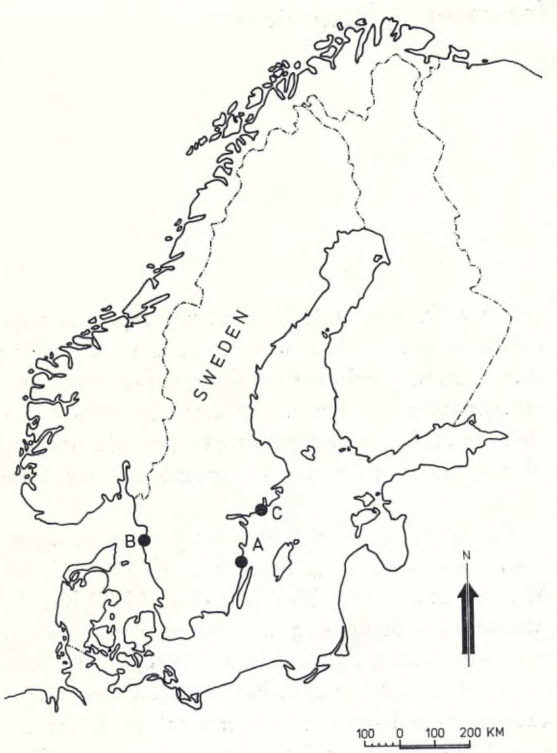


Fig. 1. Location of the three areas where the telemetry investigations were performed. Further details of this and the following figures are given in the text.

and 3 ide (*Leuciscus idus* (L.)) were equipped with ultrasonic transmitters in 1972 and 1973 and tracked at three localities on the Swedish coast (Fig. 1). Two of the sites were adjacent to hot-water effluents, one (A) being in an area influenced by the discharge from a nuclear power station, the other (B) being in the vicinity of the biggest fossil fuel operated thermal station in Sweden. The third area (C) is situated in the southern part of the archipelago of Stockholm, in the vicinity of the Askö Laboratory (University of Stockholm).

The silver eels were obtained locally from eel traps operated by commercial fishermen (at Askö and north of Oskarshamn) and so were the yellow eels (Stenungsund). Four of the trout were also obtained from fish traps, the fifth being caught by angling in the mouth of a little stream half a mile north of the point of release. The three ide were caught when migrating upstream in the fish trap barring the outlet of the Bay of Hamnefjärden (recipient of the hotwater discharge from the nuclear power station). Further details on the fish are given in conjunction with the Results section.

The smallest commercially available ultrasonic transmitter for tracking fish was used. This make is based on the description published by Henderson *et al.* (1966) and modified by Gardella *et al.* (1972). The final product, which is manufactured by Chipman Instruments (641 Charles Lane, Madison, WI 53711, U.S.A.) is a modification of the units referred to above. These tags which may be obtained without or with a thermistor incorporated (for temperature determination) measure 0.9 by 3.0 cm and have a useful life of approx. 5 days with a pulsed output signal. The range is from 300—800 m using Smith-Root receiving equipment in fresh water. The frequency has a range from 75 to 3 KHZ (upon request). The receiving equipment consisted of a Smith-Root SR-70-H hydrophone and the TA-25 sonic receiver provided by the same company.

The hydrophone is unidirectional and handheld and its sensitivity of directivity increases with the distance from the transmitter. Its frequency range is from 25—88 KHZ, with an optimum at 74. The beam pattern is conical — 8 degrees at 74 KHZ, and the weight is approx. 1.5 kilos. The hydrophone was used in conjunction with the TA-25 sonic receiver which is battery operated and portable (weight approx. 1.25 kilos) for use from a boat or in the field. The battery life (rechargeable) is good for some 10 hours of continuous tracking. The receiver is tuneable in frequency within the range 25—80 KHZ. The built in speaker is adequate for most tracking application but in a high background noise environment earphones should be used (a plug is provided on the front panel).

There are two ways of applying a sonic tag to a fish, viz. internally or externally. The principal criteria to determine the merits of the two methods were discussed by Koo and Wilson (1972). Jones and Leggett (in Koo and Wilson 1972) found that adult shad with sonic tags attached under their dorsal fins made atypical downstream movements, while shad with internally carried tags continued their upstream migration shortly after release. Preliminary studies with a perch off

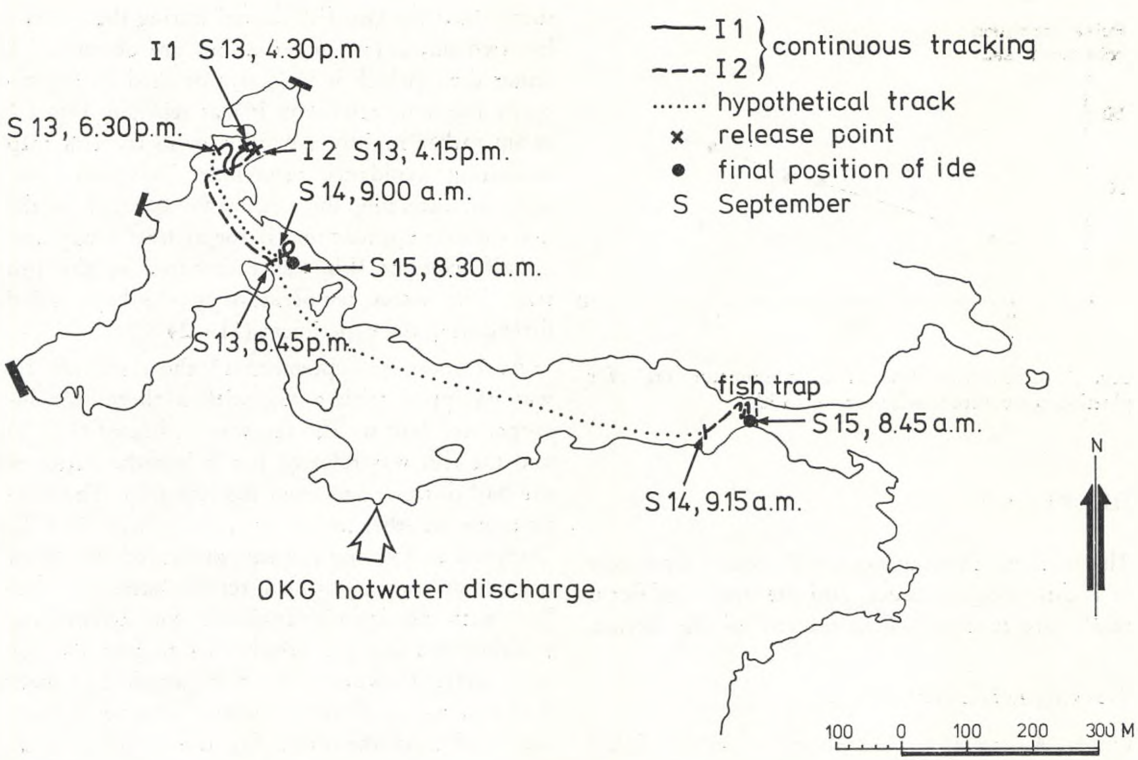


Fig. 2. Tracks of two ide released in the Hamnefjärden Bay, recipient of the hotwater discharge from Oskarshamn nuclear power station.

the Askö Laboratory (Nyman 1973) indicated that the thickness of a fish's body had no noticeable effect on the reception distance. In holding experiments at the Institute of Freshwater Research, Drottningholm, the retention time of tags in stomachs of brown trout, perch and eel was estimated. Few tags were shed in the first week indicating that sonic transmitters may be retained for at least a period of time as long as the useful life of a transmitter battery. However, tag shedding occurred in two yellow eels after three days only. No atypical behaviour or irritation from the tags was recorded, and consequently, stomach tags were used in all subsequent tracking operations.

All fish were anesthetized with MS 222 prior to inserting the tag through the mouth and they were allowed to recover for at least half an hour before release.

The directional sensitivity of the hydrophone made possible determination of the position of the fish by employing triangulation using prominent shore features as reference points. Fish were tracked either continuously or at 5 to 15 min intervals, the method used depending on the number of active fish tracked simultaneously. After fish were released the tracking equipment remained at the release point until the signal became weak indicating the fish was leaving. This procedure decreased the possibility of disturbing the fish.

The tags with thermistors were calibrated at a minimum of three different temperatures in water sampled where the fish were to be released. Since the repetition rate of the output signal was counted by ear only, there is a somewhat greater accuracy of the temperature data obtained at low temperatures (slow repetition rate). To compensate for this discrepancy all temperature recordings in "hot water" (above 15 degrees centigrade) were based on averages from three counts.

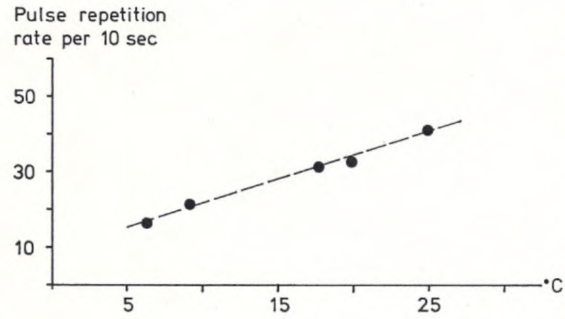


Fig. 3. Calibration line of a "thermistor tag" for plotting temperature selection of an ide.

## III. RESULTS

The tracking experiments are described separately in a chronological order, and the most significant results are summarized at the end of this section.

### *Tracking experiment 1*

On 16 August 1972 a small silver eel (0.6 kilos) with a standard tag was released off the Askö Laboratory. The eel (E 1) was tracked from a large speedboat. No activity was recorded in the first forty minutes, after which contact with the fish was interrupted. After another hour and a half contact was again established and the eel was tracked for a half hour heading southeast. It had covered some 2 kilometres from the point of release. Two months later the eel was recovered by an eel fisherman in Denmark, a distance, following the shortest way, of approx. 600 kilometres. Obviously, the tag had not influenced the migratory behaviour of the fish.

### *Tracking experiment 2*

Three ide were obtained in the fish trap blocking the outlet of the Hamnefjärden Bay on 13 september 1972. The fish were 28.0, 29.0 and 29.5 cm in total length, respectively. Two were tagged by finclipping and were fed with transmitters and released at 4 p.m. the same day. Fish I 1 had a standard tag transmitting at an optimal frequency of 54 KHZ, fish I 2 was also equipped with a standard tag transmitting at 72 KHZ. Fig. 2 shows how the two fish moved during the following two days. Two observations are obvious. I 1 either died (which is unlikely) or shed its tag already the same afternoon it was released. Fish I 2 swam rather rapidly downstream to the fish trap exhibiting avoidance behaviour. This fish, possibly on detecting the net, swam parallel to the net without approaching it for at least a day and a half. Neither fish was recaptured in the fish trap. The water temperature in the bay varied little during the experiment (21—24°C).

At 7 p.m. on September 13 the third ide I 3 was equipped with a tag with a thermistor incorporated into it. The tag was calibrated (Fig. 3) and the fish was released just below the outlet of the bay downstream from the fish trap. The temperature at the point of release was 21.6°C. Darkness and strong currents prevented the use of a boat for tracking, which, on this exposed shoreline with no islands available for determining position, reduced the possibilities to plot the fish accurately. However, the fish which had been acklimatized in 23 degree water for several hours remained near the shore for five minutes, after which it rapidly moved out to the centre of the current, which gave a count of 37/10 sec, consequently 22°C. The ide swam upstream and moved to within some ten metres of the trap. The pulse frequency varied between 36 and 42 indicating that the fish chose a narrow range of temperature. At $7^{50}$ p.m. tracking was discontinued. The following day no contact with the fish could be established, and because it was tagged it may be confirmed that the ide never entered the trap.

### *Tracking experiment 3*

On March 5 1973 two silver eels with standard transmitters were released in the northwest part of the Hamnefjärden Bay. No hotwater had been discharged for some time and whereas the marginal areas of the bay still contained some heat (5—6°C) the central part of the bay had water temperatures in the 2 to 3 degrees range due to the circulation of cold bottom water. At the release point the surface temperature was 6.5, the bottom temperature 5.2°C. One eel (E2) burrowed itself in the muddy sea-bed at a depth of 2 metres, the other (E3) swam close to the bottom a short

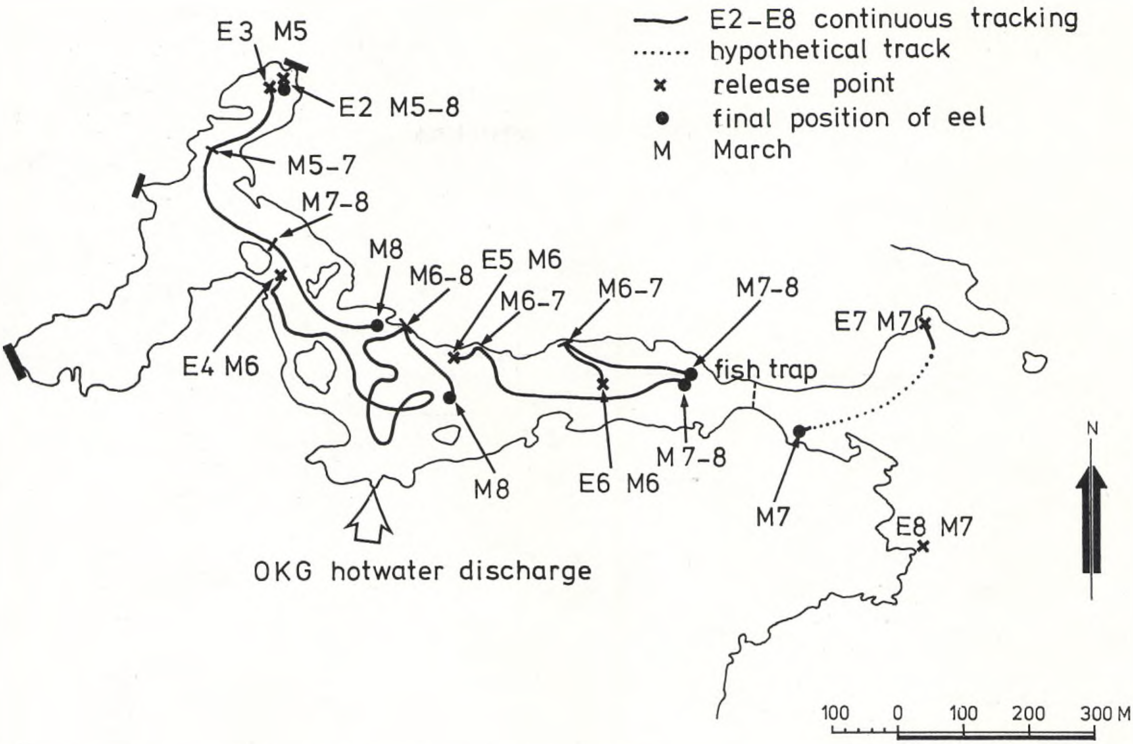


Fig. 4. Tracks of seven silver eels released in the Hamnefjärden Bay.

distance and hid beneath a rock (Fig. 4). No further activity was evidenced during that day, and the following day, with no noticeable change in temperature, both remained in the same place as before. On March 6 three more eels (two with thermistors and one with a standard transmitter) were released. The points of release are indicated in Fig. 4: E4 in a narrow straight northwest of the outlet of the water discharge, E5 roughly 200 metres downstream from the outlet and E6 200 metres upstream of the fish trap, in the middle of the current. All three took cover beneath big rocks near the shore, E4 after swimming around for three hours.

On March 7 the power station started operating and the temperature of the effluent water rose gradually. When the temperature had risen to 8°C eel No. E5 moved with the current and stopped roughly 100 metres upstream of the fish trap where it concealed itself beneath some rocks. At 3 p.m. that day E6 also swam downstream and stopped at the same place as E5. Not until the following day, March 8, when the temperature had reached 9.9°C, E4 started to move and it swam into the central part of the plume where the temperature was 12.6°C. Neither E2 nor E3 had moved on March 7 and the temperature in that part of the bay was still decreasing (5.1 at the surface). At 9 p.m. the trend of falling temperatures was reversed and the temperature reached 7°C at the position where E3 was hiding. E3 moved some 150 metres in the direction of the hotwater effluent, *i.e.* towards a rising temperature gradient, and it settled on the bottom at a temperature of 9.5°C. Four hours later (1 a.m. March 8) it moved another 200 metres (10.5°C). In the afternoon of March 8 when the trackings were terminated four eels out of five thus had become activated by a rising temperature gradient, whereas the fifth still remained in the same place as where it was released.

On March 7 two more eels (both with thermistors) were released on the coast, 250 metres on either side of the outlet of the Hamnefjärden Bay.

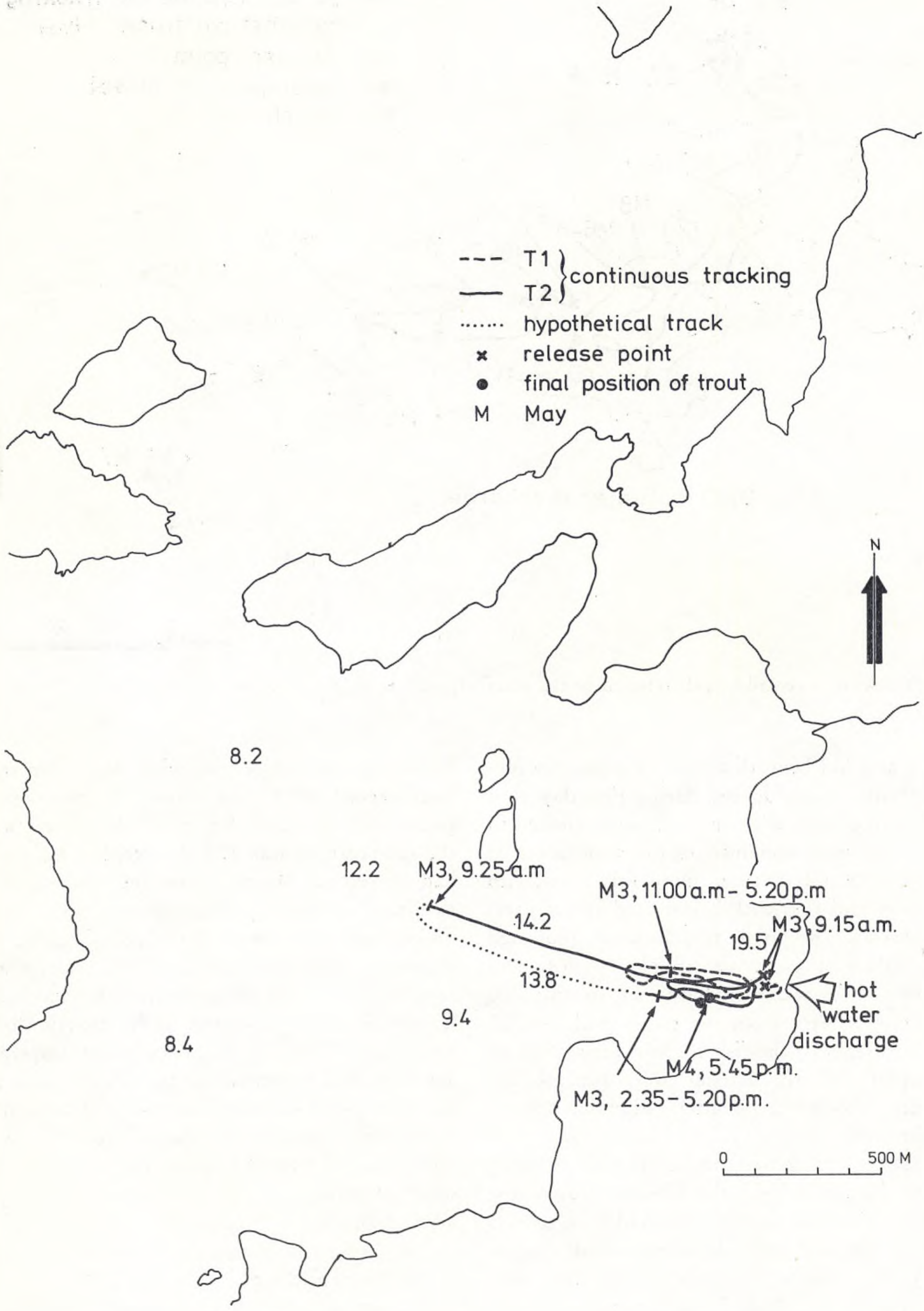


Fig. 5. Tracks of two sea-run brown trout off the Stenungsund power station.

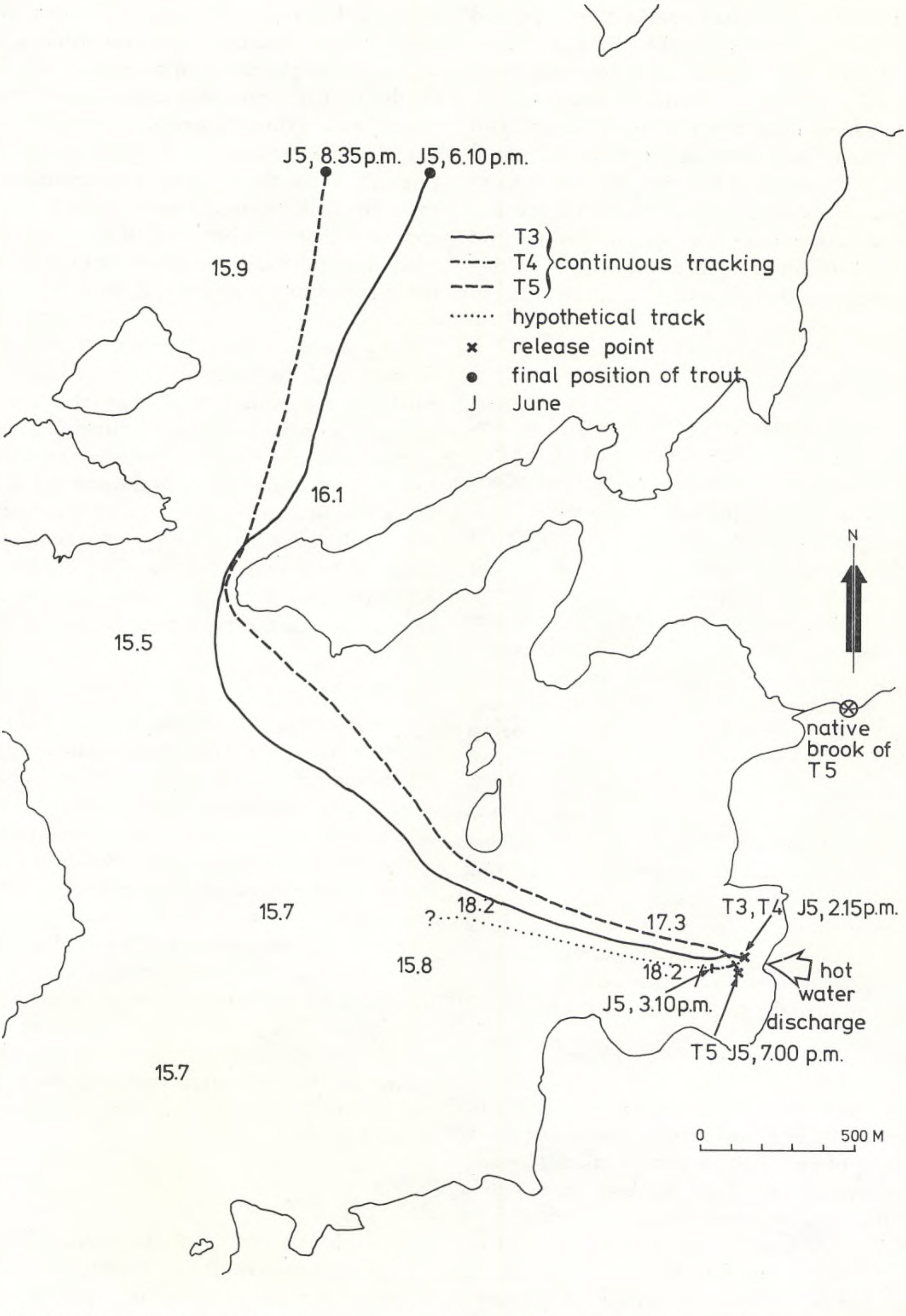


Fig. 6. Tracks of two sea-run and one freshwater-caught brown trout off the Stenungsund power station.

The surface temperature 100 metres downstream from the outlet of the bay was 8.4°C as compared to 3.3 and 3.1, respectively, where E7 and E8 were released. E7 swam rapidly away from the shore and within a couple of minutes no signal was received. E8 remained where it was released. Two hours later E7 was found again and it had moved to within 50 metres of the trap and hid close to the shore at a temperature of 9.2°C. E8 had disappeared in the meantime, and neither eel was discovered the following day despite the fact that the temperature had risen to 10.7 at the outlet of the bay.

*Tracking experiment 4*

Two sea-run brown trout, 44 and 37 cm total length, were released below the outlet of the hotwater effluent from Stenungsund thermal power station. The tracks of the fish are plotted in Fig. 5. A few temperature recordings are plotted in the map to indicate the approx. area influenced by the hotwater. All temperature observations, except for those from the transmitters, refer to surface conditions. One trout (T1) with a thermistor swam directly up to the hottest part of the discharge, the other fish (T2) with a standard transmitter rapidly left the heated area and contact was lost after 10 minutes. An hour later both trout were swimming in the most heated part of the discharge canal, mostly in temperatures of 18—19°C. The temperature of the hotwater plume varied from 20.5 to 12.8 degrees, whereas ambient temperatures in the surrounding bays averaged roughly 8 degrees. The fish were released on May 3 1973, and the following afternoon both were still present. Several other trout were rising to the surface in the heated area.

*Tracking experiment 5*

Three brown trout (40, 32 and 31 cm respectively) were released in heated effluent water just below the outlet of Stenungsund power station (*cf.* tracking experiment 4). Two of them (one with a thermistor, one with a standard transmitter) were released at $2^{15}$ p.m. on June 5 1973. Ambient sea surface temperature was in the 15—16 degree range, and the water in the effluent had a maximum at 22.3°C. Both these trout were caught in the same trap as those mentioned in the preceding experiment, namely 10 kilometres south of the power station. The trout remained within a small section of the plume for an hour, after which one of them (T3) swam downstream and left the heated area. Three hours later it had moved roughly 4 kilometres in a northerly direction (Fig. 6). When the tracking was terminated the trout swam in 16-degree water with a speed of approx. 4 kilometres per hour. In the meantime the other trout (T4) had also left the area and neither fish returned to the area during the following two days.

Early the same day another trout was caught on hook and line in a small stream half a mile north of the outlet. At 7 p.m. this fish was equipped with a standard transmitter and released in the same place as the others. It immediately left the heated area (T5), swam past the shallow bay where its native brook emptied and continued due north the same way as that taken by T3. After an hour and a half it had moved some 4 kilometres and the tracking was terminated. No other trout were observed in the heated area.

*Tracking experiment 6*

On June 6 two yellow eels (E9 and E10) were to be released at Stenungsund power station, but cleaning of the cooling system had caused a pronounced discolouration (orange red) of the water, and under these atypical conditions the experiment was postponed. The following day at 8 a.m. the water was clear again and the two eels (66 and 67 cm), one with thermistor, one with a standard tag, were released at 9 a.m. (Fig. 7). The temperature at the point of releases was 19°C. At $12^{30}$ p.m. the entire heated area was clear and the temperature had risen to 24 in the most heated part of the plume. Both eels moved to within 10 metres of the iron screens blocking the entrance to the outlet and remained there also during the following day.

*Summary of results*

1) Eels, brown trout and ide tagged with stomach transmitters do not display any atypical behaviour and tag shedding appears a minor problem.

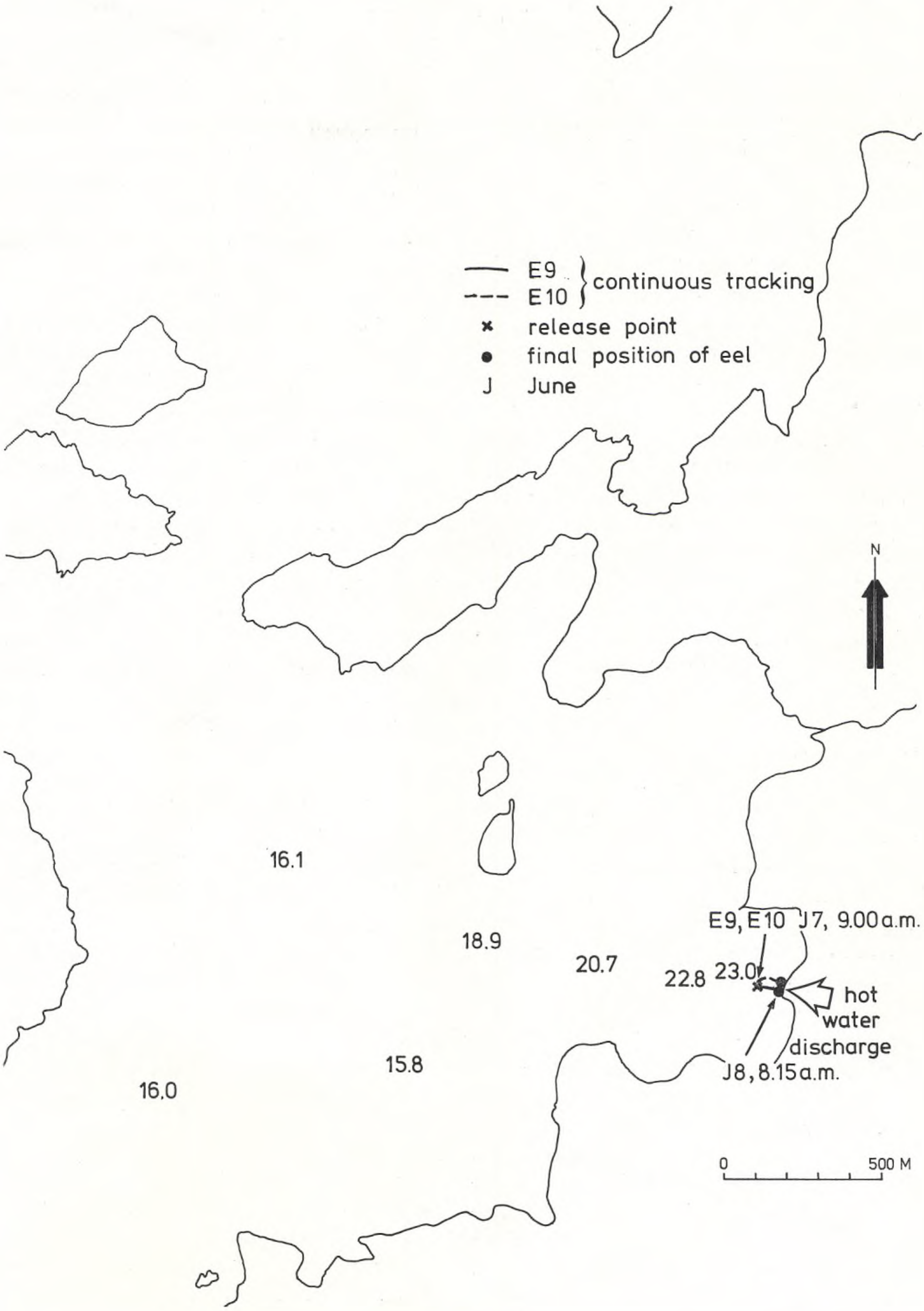


Fig. 7. Tracks of two yellow eel attracted by the hot water discharged from the Stenungsund power station.

2) Ide appear attracted by hot water (21—24°C) but at least in the autumn the attraction is limited in duration, causing an avoidance reaction, possibly after less than a day.
3) Ide display net avoidance behaviour both at day and night, and that reaction is obviously stronger than the motivation to leave an unfavourable environment (from a temperature point of view).
4) Brown trout (sea-run) are attracted by heated effluents in the winter, and select temperatures in the 18—19 degree range.
5) Brown trout avoid elevated temperatures in the summer when ambient sea temperatures have risen to 15—16°C.
6) Yellow eels are attracted by heated effluents in the summer (at least up to 24°C).
7) Silver eels become activated by a rising temperature gradient in the 7—10 degree range in the winter, and when confronted by a hot-water plume with a temperature exceeding 10°C show a rather strong tendency to remain in the heated area. At temperatures lower than 10°C attraction appears weak.

## IV. DISCUSSION

Field observations tend to confirm that fish escape lethal temperatures near heated effluent outfalls, a conclusion which is supported by results obtained in temperature gradients (*e.g.* ALABASTER 1969).

The temperature increases over ambient that prevail at Oskarshamn nuclear power station and at the thermal power station at Stenungsund rarely if ever reach the level at which they produce conditions which are lethal to fish. This fact of course depends on the ability of at least adult fish to escape a sharp temperature gradient by swimming away from the area. However, the heated plume may cause marked differences in the composition of the species present, not only by attracting species with high temperature optima but also by favouring species which are capable of withstanding great temperature fluctuations, and even by altering the genetic composition of certain species (NYMAN 1975). Apart from these distributional changes the spawning grounds of coldwater adapted fish may be destroyed, and in that respect a heated plume may have considerable adverse effect.

If we look at the problem from a commercial point of view the situation is more serious. Even subtle changes in temperature are capable of either attracting or repelling fish, and this problem may be particularly difficult where migratory species are concerned. Heated effluent outfalls in rivers, sounds or on an exposed coast thus may disturb a migratory pattern, and affect the local fishery, both the commercial and recreational.

On the southeast and west coasts of Sweden two migratory fishes account for a big part of the near-shore fishery, the silver and yellow eels on the commercial side, and the sea-run brown trout for the sports fishery. From this investigation it is clear that sea-run trout are attracted by the heated water in winter, and this observation is substantiated by information from local fishermen and divers also. It is equally evident that no mass attraction will occur because of the trout's predatory habits and strong territorial behaviour. Consequently, sea-run brown trout are likely to be repelled by heated effluents when ambient sea temperatures exceed 15°C, but attracted in the winter. It is unlikely that hotwater outfalls will cause any serious damage to trout migrations when the effluent water is discharged in an exposed marine environment. Discharges into rivers or restricted freshwater basins may, on the other hand, have detrimental effect on a trout population for obvious reasons.

LARSEN (1970) showed that there exists a direct positive relationship between the water temperature and the density of yellow eel populations. This information was also confirmed by NYMAN (1972) who examined the influence of temperature on feeding and behaviour in young yellow eels. These studies showed how extremely sensitive the yellow eel stage is to changes in temperature. Not only could a general increase in activity be attributed to a temperature increase, but feeding, swimming behaviour and territoriality seemed largely guided by the temperature and the time alloted for acklimatization. Bearing these data in mind it is hardly difficult to realize the fact that the yellow eel is both activated and attracted by a hotwater effluent. This observation is also confirmed by divers at Stenungsund who noted numerous eels in the heated area. Obviously, the only thing limiting the density of the attracted eel is the pronounced territoriality, and in the long run, the availability of prey. The silver eel phase is evidently less vulnerable to attraction, and even though silver eels become activated and attracted by a rising temperature gradient in the 7—10 degree range in the winter, there is no evidence to support a long-term attraction. In late summer dwindling silver eel catches off Oskarshamn power plant actually indicate that migrating eels are repelled by the heated plume (K.-E. BERNTSSON, personal communication).

To summarize, eels are activated and attracted by hotwater effluents in winter, yellow eel in summer as well.

The ide, being a cyprinid fish, is considered to belong to the warmwater fauna, relatively speaking. Thus it is not surprising that it is attracted by hotwater effluents. Actually, cyprinid species account for the bulk of fish entering the heated Hamnefjärden Bay on the east coast of Sweden, namely roach, bleak, white bream and ide (E. NEUMAN, personal communication). The attraction of the ide appears of short duration, which may be due in part to net avoidance. Shortage of food organisms may however also influence this reaction which may explain the slight decrease in the number of fish entering the bay after the heated effluents have been discharged into it.

Thus it may be concluded that the technique of underwater telemetry employing stomach ultrasonic transmitters allows a direct approach of working on-site and using local fish when studying the effects of elevated temperatures on fish behaviour.

## V. SUMMARY

To observe the influence of heated discharges from thermal power stations on fish behaviour ultrasonic tracking was employed.

Ide (*Leuciscus idus* (L.)) and silver eel (*Anguilla anguilla* (L.)) were studied off the nuclear power station at Oskarshamn (OKG) on the Swedish east coast, sea-run brown trout (*Salmo trutta* L.) and yellow eel were studied off Stenungsund thermal power station on the west coast of Sweden.

The tags were manufactured by CHIPMAN Instruments (Madison, Wisconsin U.S.A.) and were both the standard type and those with thermistors incorporated for temperature determinations. The hydrophone/sonic receiver employed were of SMITH-ROOT make, namely SR-70-H/TA-25.

The three species do not display any atypical behaviour as a result of carrying stomach tags and tag shedding is not a great problem. Ide are attracted by hot water, at least in the autumn, and the duration of the attraction appears short. Net avoidance was also observed. Yellow eels are attracted by heated discharges in summer, and although silver eel were activated and attracted in winter at 7—10°C by a rising temperature gradient, there are indications that they are repelled in the late summer and autumn which is their principal migration period.

Sea-run brown trout are attracted by hot water when ambient sea temperatures do not exceed 15—16°C. In summer they avoid the heated area.

The direct approach of underwater telemetry to study the effects of elevated temperatures on fish behaviour has many advantages, including the fact that fish appear uninfluenced both by the internal tags and the tracking system.

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# Allelic Selection in a Fish (*Gymnocephalus cernua* (L.)) Subjected to Hotwater Effluents

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## I. INTRODUCTION

Hotwater effluents of thermal power stations often cause pronounced alterations of migratory behaviour of fishes (*e.g.* reviewed in KRENKEL and PARKER 1969, NYMAN 1971). Most data behind these statements emanate from catch statistics, visual observations from skin and scuba diving and, finally, from studies on the behaviour of individual fish by employing underwater telemetry techniques (*cf.* Underwater Telemetry Newsletter — Fisheries Research Board of Canada and Huntsman Marine Laboratory, S:t Andrews, New Brunswick, Canada). The very problem of explaining patterns and mechanisms in migration at the population level has, however, not been attempted.

Genetic variability of various proteins in natural populations of fishes has proven a valuable tool for the estimation of population structure and interbreeding (*e.g.* reviewed by DE LIGNY 1969). In fact biochemical variants have been used in the last decade primarily to identify populations and areas of origin of marine fish (SICK 1965, MØLLER 1966, FUJINO and KANG 1968, HODGINS *et al.* 1969, and many others). However, little attention has been paid to evaluating the function of such polymorphisms, until KOEHN and RASMUSSEN (1967) described a one-locus, two-allele serum esterase polymorphism in populations of *Catostomus clarki*. These authors reported a clinal distribution of allelic frequency which was linearly correlated with latitude, and two years later (KOEHN 1969) it was shown that the differential selection of the two alleles at this locus could be attributed to a difference in enzymatic capacity at various temperatures of the alleles. In 1971 (NYMAN and SHAW) a similar clinal distribution of serum esterase alleles in *Salvelinus alpinus* was reported, and there was strong evidence that the same functional mechanism existed in this case also. The latter finding proved useful in identifying sympatric populations and species within the Arctic char species complex, and besides made possible detection of introgression (NYMAN 1972, NYMAN and FILIPSSON 1972). Further evidence of fish esterase function was provided by KOEHN *et al.* (1971). Thus there is a probability that at least certain esterases display a variability in molecular function which may be related to the need to cope with a wide range of temperatures. For the reasons stated above it seemed obvious that a combination of several populations — identifiable on the basis of significant gene frequency differencies — and some sort of temperature-dependent enzyme locus would provide a means of evaluating differences in migratory patterns induced by a flow of hotwater.

This report is meant to analyse what implications the hotwater effluents of a nuclear power plant may have on the migratory patterns and genetic transformation of local ruff (*Gymnocephalus cernua* (L.)) populations.

## II. DESCRIPTION OF THE STUDY AREA

Oskarshamn Nuclear Power Station (OKG) is situated on Sweden's Baltic coast, roughly on the same latitude as the northernmost tip of the island of Öland. Its power output in 1973 was approximately 400 MW. The cooling water discharge was some 20 cu.m./sec with a temperature increase over ambient of some 10 degrees centigrade. The

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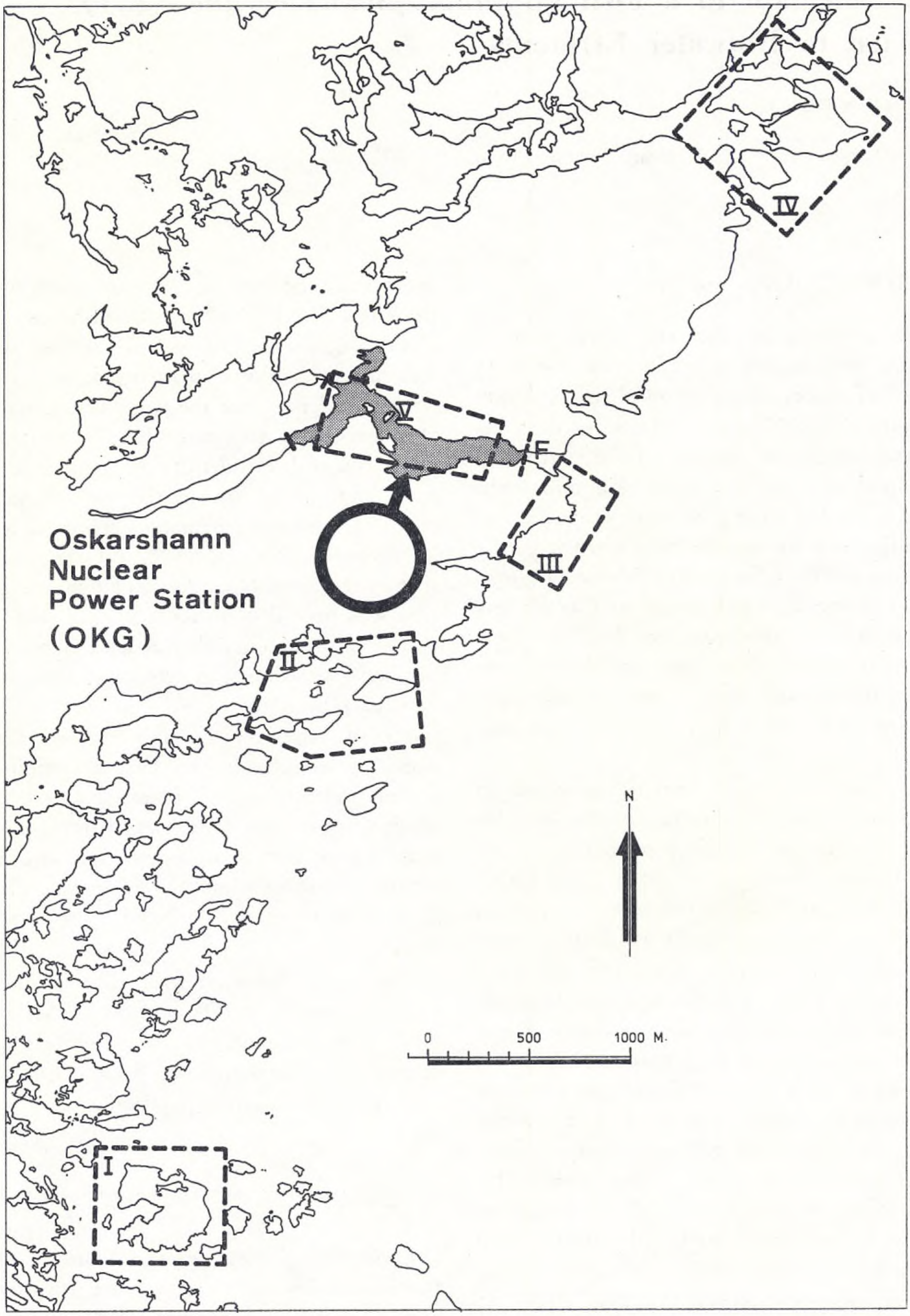


Fig. 1. The location of the six sampling stations mentioned in the text. The stippled area delimits Hamnefjärden Bay, the circle shows the site of Oskarshamn Nuclear Power Station (OKG) and the arrow indicates where the hot-water discharge from the plant enters the bay.

first unit started operating in February 1972, but initial technical problems caused a delay and it was not until September the same year that regular operations commenced. The location of the plant is shown in Fig. 1. The hotwater discharge empties in a shallow bay, which is connected to the Baltic by a narrow straight. This passage was blocked during the period of the investigation by a fence which permitted catching and recording of practically all upstream and downstream migrating fish. The counting fence was operated by employees of the National Swedish Environment Protection Board.

The fish fauna of the near-shore region in this part of the Baltic is characterized by a mixture of freshwater and marine forms, and the low salinities of this brackish basin (0.70 per cent) implies a pronounced "salt stress" to the organisms. Pike (*Esox lucius* L.), perch (*Perca fluviatilis* L.), roach (*Rutilus rutilus* L.), white bream (*Blicca bjoerkna* (L.)), ruff (*Gymnocephalus cernua* (L.)) and bleak (*Alburnus alburnus* (L.)), all of them freshwater forms, dominate the near-shore region, with flounders, sculpins, herring and cod contributing on the saltwater side. Yellow eels occur along the entire coast and migrating silver eels account for the bulk of the local commercial fishery. Atlantic salmon and sea-run brown trout occasionally contribute also.

## III. MATERIALS AND METHODS

Eight localities were sampled for ruff. Two of them (W and E) were on Lake Mälaren, west of Stockholm, the other six are shown in Fig. 1. Table 1 displays the total number of ruff analysed.

Five population samples of white bream, representing a total of 512 individual fishes were also included (Table 2).

Most fish were obtained either by nets, by traps at the counting fence or by dynamite (in the Hamnefjärden Bay only).

All fish were frozen as soon as possible after being taken ashore and kept at roughly -15 degrees centigrade until analysed. Later they were thawed, and haemolyzed blood was obtained by severing the heart. A detailed description of methods for

Table 1. *Total number of ruff analysed.*

| Location and year sampled | | Number of fish |
|---|---|---|
| W | (western part of Lake Mälaren), 1971 | 49 |
| E | (eastern part of Lake Mälaren), 1967, 1970 | 180 |
| F | (counting fence at OKG), 1972, 1973 | 133 |
| I | (see Fig. 1), 1972, 1973 | 33 |
| II | (see Fig. 1), 1972, 1973 | 82 |
| III | (see Fig. 1), 1972, 1973 | 24 |
| IV | (see Fig. 1), 1972, 1973 | 44 |
| V | (see Fig. 1), 1972, 1973 | 327 |
| Total | | 877 |

Table 2. *Total number of white bream analysed.*

| Location and year sampled | | Number of fish |
|---|---|---|
| F | (see Fig. 1), 1972 | 115 |
| I | (see Fig. 1), 1972 | 100 |
| II | (see Fig. 1), 1972 | 65 |
| IV | (see Fig. 1), 1972 | 195 |
| V | (see Fig. 1), 1972 | 37 |
| Total | | 512 |

obtaining fresh and haemolyzed blood samples has been previously given (Nyman 1967).

Horizontal electrophoresis in starch gel was used to resolve the various allelic forms of esterase. Gels consisted of 11 % hydrolyzed starch in a total volume of 300 ml of the gel buffer described by Ashton and Braden (1961).

During electrophoresis, gels were cooled by tap water (6—10°C), and after electrophoresis the gel was sliced horizontally, each slab being incubated in 100 ml of a 0.02 M solution of sodium phosphate (pH 7.0) containing α-naphthyl acetate and Fast Red TR salt for visualizing esterase activity. At least 45 individual blood samples were analysed simultaneously.

## IV. RESULTS

The serum esterase polymorphism present in ruff is previously reported (Nyman 1965). Too few individuals of ruff then prevented tests of the hypothesis of a genetic mechanism involving two codominant autosomal alleles at a single locus, but

Table 3. *Distribution of esterase phenotypes in samples of ruff from eight localities.*

| Fish origin | Phenotype F | Phenotype FS | Phenotype S | Total | f(F) gene freq. | 95 % conf. int. |
|---|---|---|---|---|---|---|
| 1) E — Lake Mälaren 1967, 1970 | 38 (35.8) [1] | 88 (91.1) | 59 (58.0) | 185 | 0.44 | 0.49—0.39 |
| W — Lake Mälaren 1971 | 13 (12.7) | 24 (24.5) | 12 (11.8) | 49 | 0.51 | 0.61—0.41 |
| 2) F — counting fence 1972, immigrating fish | | | | | | |
| hotwater | 12 (11.3) | 2 (3.4) | 1 (0.3) | 15 | 0.87 | 0.99—0.75 |
| no hotwater | 0 | 1 | 0 | 1 | — | — |
| F — counting fence 1972, emigrating fish | | | | | | |
| hotwater | 7 (6.7) | 9 (9.7) | 4 (3.5) | 20 | 0.58 | 0.74—0.42 |
| no hotwater | 4 | 0 | 0 | 4 | — | — |
| F — counting fence 1973, immigrating fish | | | | | | |
| hotwater | 4 | 1 | 0 | 5 | 0.90 | 1.00—0.71 |
| no hotwater | 0 | 0 | 0 | 0 | — | — |
| F — counting fence 1973, emigrating fish | | | | | | |
| hotwater | 25 (24.5) | 32 (32.6) | 11 (10.9) | 68 | 0.60 | 0.68—0.52 |
| no hotwater | 13 (12.8) | 6 (6.4) | 1 (0.8) | 20 | 0.80 | 0.93—0.67 |
| 3) V — Hamnefjärden 1972 | | | | | | |
| hotwater | 31 (31.7) | 36 (34.1) | 8 (9.2) | 75 | 0.65 | 0.73—0.57 |
| no hotwater | 3 (3.5) | 7 (6.0) | 2 (2.5) | 12 | 0.54 | 0.74—0.34 |
| V — Hamnefjärden 1973 | | | | | | |
| hotwater | 59 (58.3) | 36 (36.8) | 6 (5.8) | 101 | 0.76 | 0.82—0.70 |
| no hotwater | 59 (58.7) | 63 (63.2) | 17 (17.0) | 139 | 0.65 | 0.71—0.59 |
| 1972+1973 | | | | | | |
| hotwater | 90 (91.2) | 72 (71.0) | 14 (13.8) | 176 | 0.72 | 0.77—0.67 |
| no hotwater | 62 (61.8) | 70 (69.6) | 19 (19.6) | 151 | 0.64 | 0.69—0.59 |
| 4) I — see Fig. 1 | | | | | | |
| 1972 | 2 (2.0) | 5 (5.0) | 3 (3.0) | 10 | 0.45 | 0.67—0.23 |
| 1973 | 8 (6.2) | 8 (11.5) | 7 (5.3) | 23 | 0.52 | 0.66—0.37 |
| 1972+1973 | 10 (8.3) | 13 (16.5) | 10 (8.3) | 33 | 0.50 | 0.62—0.38 |
| II — see Fig. 1 | | | | | | |
| 1972 | 39 (37.8) | 26 (28.0) | 6 (5.2) | 71 | 0.73 | 0.81—0.65 |
| 1973 | 7 (5.9) | 2 (4.3) | 2 (0.8) | 11 | 0.73 | 0.92—0.54 |
| 1972+1973 | 46 (43.7) | 28 (32.3) | 8 (6.0) | 82 | 0.73 | 0.80—0.66 |
| III — see Fig. 1 | | | | | | |
| 1972 | 2 (2.5) | 6 (5.0) | 2 (2.5) | 10 | 0.50 | 0.72—0.28 |
| 1973 | 12 (11.1) | 1 (2.7) | 1 (0.2) | 14 | 0.89 | 1.00—0.77 |
| 1972+1973 | 14 (12.8) | 7 (9.5) | 3 (1.7) | 24 | 0.73 | 0.86—0.60 |
| IV — see Fig. 1 | | | | | | |
| 1972 | 13 (11.9) | 4 (6.3) | 2 (0.8) | 19 | 0.79 | 0.92—0.66 |
| 1973 | 11 (11.6) | 12 (10.9) | 2 (2.6) | 25 | 0.68 | 0.81—0.55 |
| 1972+1973 | 24 (23.4) | 16 (17.3) | 4 (3.2) | 44 | 0.73 | 0.82—0.64 |

[1] The numbers in parentheses indicate zygotic frequency assuming one-locus, two-allele panmictic population.

this was later verified (NYMAN 1969). In the same paper a similar model of genetic background was suggested for the white bream esterase polymorphism, which up to that date was unknown.

The distribution of esterase phenotypes within each of the 8 population samples of ruff, the estimated allelic frequencies and agreement with Castle-Hardy-Weinberg equilibrium assuming a one-locus, diallelic polymorphism in panmictic populations are shown in Table 3.

The two samples from western and eastern Lake Mälaren are not significantly different (1), but there is an indication that the western sample is derived from a population with a higher gene frequency than the eastern one. In this context it should be pointed out that the western sampling

Table 4. *Distribution of esterase phenotypes in samples of white bream from five localities in 1972.*

| Fish origin | Phenotype | | | Total | f(F) gene freq. | 95 % conf. int. |
|---|---|---|---|---|---|---|
| | F | FS | S | | | |
| F — counting fence ............ | 6 (7.8) [1] | 48 (44.3) | 61 (63.0) | 115 | 0.26 | 0.32—0.20 |
| V — Hamnefjärden ............ | 1 (1.2) | 11 (10.9) | 25 (24.9) | 37 | 0.18 | 0.27—0.09 |
| I — see Fig. 1 ................ | 9 (6.8) | 33 (38.5) | 58 (54.8) | 100 | 0.26 | — |
| II — see Fig. 1 ................ | 3 (3.1) | 23 (22.3) | 39 (39.5) | 65 | 0.22 | — |
| IV — see Fig 1 ............... | 10 (7.8) | 58 (62.4) | 127 (124.8) | 195 | 0.20 | — |

[1] The numbers in parentheses indicate zygotic frequency assuming one-locus, two-allele panmictic population.

point is warmer than the eastern because of the presence of heated effluents from two thermal power stations. Part two (2) of Table 3 indicates the gene frequency of the fish caught while migrating either upstream toward the source of hot water, or, when the station was closed down, migrations upstream a coldwater current. Also, fish leaving the Hamnefjärden Bay when influenced by hot or cold water were sampled. Besides, this part of the table displays the situation in two consecutive years (1972 and 1973). An obvious conclusion can be drawn from part two, *viz.* there is a significantly higher gene frequency (at the 95 % level) of the fish attracted by hotwater than of those favouring cold water. This observation is realized by the fact that ruff which are either attracted by hot water or try to avoid cold water have higher gene frequencies than those migrating upstream a cold current or leaving the bay when hot water is being discharged.

Part three of the table depicts the situation in the Hamnefjärden Bay. It appears that fish caught in the bay during hotwater releases both in 1972 and 1973 have higher gene frequencies than those obtained in coldwater intervals, and this difference is almost significant at the 95 % level if the results of the two years are combined.

The last part of the table (4) displays the gene frequency structure at four sampling points on the coast, to the north, south and immediately outside the outlet of Hamnefjärden Bay. Two observations are obvious. Sampling stations I, II and IV have not changed significantly from 1972 to 1973, whereas a pronounced change has taken place at station III, resulting in a significantly higher gene frequency in the latter year.

The white bream samples obtained in 1972 (Table 4) indicates small differencies in gene frequencies, and even the two extremes (V and F) are nonsignificant.

## V. DISCUSSION

Effects of artificially heated water on fish in situ in brackish and marine environments have been little studied. Temperature, being a very complex factor, acts on all stages of the physiology and ecology of a fish, and at present we are having great difficulty predicting with any confidence what effect a hotwater discharge will have on the migration pattern of a certain species of fish. Normally multivariate techniques must be used to ascertain a sound ecological approach to the problem, but in this case another source of information is proposed, namely the population analysis at the gene level, which permits detection of induced migratory patterns as changes in gene frequencies in population samples at various localities.

The migratory patterns of ruff influenced by heated effluents from OKG nuclear power station may be outlined accordingly: Individuals attracted by hot water in 1972 and 1973 had high frequencies of the "fast" allele of the plasma esterase polymorphism (0.87 and 0.90, respectively), whereas fish leaving the heated area displayed significantly lower values (0.58 and 0.60). This observation was reflected in the samples from the bay also, where in both years the gene frequencies were higher when heated water filled the bay, but the difference was nonsignificant. The low gene frequency of the sampling station just outside the outlet (III) indicates that in 1972 ruff attracted by hot water, *i.e.* those with a high frequency, probably were migrators from other "high-frequency populations" in the vicinity, *e.g.* II and IV. If the hypothesis of migrators from those "populations" is false the situation at sampling point III in 1973 should show a declining frequency of allele F because of predominantly heated conditions during that year. As can be seen in Table 3 the reversed situation has occurred. The frequency of 0.89 indicates an immigration of fish from adjacent populations in 1973, the presence of which had genetically altered the stock available at position III. Consequently fish from either or both II and IV had changed the gene frequency of the ruff at III significantly within a period of one year. Samples at II and IV in 1973 did not indicate any decrease in gene frequency. This probably means either that a relatively small proportion of the resident populations had responded by migrating, or, that the fish attracted came from a very limited area. There is, however, an alternative explanation to this phenomenon. Fish capable of spawning at position III in 1972 may have produced offspring selected for hotwater conditions, *i.e.* with a high gene frequency. It is, however, not very likely that selection should take place when the fish are half a year old or even older, but assuming that this really happened already in the winter 1972/73, these fish would be no more than a year old by the summer 1973 when the sampling stopped. No such young fish were sampled. This type of selection on adjacent spawning grounds may, however, bias future sampling.

It is also noteworthy that the gene frequency of ruff present in Hamnefjärden Bay (V) both under heated and normal conditions is higher in 1973 than in 1972. It would be interesting to sample this site in a few years time, when (possibly) a new ecological balance is established, in order to see which frequency is optimal in this artificial environment.

These results show how a species which is generally considered a coldwater form has adapted genetically in only one year to an induced environmental change by attracting individuals from other populations in the vicinity with a genetic configuration more relevant to the new conditions. By analogy with the results obtained in the warmer Lake Mälaren (Table 3: 1) it is, however, not likely that a high frequency of the "fast" allele *per se* is a prerequisite for adaptation to high temperatures, but obviously some other parameter as well influences the final frequency level. Another problem, which probably reduces the effect of this selective migration, is the rather narrow temperature limits within which the species is capable of spawning and producing offspring. Thus it is unlikely that a hotwater adapted population of ruff will ever become self-sustaining, also, the rapid temperature fluctuations in this artificial environment may affect adversely the fish present. In this species it may be assumed, finally, that hotwater effluents are likely to have little total effect, and the possible eradication of local spawning grounds will be counterbalanced by the more rapid nutrient turn-over favouring certain hotwater adapted individuals.

The presumed potential of the ruff to adapt to a wide range of temperatures, at least when adult, is well-known. In Lake Vättern, for instance, ruff occur from the littoral zone down to a depth of at least 95 m (Svärdson 1974). The general aspect of a polymorphic esterase versus a monomorphic one was discussed by Nyman and Shaw (1971). These authors found that at the latitude where the "monomorphic" brook trout reached the limits of its geographical distribution, the Arctic char (with a homologous allele) extended much further to the north by switching to an alternative "cold-water" allele. The case reported in the present paper, where a species responds to environmental changes by changing the frequency of certain genes is verified in other vertebrates also (*e.g.* Krebs *et al.* 1973). These authors found genetic changes at two loci (transferrin and leucine aminopeptidase) in *Microtus* populations in association with population density changes. The frequency of the $LAP^{S}$ allele, for instance, dropped some 25 per cent in males, beginning at the time of high losses, and heterozygous females ($Tf^{C}/Tf^{E}$) were found to be much more common in dispersing populations than in resident ones. Another interesting contribution was made by Redfield (1972). In the Blue Grouse Redfield found genetic differences in stocks at different densities and also evidence of selection going on within one winter. Also, it should be noted that this selection might occur if initially two different genomes were present in the ruffe populations, concealed by extensive introgression between two sibling species. The existence of sibling species of ruffe was recently evidenced by ALEKSANDROVA (1974).

This type of study may be employed for any organism which is large enough to enable protein sampling and numerous enough to provide sufficient samples for statistical treatment without severely affecting the local populations. Another prerequisite is the presence of several local populations and, finally, a suitable genetically controlled protein polymorphism. In white bream it is questionable whether there is any division into local populations or else the differences encountered would require very large samples to ascertain statistical significance. If the latter situation prevails sampling would most certainly affect the population structures and preclude a valid evaluation.

In order to predict the effect of thermal loading upon fish this type of analysis may prove efficient to elucidate changes in migratory patterns, particularly in combination with netting operations ("survey nets") and underwater telemetry.

## VI. SUMMARY

Analyses of samples of ruff (*Gymnocephalus cernua* (L.)) subjected to the hotwater effluents of a nuclear power station (OKG) on the Baltic coast of Sweden have demonstrated changes in migratory patterns depending upon the genetic composition of certain individuals better adapted to the new environment. Thus gene frequency data indicated significant differences between fish attracted by hot water and those trying to avoid it. Also, the population immediately outside the outlet of the thermally influenced Hamnefjärden Bay was transformed genetically by immigrants from adjacent populations, and in one year the frequency of the most anodal allele had increased from 0.50 to 0.89 at this sampling point. Some biological implications of such artificial temperature changes causing allelic selection are discussed.



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# Pike as the Test Organism for Mercury, DDT and PCB Pollution. A Study of the Contamination in the Stockholm Archipelago

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Appendix:

## Statistical Methods

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The waters outside Stockholm have long since been known to be polluted by effluents from the city and its surroundings (JOHANSSON 1945). More recent investigations have shown increased concentrations of nutrients in the outer parts of the studied area (WAERN 1964/65, WAERN and PEKKARI 1973), in comparison with the Baltic.

Since 1964, many mercury, DDT and PCB analyses have been carried out on fish from Swedish waters. The first values for mercury in fish from the Stockholm archipelago were published in 1967 (JOHNELS *et al.* 1967 a, WESTÖÖ 1967) and for DDT and PCB substances in 1969 (JENSEN *et al.* 1969).

## I. INTRODCTION

In order to describe the pollution and the disperson of pollutants (phosphate, nitrate etc.) in a recipient body of water, analysis of water samples is normally used. Analysis of a large number of samples taken on several occasions from many places can give a detailed picture of the pollution. However, if pollution from substances accumulating in living matter is to be studied it would be preferable for the following reasons, to use a standard organism as a test object to study the pollution pattern. The concentrations of inorganic and organic matter vary with time in natural water. If the substance to be studied accumulate in organisms, the amount of organic matter and dead or living biological matter and the variation in composition of zoo- and phytoplankton in the water will influence the analytical results. The ability of many pollutants to accumulate in plankton is very clearly documented (SÖDERGREN 1968 and 1971, JENSEN *et al.* 1972 b). Differences in the composition of the species in the water can result in different accumulation capacities. For example, the life span can vary for each species, giving longer or shorter accumulation periods. This makes it difficult to compare water samples. Furthermore, external variations in currents and winds can very rapidly influence the geographical representation of a water sample. If one stationary species is used throughout the studied area, physiological variations between species and external variations such as winds and currents will be minimized. These, in addition to the analytical difficulties involved in analyzing water are the reasons why organisms have been used as the test samples.

It should be noted, however, that the levels of the accumulated substances in organisms are influenced by the degree of biological activity and the biomass in the water (JOHNELS *et al.* 1967 b). Thus if two equal volumes of water, one with a high and the other with a low biomass, are exposed to the same amount of the substance, the highest level per unit weight organic matter will be expected in the latter sample. If nutrients (which act as fertilizers and increase the density of the biomass) are released in the same area as the accumulating substances, as happens in the conditions existing in the Stockholm archipelago the higher density of the biomass ought to moderate the levels of the accumulating substances.

The pike (*Esox lucius* L.) chosen as the test organism in the present study has some valuable qualities previously pointed out (Johnels and Westermark 1966, Johnels *et al.* 1967 a, Johnels *et al.* 1968).

1. Pike have stationary habits (Ekman 1915, Hessle 1934) and they are thus representative for the sampling area. This is necessary in a comparative study of small areas within a larger one.

2. Pike grow throughout their lifetime. Males, however, have a slower growth rate than females after the age of two years (Gottberg 1915, Segerstråle 1948, Frost and Kipling 1958/59, Svärdson 1964). This implies that the weight approximately follows the age, for the individual sexes. Furthermore, the local variation in the growth is moderate (Svärdson 1964). This is important and should be considered when levels of mercury for a standardized weight (see below) of the fish are used when comparing different localities.

3. Pike are distributed over the entire investigated area and have also a vast geographical distribution, thus making it possible to make comparisons with other areas.

4. Pike can have a considerable lifespan and have a long half-life for mercury — about 1—2 years (Tillander *et al.* 1969, Järvenpää *et al.* 1970). This is an advantage in an investigation into the general distribution pattern in an area where currents and winds can vary from day to day. Thus one analytical value for mercury will give an integrated evaluation of the pollution over a considerable time.

Since the pike is a predatory fish the levels of the substances studied here may depend on the degree of accumulation in the food species, which can vary in the studied area. Since no precise data on the choice of prey in the different regions are available, the variation due to diet has not been considered.

## II. MATERIALS AND METHODS

The water outside Stockholm is brackish (salinity 1—5 ‰) and normally covered with ice for 2—4 months every year. The pike have been collected over the period December 1968—April 1969 by fishermen at 46 separate localities at different time at different localities, see Fig. 1, and have as a rule been deep-frozen before transport to the laboratory. No fish reached the laboratory in a state of decay. As a rule five fishes have been caught at every locality. The fish have been sexed and weighed. Samples for analysis have been taken from the dorsolateral part of the lateral muscle, ventraly to the dorsal fin and under the red lateral muscle.

A correlation between the weight/age of pike and the amount of mercury has been shown (Westermark (*et al.*) 1965, Johnels *et al.* 1967 a). It has been indicated that in relation to their weight males accumulated mercury faster than females (Berglund and Olsson 1970). In only a few cases in this report is the material sufficient to form a basis for a meaningful comparison between sexes.

The weights of the individual pike varied within and between the localities, but the mercury level in a pike depends on the weight/age. Thus regression analysis has been used to determine the amount of mercury in a pike of a standardized weight (here 1 kilogram), then used for comparison between the different localities. As mentioned, only five pike were collected from each locality. If, for instance, the pike from one locality all have approximately the same weight, the possibility of determining the true regression line is small. The greater the difference between these weights in grams (the X-values) and the standard weight (1000 g), the more uncertain is the mercury content (Y-value) found at X= 1000.

In order to obtain a more substancial material which will minimize this disadvantage, the various

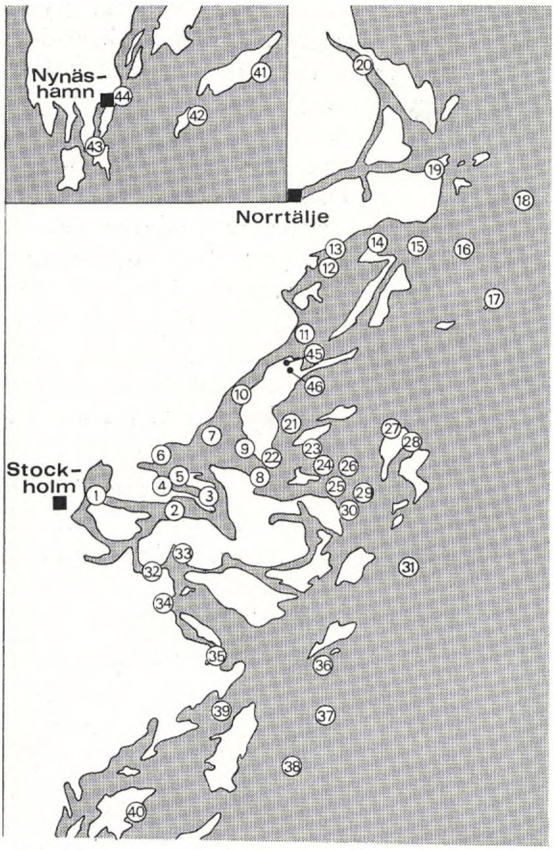


Fig. 1. Sampling localities in the Stockholm archipelago.

localities have — as much as possible — been grouped into regions (see Table 1 and Fig. 2). This division into regions has chiefly been carried out on the basis of the distance from the assumed main pollution source, the City of Stockholm and the geographical position.

To make it possible to follow the variation in contamination, the regions have been combined into 3 main areas:

1. The northern fairway (regions I—VII)
2. The main fairway (regions I—II, VIII—X, XIII)
3. The southern fairway through Lännersta (regions I, XI—XVII).

In addition, material has been collected from 2 lakes on an island situated in the Stockholm archipelago (loc. 45—46).

The mercury has been determined by means of activation analysis (LJUNGGREN *et al.* 1971). All analytical values represent the level of total mercury. Investigation has, however, shown that on average 99 % of the total mercury in pike muscle consists of methyl mercury (WESTÖÖ and RYDÄLV 1969). For chlorinated hydrocarbons, gaschromatography with electron capture detection has been used. Extraction of the archipelago samples (loc. 1—44) was performed using hexane in a soxhlet apparatus (JENSEN and WIDMARK 1967) while the samples from the two island lakes (loc. 45—46) were extracted by a method reported recently (JENSEN *et al.* 1972 a). No comparisons of levels are made between the fresh water pike and the pike from the brackish water. The values for the levels of chlorinated hydrocarbons are given both with respect to fresh tissue and to extractable fat, while all statistical calculations have been carried out on the levels in extractable fat due to certain resultant advantages (HOLDEN 1962, JENSEN *et al.* 1969).

## III. RESULTS

### *Mercury levels in pike*

The present levels of mercury generally agree with those previously published (JOHNELS *et al.* 1967 a, WESTÖÖ 1967, WESTÖÖ and RYDÄLV 1969). Since

Table 1. *Sampling localities within the regions.*

| Region | Localities |
|---|---|
| I | 1—6 |
| II | 7—10 |
| III | 11 |
| IV | 12—15 |
| V | 16—18 |
| VI | 19 |
| VII | 20 |
| VIII | 21—24 |
| IX | 25—30 |
| X | 31 |
| XI | 32 |
| XII | 33—35 |
| XIII | 36—38 |
| XIV | 39 |
| XV | 40 |
| XVI | 41—42 |
| XVII | 43 |

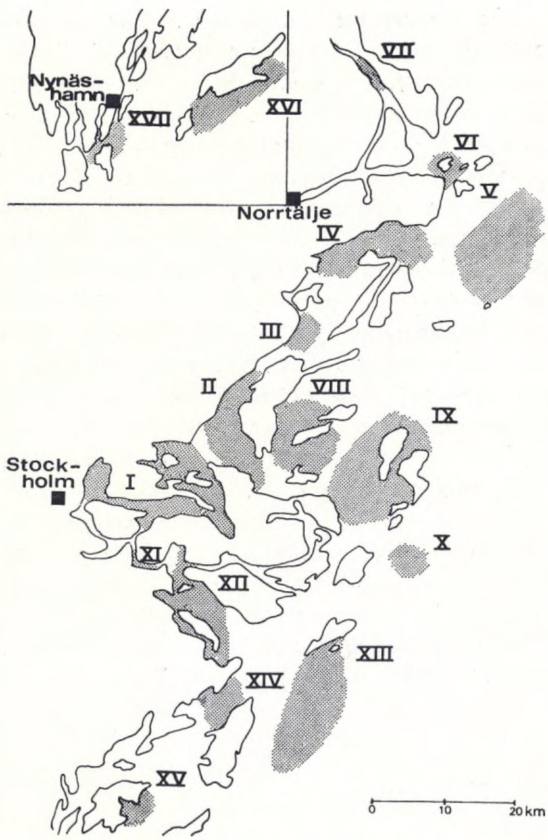


Fig. 2. Studied areas in the Stockholm archipelago.

the mercury exposure may vary from year to year, previously published results have not been dealt with in this paper with one exception. Because of treacherous ice conditions in 1969, no material was collected in the outer part of the southern archipelago. This area (region XVI, see Table 1, Fig. 2) plays an essential role in the estimation of the spread of pollutants, and therefore 10 values for methyl mercury in pike from February 1968, published by WESTÖÖ and RYDÄLV 1969, have been used. This is also why no levels of DDT and PCB substances were obtained from region XVI. Extensive changes in the mercury pollution situation during 1968 in the area SE Utö have been regarded as improbable.

The mean values and 95 % confidence interval for mercury in females and males, considered separately, from the same region have been calculated (see Table 2). For the statistical methods, see the Appendix, part V. The differences between the sexes have been tested when 5 or more specimens of each sex have been available. For the statistical method, see the Appendix, part II and IV. As no difference was established ($p > 0.05$) females and males have been considered together.

In Table 2 the mean value and confidence interval for both sexes together from the separate

Table 2. *Mean levels of mercury and 95 % confidence interval in fresh tissue for a standard pike (1 kilo) in different regions. The values for the sexes separately have been calculated when 5 or more specimens are available. n=number of specimens used in the test. Regarding three fishes in region I, the sex was not determined.*

| Region | n | ♂♂+♀♀ Mercury in ppm | n | ♀♀ Mercury in ppm | n | ♂♂ Mercury in ppm |
|---|---|---|---|---|---|---|
| I | 27 | $1.16 \pm 0.28$ | 15 | $0.80 \pm 0.68$ | 9 | $1.34 \pm 0.29$ |
| II | 17 | $1.03 \pm 0.19$ | 14 | $1.08 \pm 0.25$ | | |
| III | 5 | $0.67 \pm 0.17$ | | | | |
| IV | 20 | $0.52 \pm 0.07$ | 17 | $0.52 \pm 0.07$ | | |
| V | 14 | $0.57 \pm 0.09$ | 7 | $0.53 \pm 0.21$ | 7 | $0.60 \pm 0.16$ |
| VI | 5 | $0.81 \pm 0.21$ | | | | |
| VII | 5 | $0.69 \pm 0.30$ | | | | |
| VIII | 20 | $0.81 \pm 0.17$ | 10 | $0.78 \pm 0.37$ | 10 | $0.85 \pm 0.13$ |
| IX | 30 | $0.75 \pm 0.09$ | 16 | $0.74 \pm 0.14$ | 14 | $0.77 \pm 0.12$ |
| X | 5 | $0.68 \pm 0.18$ | | | | |
| XI | 5 | $1.95 \pm 1.93$ | | | | |
| XII | 15 | $0.85 \pm 0.11$ | 10 | $0.84 \pm 0.18$ | 5 | $0.86 \pm 0.22$ |
| XIII | 15 | $0.57 \pm 0.16$ | 10 | $0.54 \pm 0.32$ | 5 | $0.60 \pm 0.29$ |
| XIV | 5 | $0.82 \pm 0.23$ | | | | |
| XV | 5 | $0.50 \pm 0.20$ | | | | |
| XVI | 10 | $0.30 \pm 0.05$ | | | | |
| XVII | 8 | $0.44 \pm 0.16$ | | | | |

Table 3. *Tests of differences between mean levels of mercury in pike, females and males separately and together in different regions. Results given by — if $p \geq 0.05$, × if $0.01 \leq p < 0.05$, ×× if $0.001 \leq p < 0.01$, ××× if $p < 0.001$.*

| Region | ♀♀+♂♂ | ♀♀ | ♂♂ |
|---|---|---|---|
| I—II | — | — | |
| I—III | ×× | | |
| I—IV | ××× | | |
| I—V | ××× | | |
| I—VIII | × | — | ×× |
| I—IX | ×× | | |
| I—X | ×× | | |
| I—XI | — | | |
| I—XII | × | | |
| I—XIII | ×× | — | ×× |
| I—XIV | — | | |
| I—XV | ×× | | |
| I—XVII | ××× | | |
| II—III | × | | |
| II—IV | ××× | ××× | |
| II—V | ××× | | |
| II—VIII | — | — | |
| II—IX | × | × | |
| II—X | × | | |
| II—XIII | ×× | | |
| III—IV | — | | |
| III—V | — | | |
| III—VI | — | | |
| IV—V | — | — | |
| IV—VI | × | | |
| IV—VII | — | | |
| V—VI | × | | |
| V—VII | — | | |
| V—X | — | | |
| V—XIII | — | | |
| VI—VII | — | | |
| VIII—IX | — | — | — |
| VIII—X | — | | |
| VIII—XIII | × | | |
| IX—X | — | | |
| IX—XIII | — | | |
| X—XIII | — | | |
| XI—XII | — | | |
| XI—XIII | — | | |
| XI—XIV | — | | |
| XI—XV | — | | |
| XI—XVI | — | | |
| XI—XVII | — | | |
| XII—XIII | ×× | — | — |
| XII—XIV | — | | |
| XII—XV | × | | |
| XII—XVI | ××× | | |
| XII—XVII | ×× | | |
| XIII—XIV | — | | |
| XIII—XV | — | | |
| XIII—XVI | ×× | | |
| XIII—XVII | — | | |
| XIV—XV | × | | |
| XIV—XVI | ×× | | |
| XIV—XVII | × | | |
| XV—XVI | × | | |
| XV—XVII | — | | |
| XVI—XVII | — | | |

regions are also given. The difference between the regions has been tested (see Table 3).

To test whether there was any discrepancy in the significant differences between the regions when comparing females and males, together and individually, the p-value was also calculated for differences between the regions using the separate sexes (see Table 3). In no case was a difference established for one sex which was not found for both sexes together. On the other hand differences which were found for both sexes together were not always found for one single sex. This may depend on the limitation of the material.

Tables 2, 3 and Fig. 3 show that there is a decrease in the mercury levels in pike when going from the inner to the outer part of the archipelago. All over the studied area (with one exception, region XVI), the levels of mercury were above 0.4 ppm which seems to be the upper limit of the natural levels in the Baltic. The mercury level in region XVI does not seem to be influenced by industrial or municipal effluents. The levels here agree with these recorded for pike from uninfluenced localities along the Baltic coast (Westöö and Rydälv 1969 and 1971).

## IV. CHLORINATED HYDROCARBONS

### *Levels of chlorinated hydrocarbons in pike*

It has been suggested that the DDT levels in field collected, fully grown, mosquito fishes (*Gambusia affinis*), do not depend on the weight (Murphy 1971). In the same paper, a negative correlation to weight was found in a short time (48 hours) experiment. A positive correlation between age and levels of PCB in fresh tissue of lake trout has been found (Bache *et al.* 1972). In 1970 a positive correlation between weight and levels of DDT in fresh tissue of lake trout was found but when it is the DDT levels in fat which are considered, the differences between the size groups become considerably less (Reinert 1970). To examine the conditions for pike, the relation between weight and levels of DDT and PCB substances within the regions have been statistically tested for the sexes individually and together, where at least 5 specimens of each sex are available (see Table 4). For the statistical method used, see the Appendix, part III.

Table 4. *Tests of dependence between levels of DDT and PCB substances in extractable fat of pike and the weight of those pike.* $r$ = *correlation coefficient,* $b$ = *regression coefficient,* $\alpha$ = *value for Y (the level) at X (the weight) = 0. If* $b \neq 0$ *there is a significant dependence.* $p < 0.05$ +, $p < 0.01$ ++, $p < 0.001$ +++.

| | | ♂♂+♀♀ | | | | | | ♀♀ | | | | | | ♂♂ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | | n | sDDT | DDE | DDD | DDT | PCB | n | sDDT | DDE | DDD | DDT | PCB | n | sDDT | DDE | DDD | DDT | PCB |
| I | r | 28 | 0.54 | 0.37 | 0.68 | 0.67 | 0.30 | 16 | 0.43 | 0.28 | 0.53 | 0.60 | 0.44 | 9 | 0.83 | 0.66 | 0.76 | 0.88 | 0.23 |
| | b | | 6.91 | 2.86 | 0.92 | 2.68 | 3.33 | | 7.97 | 3.33 | 0.92 | 3.26 | 6.45 | | 2.72 | 1.20 | 0.39 | 0.96 | 1.54 |
| | α | | 3.37 | 3.19 | 0.30 | –0.42 | 12.05 | | 1.47 | 2.32 | 0.47 | –1.65 | 2.74 | | 7.40 | 4.58 | 0.88 | 1.43 | 13.76 |
| | | | ++ | | +++ | +++ | | | | | + | + | | | ++ | | + | ++ | |
| II | r | 17 | 0.50 | 0.44 | 0.26 | 0.54 | 0.43 | 14 | 0.47 | 0.49 | 0.15 | 0.47 | 0.45 | | | | | | |
| | b | | 3.51 | 1.36 | 0.28 | 1.70 | 3.03 | | 3.27 | 1.56 | 0.16 | 1.43 | 3.17 | | | | | | |
| | α | | 9.93 | 5.53 | 1.55 | 2.05 | 9.77 | | 10.51 | 5.05 | 1.83 | 2.69 | 9.41 | | | | | | |
| | | | + | | | + | | | | | | | | | | | | | |
| III | r | 5 | 0.28 | 0.03 | 0.86 | 0.32 | –0.22 | | | | | | | | | | | | |
| | b | | 3.03 | 0.24 | 1.92 | 0.78 | –1.87 | | | | | | | | | | | | |
| | α | | 20.45 | 12.53 | 0.59 | 5.65 | 16.51 | | | | | | | | | | | | |
| IV | r | 20 | 0.05 | 0.21 | –0.12 | –0.13 | –0.09 | | | | | | | | | | | | |
| | b | | 0.26 | 0.58 | –0.12 | –0.25 | –0.17 | | | | | | | | | | | | |
| | α | | 18.33 | 8.60 | 2.63 | 5.91 | 8.20 | | | | | | | | | | | | |
| V | r | 15 | –0.36 | –0.42 | –0.32 | –0.28 | –0.28 | 7 | –0.51 | –0.59 | –0.45 | –0.44 | –0.40 | 8 | –0.03 | –0.14 | –0.22 | 0.10 | 0.26 |
| | b | | –2.62 | –1.16 | –0.26 | –1.03 | –0.81 | | –2.05 | –0.77 | –0.25 | –0.91 | –0.71 | | –1.47 | –2.73 | –1.27 | 2.69 | 5.38 |
| | α | | 14.76 | 5.84 | 1.54 | 6.52 | 6.48 | | 12.78 | 4.52 | 1.54 | 6.01 | 5.96 | | 14.60 | 7.81 | 2.45 | 3.44 | 1.21 |
| VI | r | 5 | –0.24 | 0.19 | –0.20 | –0.42 | 0.43 | | | | | | | | | | | | |
| | b | | –9.98 | 1.77 | –0.54 | –14.32 | 36.07 | | | | | | | | | | | | |
| | α | | 18.04 | 2.68 | 1.11 | 15.69 | –12.97 | | | | | | | | | | | | |
| VII | r | 5 | 0.68 | 0.40 | 0.94 | 0.62 | –0.06 | | | | | | | | | | | | |
| | b | | 13.22 | 3.47 | 2.92 | 5.64 | –1.55 | | | | | | | | | | | | |
| | α | | 13.44 | 9.18 | 0.35 | 3.07 | 15.72 | | | | | | | | | | | | |
| | | | | | + | | | | | | | | | | | | | | |

| | | ♂♂+♀♀ | | | | | | ♀♀ | | | | | | ♂♂ | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Region | | n | sDDT | DDE | DDD | DDT | PCB | n | sDDT | DDE | DDD | DDT | PCB | n | sDDT | DDE | DDD | DDT | PCB |
| VIII | r | 20 | 0.54 | 0.49 | 0.56 | 0.51 | −0.01 | 10 | 0.54 | 0.61 | 0.48 | 0.42 | 0.19 | 10 | 0.70 | 0.67 | −0.02 | 0.70 | 0.60 |
| | b | | 4.71 | 1.96 | 0.74 | 1.61 | −0.04 | | 4.33 | 2.00 | 0.64 | 1.33 | 0.59 | | 14.39 | 7.30 | −0.00 | 4.52 | 5.89 |
| | α | | 8.52 | 4.48 | 1.09 | 2.49 | 9.26 | | 8.39 | 3.63 | 1.29 | 2.99 | 6.43 | | 0.57 | 0.26 | 1.86 | 0.44 | 4.64 |
| | | | + | + | ++ | + | | | | | | | | | + | + | | + | |
| IX | r | 30 | 0.34 | 0.42 | 0.41 | 0.09 | 0.03 | 16 | 0.38 | 0.50 | 0.38 | 0.11 | 0.07 | 14 | 0.17 | 0.10 | 0.26 | 0.19 | −0.34 |
| | b | | 1.39 | 0.84 | 0.31 | 0.16 | 0.12 | | 1.32 | 0.84 | 0.24 | 0.17 | 0.22 | | 1.87 | 0.60 | 0.45 | 0.93 | −3.69 |
| | α | | 11.31 | 4.63 | 1.12 | 4.84 | 9.29 | | 11.64 | 4.69 | 1.48 | 4.64 | 9.43 | | 10.57 | 4.82 | 0.70 | 4.26 | 12.88 |
| | | | | + | + | | | | | | | | | | | | | | |
| X | r | 5 | 0.68 | 0.61 | 0.72 | 0.61 | −0.52 | | | | | | | | | | | | |
| | b | | 8.15 | 3.33 | 1.71 | 2.85 | −1.54 | | | | | | | | | | | | |
| | α | | 3.10 | 1.55 | −0.49 | 1.87 | −5.69 | | | | | | | | | | | | |
| XI | r | 5 | 0.44 | 0.26 | 0.28 | 0.51 | −0.16 | | | | | | | | | | | | |
| | b | | 2.06 | 0.93 | 0.18 | 0.89 | −0.67 | | | | | | | | | | | | |
| | α | | 9.93 | 5.64 | 1.31 | 2.01 | 16.19 | | | | | | | | | | | | |
| XII | r | 15 | 0.22 | 0.32 | 0.40 | −0.06 | −0.12 | 10 | 0.29 | 0.45 | 0.41 | −0.11 | 0.63 | 5 | 0.54 | 0.71 | 0.62 | 0.21 | −0.45 |
| | b | | 3.19 | 2.40 | 0.70 | −0.32 | −1.67 | | 3.05 | 2.33 | 0.71 | −0.38 | 2.62 | | 15.46 | 10.40 | 1.48 | 2.42 | −12.79 |
| | α | | 9.64 | 3.81 | 0.71 | 4.70 | 11.90 | | 8.03 | 2.72 | 0.58 | 4.42 | 3.07 | | 0.32 | −2.24 | 0.15 | 2.55 | 30.00 |
| XIII | r | 15 | 0.25 | 0.16 | 0.29 | 0.26 | 0.24 | 10 | 0.46 | 0.42 | 0.45 | 0.42 | 0.45 | 5 | −0.54 | 0.43 | −0.41 | −0.62 | −0.17 |
| | b | | 1.86 | 0.41 | 0.24 | 1.09 | 0.91 | | 3.76 | 0.99 | 0.42 | 2.06 | 1.94 | | −13.46 | −5.01 | −1.11 | −6.08 | −1.58 |
| | α | | 9.86 | 3.85 | 0.94 | 4.65 | 4.58 | | 4.90 | 2.34 | 0.44 | 2.08 | 1.75 | | 25.71 | 9.33 | 2.36 | 12.20 | 8.17 |
| XIV | r | 5 | −0.49 | −0.48 | −0.16 | −0.52 | −0.87 | | | | | | | | | | | | |
| | b | | −0.31 | −2.27 | −0.37 | −6.36 | −10.34 | | | | | | | | | | | | |
| | α | | 24.72 | 7.70 | 1.95 | 14.20 | 19.04 | | | | | | | | | | | | |
| XV | r | 5 | −0.21 | −0.27 | −0.29 | −0.16 | −0.07 | | | | | | | | | | | | |
| | b | | −7.17 | −2.10 | −0.98 | −3.33 | −2.85 | | | | | | | | | | | | |
| | α | | 26.04 | 8.28 | 3.32 | 12.86 | 14.04 | | | | | | | | | | | | |
| XVII | r | 9 | 0.27 | 0.20 | 0.33 | 0.18 | 0.17 | 6 | 0.28 | 0.18 | 0.25 | 0.60 | 0.31 | | | | | | |
| | b | | 0.78 | 0.54 | 0.09 | 0.13 | 0.28 | | 0.78 | 0.44 | 0.07 | 0.28 | 0.53 | | | | | | |
| | α | | 11.19 | 6.74 | 1.29 | 2.20 | 6.08 | | 10.54 | 6.69 | 1.40 | 1.43 | 4.63 | | | | | | |

Table 5. *Mean levels, in ppm, and 95 % confidence intervals of DDT and PCB substances in extractable fat of pike of different sexes from regions where five or more specimens of each sex are available. n = number of specimens. Significant differences between the levels of each sex are given by — if $p \geq 0.05$, + if $0.01 \leq p < 0.05$, ++ if $0.001 \leq p < 0.01$, +++ if $p < 0.001$.*

| Region | Sex | n | sDDT | DDE | DDD | DDT | PCB |
|---|---|---|---|---|---|---|---|
| I | ♀♀ | 16 | 22±9 | 11±6 | 2.8±0.9 | 6.6±2.8 | 19±8 |
| | ♂♂ | 9 | 11±2 | 6.1±1.4 | 1.4±0.4 | 2.6±0.8 | 16±5 |
| | | | + | — | ++ | + | — |
| V | ♀♀ | 7 | 9.2±4.8 | 3.2±1.5 | 1.1±0.7 | 4.4±2.4 | 4.7±2.1 |
| | ♂♂ | 8 | 13±7 | 5.4±2.5 | 1.3±0.7 | 5.9±3.5 | 6.1±2.6 |
| | | | — | — | — | — | — |
| VIII | ♀♀ | 10 | 17±6 | 7.8±2.7 | 2.6±1.1 | 5.8±2.6 | 7.7±2.5 |
| | ♂♂ | 10 | 14±6 | 7.3±3.1 | 1.9±0.7 | 4.2±1.9 | 11±3 |
| | | | — | — | — | — | — |
| IX | ♀♀ | 16 | 14±3 | 6.1±1.5 | 1.9±0.6 | 4.9±1.3 | 9.8±2.7 |
| | ♂♂ | 14 | 13±3 | 5.4±1.3 | 1.2±0.4 | 5.2±1.1 | 9.1±2.4 |
| | | | — | — | + | — | — |
| XII | ♀♀ | 10 | 12±4 | 5.8±2.1 | 1.5±0.7 | 3.9±1.5 | 6.5±1.7 |
| | ♂♂ | 5 | 17±11 | 8.7±5.9 | 1.7±0.9 | 5.1±4.6 | 17±11 |
| | | | — | — | — | — | — |
| XIII | ♀♀ | 10 | 13±6 | 4.4±1.6 | 1.3±0.6 | 6.4±3.4 | 5.8±3.0 |
| | ♂♂ | 5 | 14±8 | 4.8±3.6 | 1.4±0.8 | 6.7±3.1 | 6.7±2.9 |
| | | | — | — | — | — | — |

Table 6. *Mean values of weight and mean levels of DDT and PCB substances in ppm of extractable fat and fresh tissue in pike from different regions. For levels in extractable fat the 95 % confidence interval are given. n = number of specimens analyzed.*

| Region | n | Mean weight in g | ppm in extractable fat sDDT | DDE | DDD | DDT | PCB | ppm in fresh tissue sDDT | DDE | DDD | DDT | PCB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 30 | 1908 | 18±6 | 9.1±3.3 | 2.3±0.7 | 5.1±1.8 | 20±5 | 0.11 | 0.059 | 0.014 | 0.031 | 0.11 |
| II | 17 | 1437 | 15±3 | 7.5±1.3 | 2.0±0.4 | 4.5±1.3 | 14±3 | 0.071 | 0.036 | 0.0094 | 0.021 | 0.069 |
| III | 5 | 1569 | 25±14 | 13±9 | 3.6±2.9 | 6.9±3.1 | 14±11 | 0.19 | 0.10 | 0.023 | 0.048 | 0.12 |
| IV | 20 | 1891 | 19±5 | 9.7±2.6 | 2.4±0.9 | 5.4±1.8 | 7.9±1.7 | 0.091 | 0.047 | 0.012 | 0.026 | 0.038 |
| V | 15 | 1293 | 11±4 | 4.3±1.5 | 1.2±0.4 | 5.2±1.9 | 5.4±1.5 | 0.050 | 0.019 | 0.0055 | 0.023 | 0.025 |
| VI | 5 | 607 | 12±4 | 3.8±0.9 | 0.79±0.25 | 7.0±3.2 | 8.9±7.8 | 0.061 | 0.019 | 0.0040 | 0.035 | 0.045 |
| VII | 5 | 981 | 26±7 | 13±3 | 3.2±1.1 | 8.6±3.2 | 14±9 | 0.15 | 0.071 | 0.018 | 0.047 | 0.078 |
| VIII | 20 | 1557 | 16±4 | 7.5±1.9 | 2.2±0.6 | 5.0±1.5 | 9.2±1.9 | 0.072 | 0.034 | 0.011 | 0.022 | 0.044 |
| IX | 30 | 1390 | 13±2 | 5.8±1.0 | 1.6±0.4 | 5.1±0.8 | 9.5±1.7 | 0.068 | 0.030 | 0.0079 | 0.027 | 0.049 |
| X | 5 | 905 | 11±4 | 4.6±1.9 | 1.1±0.8 | 4.4±1.6 | 4.3±1.0 | 0.062 | 0.027 | 0.0060 | 0.026 | 0.026 |
| XI | 5 | 3219 | 17±8 | 8.6±5.7 | 1.9±1.1 | 4.9±2.8 | 14±7 | 0.080 | 0.042 | 0.0094 | 0.024 | 0.070 |
| XII | 15 | 1224 | 14±4 | 6.8±2.1 | 1.6±0.5 | 4.3±1.5 | 9.9±4.0 | 0.068 | 0.034 | 0.0079 | 0.021 | 0.052 |
| XIII | 15 | 1693 | 13±4 | 4.5±1.3 | 1.3±0.5 | 6.5±2.2 | 6.1±2.0 | 0.072 | 0.026 | 0.0074 | 0.036 | 0.034 |
| XIV | 5 | 950 | 16±10 | 5.5±2.5 | 1.6±1.2 | 8.2±6.5 | 9.2±6.2 | 0.12 | 0.037 | 0.012 | 0.062 | 0.064 |
| XV | 5 | 1178 | 18±14 | 5.8±3.2 | 2.2±1.4 | 8.9±8.1 | 11±16 | 0.096 | 0.032 | 0.012 | 0.048 | 0.053 |
| XVII | 9 | 2510 | 13±5 | 8.0±4.8 | 1.5±0.5 | 2.5±1.2 | 6.7±2.9 | 0.069 | 0.040 | 0.0080 | 0.015 | 0.037 |

As can be seen in Table 4 there is a significant positive correlation between the amount of chlorinated hydrocarbons and the weight of the pike in only a few regions. No striking discrepancy seems to exist between the number of significant correlations for males and females. The few significant correlations do not however warrant a comparison between regions, based upon a calculated level of chlorinated hydrocarbons to a standard weight, especially when no general positive or negative dependence between weight and level has been found. The comparisons will therefore

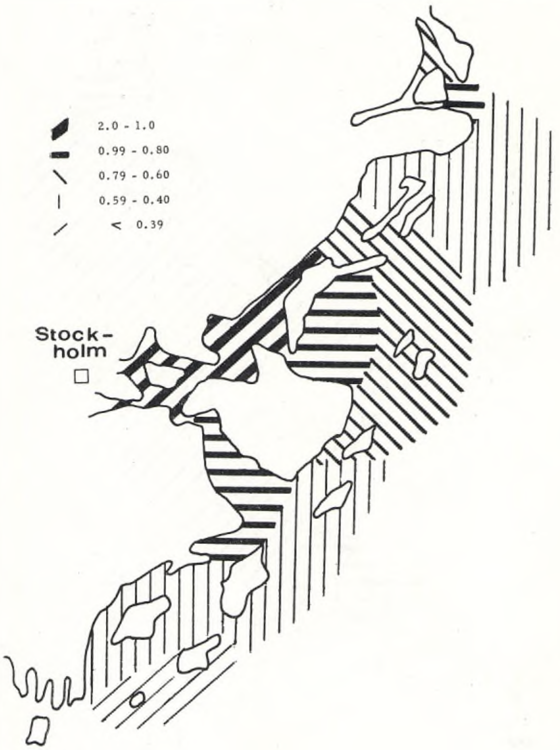


Fig. 3. Levels of mercury (mg/kg) in lateral muscle of pike from the Stockholm archipelago.

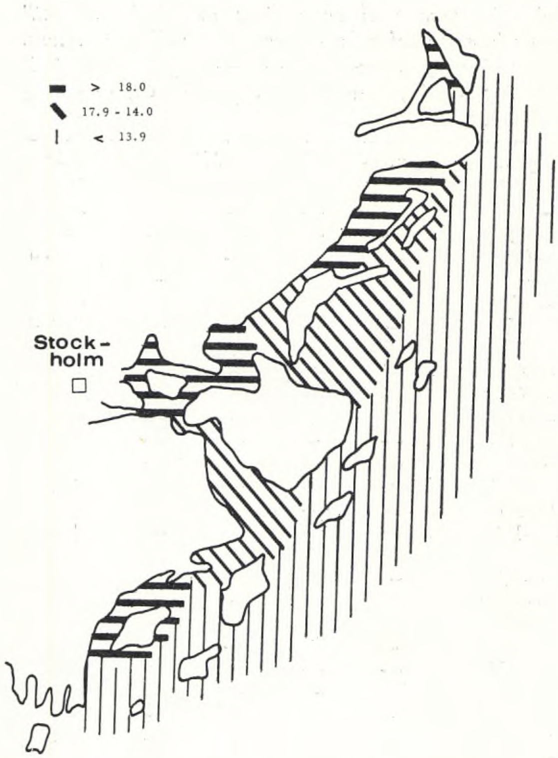


Fig. 4. Levels of sDDT (mg/kg) in extractable fat of lateral muscle in pike from the Stockholm archipelago.

be based upon the mean levels of chlorinated hydrocarbons but consideration has been taken to the mean weight of the fishes in the discussion, when comparing the levels for the regions (mean weight, see Table 6).

The mean levels of the chlorinated hydrocarbons have been calculated for females and males separately and the difference in levels has been tested in regions with at least 5 specimens of each sex (see Table 5). For the statistical method, see the Appendix, part V. For PCB, no significant difference was established. For DDT compounds, a significant difference was found in two of the six regions. An explanation for this difference in region I might be that localities 1 and 2, situated nearest the sewage treatment plants of Stockholm, are represented by 8 females and only 2 males. In the other localities (loc. 3, 4, 5 and 6) the two sexes are evenly represented (8 ♀♀ and 7 ♂♂). The mean level of the total amount of DDT compounds is, for females and males together, 25 ppm in extractable fat in localities 1 and 2, while in localities 3, 4, 5 and 6, only 13 ppm. For females alone the corresponding levels are 28 and 9.7 ppm respectively. If there is local pollution close to the sewage treatment plants, the uneven sex distribution may influence the levels for the different sexes in the whole region. As the material is rather limited, the few differences found in levels between the sexes do not warrant a separation of sexes when comparing the levels of chlorinated hydrocarbons between the regions. The results are therefore given as mean levels of both sexes together.

In Table 6 and Figs. 2—8 mean values of DDT and PCB compounds from different regions are given. The results of the statistical tests for the differences of these levels between the regions are given in Table 7. For the statistical method, see the Appendix, part V. As can be seen, a consequent, significant decrease of the PCB levels occurs when going from the inner part of the

Table 7. *Results of comparison of DDT and PCB levels in extractable fat of pike from different regions. The following symbols are used — if $p \geq 0.05$, + if $0.01 \leq p < 0.05$, ++ if $0.001 \leq p < 0.01$, +++ if $p < 0.001$.*

| Region | sDDT | DDE | DDD | DDT | PCB |
|---|---|---|---|---|---|
| I—II | — | — | — | — | — |
| I—III | — | — | — | — | — |
| I—IV | — | — | — | — | +++ |
| I—V | — | + | + | — | +++ |
| I—VIII | — | — | — | — | +++ |
| I—IX | — | — | — | — | +++ |
| I—X | + | + | + | — | +++ |
| I—XI | — | — | — | — | — |
| I—XII | — | — | — | — | ++ |
| I—XIII | — | + | + | — | +++ |
| I—XIV | — | — | — | — | + |
| I—XV | — | — | — | — | — |
| I—XVII | — | — | — | + | +++ |
| II—III | — | — | — | — | — |
| II—IV | — | — | — | — | ++ |
| II—V | — | ++ | + | — | +++ |
| II—VIII | — | — | — | — | ++ |
| II—IX | — | + | — | — | + |
| II—X | — | + | — | — | +++ |
| II—XIII | — | ++ | — | — | +++ |
| III—IV | — | — | — | — | — |
| III—V | — | — | — | — | — |
| III—VI | — | + | — | — | — |
| IV—V | + | ++ | + | — | + |
| IV—VI | + | +++ | ++ | — | — |
| IV—VII | — | — | — | — | — |
| V—VI | — | — | — | — | — |
| V—VII | ++ | ++ | ++ | — | — |
| V—X | — | — | — | — | — |
| V—XIII | — | — | — | — | — |
| VI—VII | ++ | ++ | ++ | — | — |
| VIII—IX | — | — | — | — | — |
| VIII—X | — | + | + | — | +++ |
| VIII—XIII | — | + | + | — | + |
| IX—X | — | — | — | — | +++ |
| IX—XIII | — | — | — | — | + |
| X—XIII | — | — | — | — | — |
| XI—XII | — | — | — | — | — |
| XI—XIII | — | — | — | — | + |
| XI—XIV | — | — | — | — | — |
| XI—XV | — | — | — | — | — |
| XI—XVII | — | — | — | — | — |
| XII—XIII | — | — | — | — | — |
| XII—XIV | — | — | — | — | — |
| XII—XV | — | — | — | — | — |
| XII—XVII | — | — | — | — | — |
| XIII—XIV | — | — | — | — | — |
| XIII—XV | — | — | — | — | — |
| XIII—XVII | — | — | — | ++ | — |
| XIV—XV | — | — | — | — | — |
| XIV—XVII | — | — | — | — | — |
| XV—XVII | — | — | — | — | — |

archipelago to the outer. This is also true for DDE and DDD but not for sDDT (DDT+DDD×1.11 +DDE×1.11) and DDT. In the northern fairway the amounts of DDD and DDE are higher in region VII than in regions VI and V.

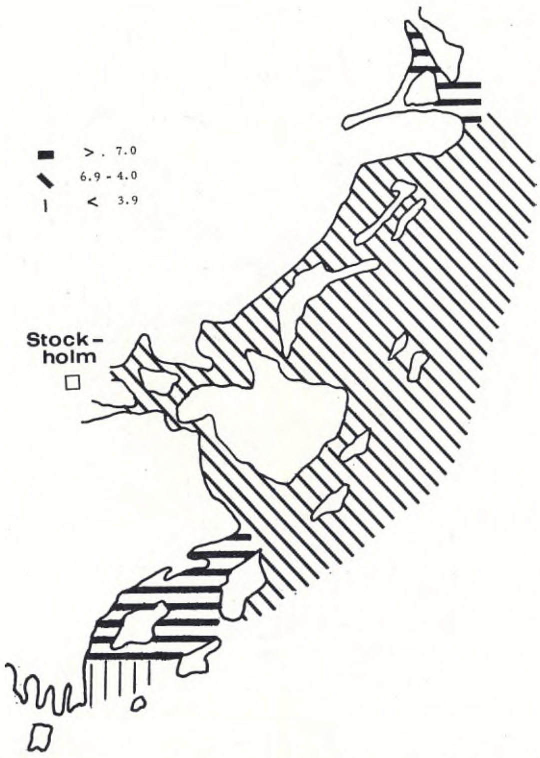


Fig. 5. Levels of DDT (mg/kg) in extractable fat of lateral muscle in pike from the Stockholm archipelago.

*Proportion of DDT, DDD and DDE*

To roughly estimate if there was any striking dependence between the weight and the proportion of the separate DDT substances (DDT, DDD and DDE), the regression line and the correlation coefficient have been calculated for a few regions (regions I, VIII, IX and XIII), for males and females, separately and together (see Table 8). Since the individual specimens cannot be assumed to be normally distributed, the test of b=0 previously used for levels as a function of weight is not applicable. The DDD and DDE values have been multiplied by 1.11 to correct for the loss in the molecular weight when DDT is metabolized.

The regression coefficients varied for the different ratios, from negative to positive values and the correlation coefficient was in most cases rather

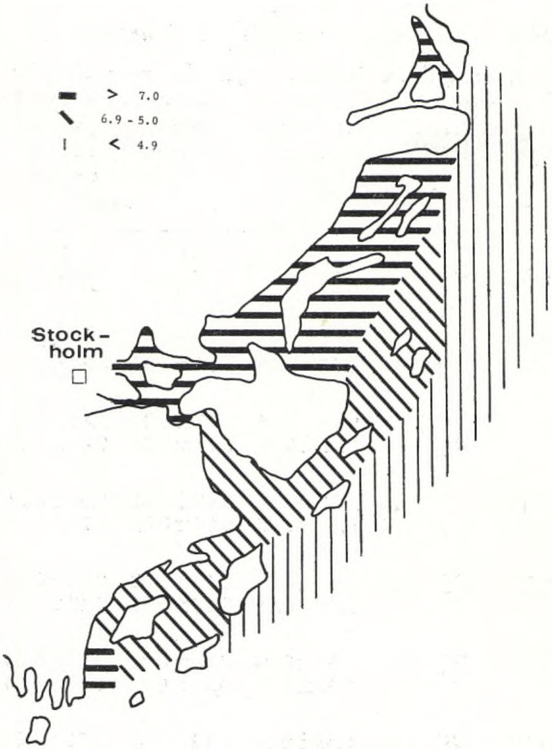


Fig. 6. Levels of DDE (mg/kg) in extractable fat of lateral muscle in pike from the Stockholm archipelago.

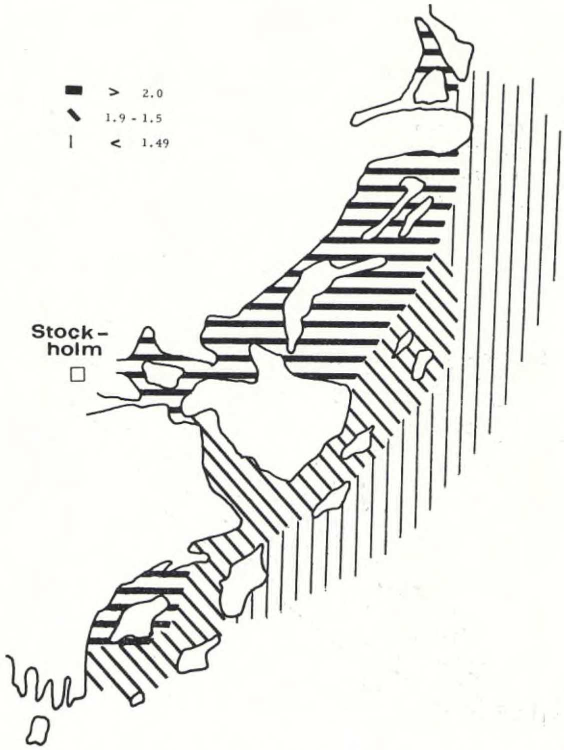


Fig. 7. Levels of DDD (mg/kg) in extractable fat of lateral muscle in pike from the Stockholm archipelago.

Table 8. *Calculated dependence between weight and the ratio* $\frac{DDx}{sDDT}$ *in a few regions, sexes separately and together. n = number of specimens, r = correlation coefficient, b = regression coefficient,* $\alpha = Y$ *at* $X = 0$.

| Region | | ♀♀+♂♂ | | | | ♀♀ | | | | ♂♂ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | $\frac{DDE}{sDDT}$ | $\frac{DDD}{sDDT}$ | $\frac{DDT}{sDDT}$ | n | $\frac{DDE}{sDDT}$ | $\frac{DDD}{sDDT}$ | $\frac{DDT}{sDDT}$ | n | $\frac{DDE}{sDDT}$ | $\frac{DDD}{sDDT}$ | $\frac{DDT}{sDDT}$ |
| I | r | 28 | −0.59 | 0.23 | 0.67 | 16 | 0.08 | −0.21 | 0.56 | 9 | −0.54 | 0.08 | 0.58 |
| | b | | −0.006 | 0.001 | 0.005 | | 0.001 | −0.001 | 0.005 | | −0.003 | 0.000 | 0.003 |
| | α | | 70.58 | 12.52 | 16.91 | | 52.53 | 17.51 | 17.68 | | 66.19 | 13.74 | 20.07 |
| VIII | r | 20 | −0.29 | 0.32 | 0.17 | 10 | −0.23 | 0.31 | −0.08 | 10 | −0.31 | 0.04 | 0.32 |
| | b | | −0.002 | 0.001 | 0.001 | | −0.001 | 0.001 | −0.000 | | −0.007 | 0.000 | 0.007 |
| | α | | 58.39 | 13.35 | 28.26 | | 54.13 | 14.22 | 32.82 | | 64.52 | 13.82 | 21.67 |
| IX | r | 30 | 0.16 | 0.29 | −0.26 | 16 | 0.26 | 0.20 | −0.27 | 14 | −0.15 | 0.29 | 0.05 |
| | b | | 0.001 | 0.001 | −0.002 | | 0.002 | 0.000 | −0.002 | | −0.004 | 0.003 | 0.001 |
| | α | | 46.60 | 11.19 | 42.22 | | 46.03 | 14.01 | 39.96 | | 51.92 | 7.32 | 40.76 |
| XIII | r | 15 | 0.08 | 0.16 | −0.11 | 10 | −0.03 | 0.17 | −0.01 | 5 | −0.07 | 0.26 | −0.00 |
| | b | | 0.001 | 0.000 | −0.001 | | −0.000 | 0.000 | −0.000 | | −0.002 | 0.002 | −0.000 |
| | α | | 38.05 | 10.57 | 51.36 | | 40.91 | 10.31 | 48.77 | | 38.90 | 9.26 | 51.78 |

low ($-0.5 < r < +0.5$). The results obtained do not warrant a comparison of the mean ratios $\frac{DDx}{sDDT}$ (where DDx stands for DDT, DDD×1.11 or DDE×1.11 and sDDT stands for DDT+DDD ×1.11+DDE×1.11) using a standard weight. Thus, the mean figures and 95 % confidence intervals of these ratios $\frac{DDx}{sDDT}$ are calculated without consideration to the individual weights of the

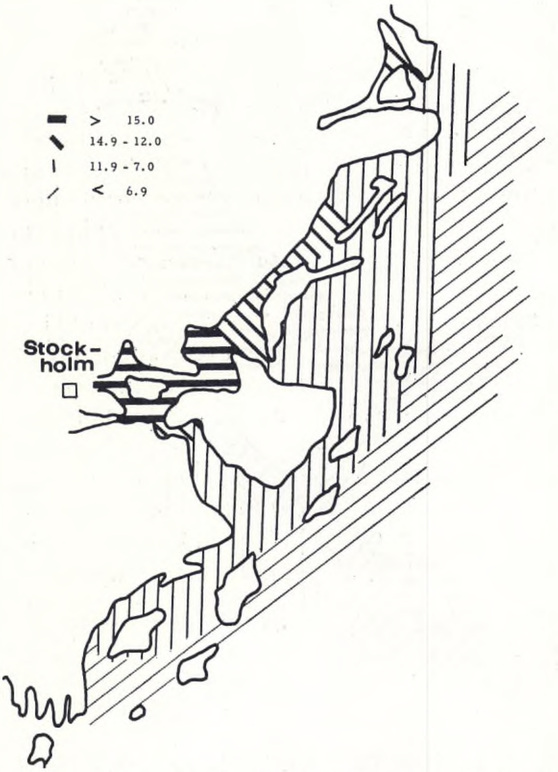


Fig. 8. Levels of PCB (mg/kg) in extractable fat of lateral muscle in pike from the Stockholm archipelago.

Table 9. *The mean ratio* $\frac{\text{DDx}}{\text{sDDT}}$ *and approximative 95 % confidence interval in female and male pike from regions where 5 or more specimens of each sex are available.* $n$ = *number of specimens analyzed. Significant differences between the levels of each sex are given by* — *if* $p \geq 0.05$, + *if* $0.01 \leq p < 0.05$, ++ *if* $0.001 \leq p < 0.01$, +++ *if* $p < 0.001$.

| Region | Sex | n | $\frac{\text{DDE}}{\text{sDDT}}$ | $\frac{\text{DDD}}{\text{sDDT}}$ | $\frac{\text{DDT}}{\text{sDDT}}$ |
|---|---|---|---|---|---|
| I | ♀♀ | 16 | 0.55 ± 0.10 | 0.14 ± 0.04 | 0.30 ± 0.07 |
| | ♂♂ | 9 | 0.62 ± 0.04 | 0.14 ± 0.02 | 0.24 ± 0.03 |
| | | | – | – | – |
| V | ♀♀ | 7 | 0.38 ± 0.04 | 0.13 ± 0.02 | 0.48 ± 0.05 |
| | ♂♂ | 8 | 0.45 ± 0.04 | 0.11 ± 0.03 | 0.44 ± 0.07 |
| | | | + | – | – |
| VIII | ♀♀ | 10 | 0.50 ± 0.04 | 0.17 ± 0.02 | 0.33 ± 0.03 |
| | ♂♂ | 10 | 0.56 ± 0.03 | 0.14 ± 0.02 | 0.29 ± 0.03 |
| | | | + | – | – |
| IX | ♀♀ | 16 | 0.49 ± 0.05 | 0.15 ± 0.02 | 0.36 ± 0.05 |
| | ♂♂ | 14 | 0.48 ± 0.05 | 0.10 ± 0.02 | 0.42 ± 0.05 |
| | | | – | +++ | – |
| XII | ♀♀ | 10 | 0.53 ± 0.04 | 0.14 ± 0.02 | 0.33 ± 0.06 |
| | ♂♂ | 5 | 0.58 ± 0.07 | 0.11 ± 0.02 | 0.31 ± 0.08 |
| | | | – | – | – |
| XIII | ♀♀ | 10 | 0.38 ± 0.07 | 0.12 ± 0.01 | 0.50 ± 0.07 |
| | ♂♂ | 5 | 0.39 ± 0.07 | 0.11 ± 0.02 | 0.50 ± 0.08 |
| | | | – | – | – |

fishes. For the statistical method, see the Appendix, part VI.

To test whether there are any differences in the mean ratios between the sexes, the mean ratios for each sex have been calculated and the differences have been tested in regions where 5 or more specimens of each sex are available, (see Table 9). For the statistical method, see the Appendix, part VII. Only $p < 0.001$ is considered as a significant difference. This is a result of the reservations in the Appendix, Statistical methods, part VII.

In only 1 out of 18 tests are the differences significant. Thus it seems adequate to use the mean ratio for both sexes together. In Table 10 the calculated mean ratios are given. The ratios in the regions have been tested against each other (see Table 11). The two tables show that there is a statistically, significant increase of the ratios $\frac{\text{DDT}}{\text{sDDT}}$ and a decrease of $\frac{\text{DDE}}{\text{sDDT}}$ in the outer part of the archipelago. In the northern fairway the same relation is found when comparing the inner region VII, with the outer regions V and VI. In the southern fairway, region XVII outside the town of Nynäshamn shows an increase in $\frac{\text{DDE}}{\text{sDDT}}$ and a decrease of $\frac{\text{DDT}}{\text{sDDT}}$ when compared to regions XIV and XV, (see Tables 10 and 11).

## V. MERCURY, DDT AND PCB COMPOUNDS IN PIKE FROM ISLAND LAKES IN THE ARCHIPELAGO

Analysis have also been carried out on 5 pike from each of two lakes Skären (loc. 45) and Kyrksjön (loc. 46) situated only 400 m apart on the island Ljusterö, downwind from Stockholm in the

Table 10. *The mean ratio and approximative 95 % confidence interval of* $\frac{DDx}{sDDT}$ *in pike from different regions, both sexes together. n = number of specimens analyzed.*

| Region | n | $\frac{DDE}{sDDT}$ | $\frac{DDD}{sDDT}$ | $\frac{DDT}{sDDT}$ |
|---|---|---|---|---|
| I | 30 | $0.57 \pm 0.07$ | $0.14 \pm 0.03$ | $0.29 \pm 0.05$ |
| II | 17 | $0.56 \pm 0.04$ | $0.14 \pm 0.02$ | $0.30 \pm 0.04$ |
| III | 5 | $0.57 \pm 0.11$ | $0.16 \pm 0.07$ | $0.27 \pm 0.07$ |
| IV | 20 | $0.57 \pm 0.05$ | $0.14 \pm 0.02$ | $0.29 \pm 0.04$ |
| V | 15 | $0.42 \pm 0.03$ | $0.12 \pm 0.02$ | $0.46 \pm 0.05$ |
| VI | 5 | $0.35 \pm 0.06$ | $0.07 \pm 0.01$ | $0.58 \pm 0.08$ |
| VII | 5 | $0.53 \pm 0.04$ | $0.14 \pm 0.03$ | $0.33 \pm 0.03$ |
| VIII | 20 | $0.53 \pm 0.03$ | $0.16 \pm 0.02$ | $0.32 \pm 0.03$ |
| IX | 30 | $0.49 \pm 0.03$ | $0.13 \pm 0.02$ | $0.38 \pm 0.04$ |
| X | 5 | $0.47 \pm 0.02$ | $0.11 \pm 0.04$ | $0.42 \pm 0.04$ |
| XI | 5 | $0.58 \pm 0.14$ | $0.13 \pm 0.03$ | $0.29 \pm 0.12$ |
| XII | 15 | $0.55 \pm 0.04$ | $0.13 \pm 0.02$ | $0.32 \pm 0.05$ |
| XIII | 15 | $0.39 \pm 0.05$ | $0.11 \pm 0.01$ | $0.50 \pm 0.05$ |
| XIV | 5 | $0.38 \pm 0.07$ | $0.11 \pm 0.03$ | $0.51 \pm 0.07$ |
| XV | 5 | $0.36 \pm 0.06$ | $0.13 \pm 0.01$ | $0.50 \pm 0.07$ |
| XVII | 9 | $0.68 \pm 0.14$ | $0.13 \pm 0.02$ | $0.19 \pm 0.12$ |

prevailing wind. The mean levels for mercury, DDT and PCB compounds are given in Table 12. The differences between the levels of these compounds have been statistically tested (see Table 12). The levels of DDT-compounds are significantly higher in Skären. For mercury and PCB, no significant differences were found but the mean values were, however, higher in Skären. One pike with a very high level of mercury increased the variance in Skären. If this single pike was omitted when testing the difference between the two lakes, a significant, higher mercury level was established in Skären (see Table 12). Two homogenates of the isopode *Asellus aquaticus*, from Skären and Kyrksjön contained 0.15 and 0.09 ppm respectively of mercury on dry weight basis.

The mean ratio DDx/sDDT in the two lakes have been calculated (see Table 13). Test of ratio differences (see Table 13) showed a significant, higher proportion of $\frac{DDE}{sDDT}$ and a lower of $\frac{DDD}{sDDT}$ in Skären.

Table 11. *Results from tests of differences of* $\frac{DDx}{sDDT}$ *between different regions. Results given as* — *if* $p \geq 0.05$, + *if* $0.01 \leq p < 0.05$, ++ *if* $0.001 \leq p < 0.01$, +++ *if* $p < 0.001$.

| Region | Significant differences $\frac{DDE}{sDDT}$ | $\frac{DDD}{sDDT}$ | $\frac{DDT}{sDDT}$ | Region | Significant differences $\frac{DDE}{sDDT}$ | $\frac{DDD}{sDDT}$ | $\frac{DDT}{sDDT}$ |
|---|---|---|---|---|---|---|---|
| I—II | — | — | — | IV—VII | — | — | — |
| I—III | — | — | — | V—VI | + | +++ | ++ |
| I—IV | — | — | — | V—VII | +++ | — | +++ |
| I—V | +++ | — | +++ | V—X | + | — | — |
| I—VIII | — | — | — | V—XIII | — | — | — |
| I—IX | + | — | ++ | VI—VII | +++ | +++ | +++ |
| I—X | ++ | — | +++ | VIII—IX | — | + | ++ |
| I—XI | — | — | — | VIII—X | ++ | + | +++ |
| I—XII | — | — | — | VIII—XIII | +++ | +++ | +++ |
| I—XIII | +++ | + | +++ | IX—X | — | — | — |
| I—XIV | +++ | — | +++ | IX—XIII | ++ | — | +++ |
| I—XV | +++ | — | +++ | X—XIII | ++ | — | + |
| I—XVII | — | — | — | XI—XII | — | — | — |
| II—III | — | — | — | XI—XIII | + | — | ++ |
| II—IV | — | — | — | XI—XIV | + | — | ++ |
| II—V | +++ | — | +++ | XI—XV | ++ | — | ++ |
| II—VIII | — | — | — | XII—XIII | +++ | — | +++ |
| II—IX | + | — | ++ | XII—XIV | +++ | — | +++ |
| II—X | +++ | — | +++ | XII—XV | +++ | — | +++ |
| II—XIII | +++ | ++ | +++ | XIII—XIV | — | — | — |
| III—IV | — | — | — | XIII—XV | — | + | — |
| III—V | + | — | +++ | XIII—XVII | +++ | — | +++ |
| III—VI | +++ | + | +++ | XIV—XV | — | — | — |
| IV—V | +++ | — | +++ | XIV—XVII | +++ | — | +++ |
| IV—VI | +++ | +++ | +++ | XV—XVII | +++ | — | +++ |

Table 12. *Mean levels in ppm of mercury in fresh tissue for a standard pike (1 kilo) and mean levels of DDT and PCB substances in extractable fat and fresh tissue in pike from two island lakes in the archipelago. For mercury, DDT and PCB substances in extractable fat, the 95 % confidence limit are given. n = number of specimens. Results of testing the differences between the two lakes are given as — if $p \geq 0.05$, + if $0.01 \leq p < 0.05$, ++ if $0.001 \leq p < 0.01$, +++ if $p < 0.001$. For Skären the mercury level has also been calculated when one very high value has not been taken into account.*

| Locality | n | Mean weight | Hg | Extractable fat | | | | | Fresh tissue | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | sDDT | DDE | DDD | DDT | PCB | sDDT | DDE | DDD | DDT | PCB |
| 45 Skären | 5 | 1557 | 1.66±1.67 | 11±5 | 6.4±3.0 | 1.5±0.7 | 1.9±1.0 | 10±6 | 0.053 | 0.032 | 0.0075 | 0.0097 | 0.050 |
| | 4 | 1536 | 1.32±0.39 | | | | | | | | | | |
| 46 Kyrksjön | 5 | 933 | 0.38±0.10 | 3.2±2.2 | 1.6±1.2 | 0.67±0.45 | 0.63±0.36 | 4.1±2.7 | 0.012 | 0.0063 | 0.0026 | 0.0025 | 0.016 |
| Differences | 5+5 | | — | + | + | + | + | — | | | | | |
| | 4+5 | | ++ | | | | | | | | | | |

Table 13. *The mean ratio* $\frac{DDx}{sDDT}$ *and approximative 95 % confidence interval in pike from two island lakes in the archipelago. n = number of specimens. The differences between the localities has been tested. Result given as — if $p \geq 0.05$, + if $0.01 \leq p < 0.05$, ++ if $0.001 \leq p < 0.01$, +++ if $p < 0.001$.*

| Locality | n | DDE/sDDT | DDD/sDDT | DDT/sDDT |
|---|---|---|---|---|
| 45 | 5 | 0.66±0.04 | 0.16±0.01 | 0.18±0.04 |
| 46 | 5 | 0.57±0.03 | 0.23±0.01 | 0.20±0.02 |
| Differences | 5+5 | +++ | +++ | — |

## VI. DISCUSSION

### *Mercury*

In the northern fairway a decrease in the levels towards the peripheral parts has been statistically demonstrated (Tables 2, 3 and Fig. 3). In area VI outside Norrtälje a significant increase, in comparison to surrounding areas IV and V, is observed. This may be an effect of local pollution. The decrease in peripheral direction from Stockholm is also statistically proved along the main fairway and the southern fairway.

The highest mean level was found in the southern fairway outside an old paper mill (region XI). The plant was closed down in 1964 but old sediments containing fibres are still in place. The use of mercury in the industrial process, which has been confirmed (Pettersson personal communication 1972), may be one probable explanation for the high mean level here. This region is also close to the two main sewage treatment plants of Stockholm (Henriksdal and Käppala), a fact which may also influence the values.

In the outer parts (regions XIII—XVII) of the southern fairway rather low mean values of mercury are observed. In region XVII, near the town Nynäshamn, no obvious increase is noted. However, one single pike caught in the harbour of Nynäshamn (loc. 44), weighing only 0.6 kg, contained 1.6 mg/kg of mercury, indicating local pollution here.

Looking at the mean values only (see Table 2) there seems to be a rapid decrease from regions XI to XII. The sound Boo channel between regions XI and XII is very narrow and the flow of polluted water, to the larger region XII, is small, which may explain the rapid decrease.

The outer parts of the fairways can be characterized as regions having good water exchange with the Baltic. Region X, in the outer part of the main fairway, showed a higher, but not a significantly higher mean level than those regions of V and XIII (the outer parts of the northern and southern fairways). This may indicate that the main flow of pollutants from Stockholm passes through the main fairway. This is in agreement with data on nutrients (phosphates, nitrates etc) in the water (WAERN and PEKKARI 1973). The levels of mercury observed give a clear indication of extensive mercury pollution of the Stockholm archipelago. Only region XVI showed low "natural" levels.

Since mercury is bioaccumulating the levels in the different regions must — among other factors — also depend on the density of the biomass into which the mercury has to be accumulated. As the density is higher in the inner part of the archipelago, the levels in this part will not be so high as those expected if the lower biomass characterizing the outer part existed throughout the studied area. At the same time, the higher density of the biomass in the inner part increases the sedimentation, and binding of mercury, organic or inorganic, to the sediments for a longer or shorter time is possible (HÅKANSSON 1972). Thus the inner part may act as a "mercury trap".

### *DDT substances*

The DDT pollution seems to be more complicated than that of mercury. There are several reports of biological and microbiological conversion of DDT to DDD (BRIDGES *et al.* 1963, KALLMAN and ANDREWS 1963, FINLEY and PILLMORE 1963, MISKUS *et al.* 1965, MENDEL and WALTON 1966, WEDEMEYER 1966, JOHNSON *et al.* 1967, KO and LOCKWOOD 1968). Aquatic microorganisms will to a smaller extent also metabolize DDT to DDE (COPE and SANDERS 1963, MATSUMURA *et al.* 1971). As in the case of mercury, the levels of the bioaccumulating DDT substances in pike ought to be influenced by the density of the biomass. Furthermore, the degree of degradation to DDD and DDE may be dependent on the degree of biological activity. Lastly, the Baltic is known to be heavily polluted with DDT substances (JENSEN *et al.* 1969 and 1972 a) — compared with the local pollution with mercury — resulting in the possibility of pollution of the archipelago from two directions, the Baltic and the City of Stockholm.

Regarding the proportion between the different DDT substances, it can be stated that DDT/sDDT >DDE/sDDT in the outer parts and DDT/sDDT <DDE/sDDT in the inner parts (see Tables 10 and 11). This is also true when comparing region VII to region VI, and region XVII to the surrounding regions, where regions VII and XVII can be regarded as inner parts of the archipelago, since they are close to the shore and local sources of pollution.

A slight increase has been shown in the DDE proportion when the salinity of the water rises (MURPHY 1970). In the Stockholm archipelago the salinity increases towards the peripheral parts and the DDE proportion decreases. This may be explained by the higher microbiological activity converting DDT into DDE and DDD more rapidly in areas with a high density of biomass — the inner part of the archipelago — compared to areas with a lower biomass — the outer part of the archipelago.

No consequent, significant, change of the sDDT levels are recorded when comparing the outer and inner parts. However, looking at the mean values only, the levels seem to be lower in the outer parts than in the inner part (see Tables 6, 7 and Fig. 4). The significant, higher levels of sDDT in region VII compared to regions V and VI cannot be explained.

The few significant differences in the levels of DDT itself may be random, but looking at the mean values only, the highest contents are often found in the peripheral parts (see Table 6 and Fig. 5).

For levels of DDE and DDD there is a consequent, significant decrease, comparing the innermost parts to the outmost (see Tables 6, 7 and Figs. 6, 7).

Since DDE is more persistent than DDD, higher levels of DDE are to be expected.

If there exists a correlation between fish weight and the level of DDT compounds, the differences in mean weight between the regions would influence the levels. Since the mean weights are mostly lowest in the outer parts of the archipelago (see Table 6), the decreases in levels in the peripheral parts could depend on a decreasing mean weight. However, some values argue against this. In region XI close to region I a very high mean weight is recorded but the mean levels for DDT substances were nevertheless lower than in region I. In regions VII and XIV on the other hand, a low mean weight is found, but the levels here were nevertheless rather high. Thus even if the mean fish weight of the regions has an influence upon the levels, this is obviously not the main explanation for the observed differences.

With respect to the degree of microbiological activity as a factor for DDT metabolism, some hypothetical explanations for the presence of DDT substances in the studied area can be given.

1) The nonsignificant differences in the mean levels of sDDT and DDT depend on chance. Thus the amounts of sDDT and DDT are the same all over the studied area but DDE and DDD decrease significantly from the inner parts to the outer.

The most probable explanation for this would be that the sources of the DDT pollution in 1968 were or had been in the inner part of the archipelago, but the higher microbiological activity here metabolizes DDT. DDD which is known to be the main constituent in a DDT degradation by aquatic microorganisms is then further converted while the rather small amount of DDE also formed is more stable and the DDE levels may thus be a result of several years of DDT pollution. An earlier DDT contamination of the inner parts of the archipelago gives rise to a decreasing level of DDE when leaving these parts.

2) The nonsignificant differences in mean levels of sDDT and DDT are true. The highest levels for sDDT are mainly found in the inner part of the archipelago while for DDT they are mainly found in the peripheral parts.

An explanation for this may be that in 1968 there was or had been a release of DDT from the inner parts, giving higher levels of DDE and sDDT here. That DDT itself does not increase can be explained by the higher degree of microbiological degradation towards the inner part. The higher mean levels of DDT itself in some peripheral parts and the generally higher $\frac{\text{DDT}}{\text{sDDT}}$ ratio here (see regions VI, XIII, XIV, XV, Tables 6 and 10) can be explained by an influence of DDT from the Baltic.

Regarding the higher DDE levels in the inner parts, it seems probable in both of the explanations that sources here have contaminated the archipelago. The higher $\frac{\text{DDT}}{\text{sDDT}}$ ratio in the peripheral parts and the fact that the Baltic is heavily contaminated by DDT, suggest an outmost pollution from the Baltic to be a contributory factor.

In 1970, the use of DDT was banned in Sweden and investigations in the future may give a basis for evaluating results of the ban.

### *PCB*

The pattern for the PCB pollution is in good agreement with that for mercury. The mean levels in pike are significantly decreasing throughout the three fairways (see Tables 6, 7 and Fig. 8). In one of the sewage treatment plants of Stockholm it has been confirmed that the sludge contains high levels of PCB (Ahling and Jensen 1970). The results obtained in the present paper are in good agreement with earlier investigations on plankton samples from the northern fairway (Jensen *et al.* 1972 b). In the latter publication, it was pointed out that antifouling paint contaminates the samples with PCB. In all the studied areas there is an intensive boat traffic — commercial shipping and pleasure boats — and the contamination of the water from antifouling paint may also influence the levels found in pike.

## VII. MERCURY, DDT AND PCB SUBSTANCES IN TWO ISLAND LAKES

The levels of contaminating compounds in pike from Skären and Kyrksjön are quite different. In neither of them, are pike able to migrate from the archipelago up to the lakes. Nor can the differences be explained by local pollutions, such as industrial or community waterborne wastes. The two lakes are situated 400 m from each other on the island Ljusterö. Skären (loc. 45) a spring lake is surrounded by only a few holiday cottages and is oligotrophic ($\varkappa_{20} \cdot 10^6$ was 130 in January 1971 and in October 1971, 140). The shore vegetation consists of sedges and sparse clumps of reed. The mean depth is about 4 m and the water surface is twice as large as that of Kyrksjön. Kyrksjön (loc. 46) is, on the other hand, surrounded by a few farms and eutrophic ($\varkappa_{20} \cdot 10^6$ was 650 in January 1971 and 750 in October 1971). The shore vegetation consisting of clumps of reed is thick and the presence of *Buteomus umbellatus* also characterizes the eutrophic conditions. The mean depth is about 2 m. Estimations in spring 1972 of the water flow in the outlet give approximately the same water flow in both the two lakes. The mean levels of compounds tested here are or seem to be higher in Skären, than in Kyrksjön. The higher mercury levels could possibly be explained by differences in the mineral background, despite the fact that the lakes are situated only 400 m from each other. In Sweden DDT has mainly been used by farmers and thus the highest levels were to be expected in Kyrksjön. But the highest levels were recorded in Skären. The presence of PCB, which is mostly used industrially, in the two lakes indicates airborne fallout. The levels also seem to be higher for PCB in Skären. In the spring of 1972, at the time of ice melting, a sample of soot taken on the ice of Lake Skären, where the soot was concentrated to pores in the ice, contained 3.4 ppm of mercury, 0.24 ppm of DDT substances and 2.9 ppm of PCB on a dry weight basis. Thus it must be reasonable to assume that the major part of mercury and DDT substances also reach the lakes from the air.

The differences in the levels of DDT substances and the possible difference in the mean levels of mercury and PCB between the lakes may be explained by the higher biomass in Lake Kyrksjön, giving lower levels of bioaccumulating substances/unit weight organic matter in Kyrksjön. There may also be a slower water exchange in the deeper lake, Skären, resulting in a longer exposition time of the water for airborne fallout. If the previously mentioned hypothesis, that the levels of the more persistent DDE gives an indication of a long term pollution and DDD which is further converted gives an indication of recent microbiological degradation of DDT, the higher $\frac{\text{DDE}}{\text{sDDT}}$ in Skären can be explained by a slower water exchange, while the higher $\frac{\text{DDD}}{\text{sDDT}}$ in Kyrksjön may be explained by the higher microbiological activity.

## VIII. SOURCES OF THE POLLUTION

The following are probable sources for the pollution of the archipelago by mercury, DDT and PCB substances.

### *Mercury*

High values of mercury have been found in the water from the sewage treatment plant at Henriksdal in Stockholm (Westermark *et al.* 1968). It has also been indicated in the present paper that a local source of pollution in the inner part of the archipelago is the paper mill in region XI. Thus it is obvious that the waters of the archipelago are polluted via waters from the inner part. It seems also probable that the pollution is airborne to some extent (Johnels *et al.* 1967 a) and the levels in soot particles mentioned earlier stress the importance of this source. A loss of mercury when burning coal and mineral oils has earlier been postulated (Westermark and Ljunggren 1968, Ljunggren *et al.* 1971).

For the island lakes, where the sewage is restricted, or non-existent, airborne fallout seems to be the most probable source.

### *DDT substances*

Because of the more complicated pattern for the occurrence of the various DDT substances, the sources are not so easy to account for. However, it seems most reasonable, from the studies on the occurence of DDE and DDD, that there are or have been sources of DDT substances in the inner part of the archipelago. On the other hand, the DDT contaminated Baltic waters may also have an effect on the levels found in the archipelago, especially in the peripheral, outmost parts. The levels in pike and those in the soot particles on the ice from the island lakes also lead to the assumption that airborne fallout takes place. Airborne fallout of DDT and PCB substances has earlier been shown (Abbot *et al.* 1965, Wheatley and HARDMAN 1965, RISEBROUGH *et al.* 1968, SÖDERGREN 1972).

*PCB substances*

The PCB found in sludge from the sewage treatment plant at Henriksdal indicates a loss of PCB to the effluent water. The loss of PCB from antifouling paint will also influence the pollution situation and as the boat traffic is most intensive in the inner part, the effect will be most pronounced here. Furthermore, the PCB levels in the island lakes and the levels in airborne soot particles indicates an influence from airborne fallout.

## IX. CONCLUSIONS

Pike has been shown to be a suitable organism for a study of the pollution pattern in an area contaminated by bioaccumulating substances. For the mercury levels, the weight of the individual fishes must be considered and the mercury level at a calculated standard weight has to be used when comparing different regions. For the DDT and PCB substances in the present material, the mean levels of the samples can be used.

In the studied area, the levels of mercury and PCB decrease from Stockholm outwards in the archipelago. Local sources in the inner part of the archipelago, and for PCB, loss from antifouling paint also, seem to be mainly responsible for the pollution. Only in the outer parts of the southern area are natural low mercury levels found. DDE and DDD also decrease and sDDT seems to decrease in the same manner as for mercury and PCB. The DDT levels do not decrease outwards. Thus the ratio $\frac{\text{DDE}}{\text{sDDT}}$ is highest in the inner part of the archipelago and this is also true for $\frac{\text{DDD}}{\text{sDDT}}$. $\frac{\text{DDT}}{\text{sDDT}}$, on the other hand, is highest in the outer part. The most probable explanation for this condition is that the archipelago is or has been contaminated from local sources in the inner parts. In addition, in view of the fact that DDT levels did not decrease in the outer parts and that the ratio $\frac{\text{DDT}}{\text{sDDT}}$ increased significantly, contamination of the outermost parts of the archipelago from the Baltic seems certain.

High levels of mercury, DDT and PCB substances in pike from one of two island lakes in the prevailing wind direction from Stockholm indicate airborne fallout. Lower levels are recorded in a neighbouring lake. The differences in the levels between the lakes may be explained by a higher biomass and a more rapid water exchange in the lake with lower values.

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APPENDIX:

# Statistical Methods

JAN GRANDELL

## I. INTRODUCTION

This is an account of the statistical methods which have been used in the analysis of the levels of mercury, DDT and PCB substances. For mercury levels in pike a dependence between weight and level has ben found previously. For levels of DDT and PCB substances in pike this dependence has not been studied. As a measure of the mercury level pike with the weight of 1 kilogram have been used as standard.

## II. REGRESSION ANALYSIS

Let $X_i$ be the weight of an arbitrary pike and $Y_i$ the mercury level. In a linear regression model $Y_i$ are assumed to be independent for different values of i and normally distributed with the mean values $\alpha+\beta X_i$ and the common variance $\sigma^2$. Thus $Y_i=\alpha+\beta X_i+\varepsilon_i$ where $\varepsilon_i$ are independent for different values of i and normally distributed with common mean value 0 and variance $\sigma^2$.

Assume that n specimens of pike have been collected having weights $X_1, \ldots, X_n$ and mercury levels $Y_1, \ldots, Y_n$.

Define: $\bar{X}=\frac{1}{n}\sum_1^n X_i$

$$\bar{Y}=\frac{1}{n}\sum_1^n Y_i$$

and put $a=\alpha+\beta\bar{X}$, and $b=\beta$ giving

$$\alpha+\beta X_i=\alpha+\beta\bar{X}+\beta(X_i-\bar{X})=a+b(X_i-\bar{X}).$$

This reformulation is suitable for the calculations.

Introduce the notations

$$A_{xx}=\sum_{1}^{n}(X_i-\bar{X})^2$$

$$A_{xy}=\sum_{1}^{n}(X_i-\bar{X})(Y_i-\bar{Y})$$

$$A_{yy}=\sum_{1}^{n}(Y_i-\bar{Y})^2.$$

Estimate a and b with the method of least squares *i.e.* $a^*$ and $b^*$ are chosen such that

$$\sum_{1}^{n}(Y_i-a-b(X_i-\bar{X}))^2$$

is minimized.

It is easy to show that

$$a^*=\bar{Y}$$

$$b^*=\frac{A_{xy}}{A_{xx}}$$

Consider $Q=\sum_{1}^{n}(Y_i-\bar{Y}-b^*(X_i-\bar{X}))^2=$

$$=A_{yy}+(b^*)^2A_{xx}-2b^*A_{xy}=A_{yy}-\frac{A^2_{xy}}{A_{xx}}$$

We have

$$E(Y_i-\bar{Y})^2=E(a+bX_i+\varepsilon_i-a-b\bar{X}-\bar{\varepsilon})^2=$$

$$=b^2(X_i-\bar{X})^2+E(\bar{\varepsilon}_i-\bar{\varepsilon})^2=b^2(X_i-\bar{X})^2+$$

$$+E(\varepsilon_i-\frac{1}{n}\varepsilon_i-\frac{1}{n}\sum_{k\neq i}\varepsilon_k)^2=b^2(X_i-\bar{X})^2+$$

$$+(1-\frac{1}{n})^2\sigma^2+\frac{n-1}{n^2}\sigma^2=b^2(X_i-\bar{X})^2+\frac{n-1}{n}\sigma^2.$$

From this it follows that $EA_{yy}=b^2A_{xx}+(n-1)\sigma^2$.

In a similar way it can be shown that

$$E(Y_i-\bar{Y})(Y_j-\bar{Y})=b^2(X_i-\bar{X})(X_j-\bar{X})-\frac{1}{n}\sigma^2$$

if $i\neq j$.

From this it follows that

$$EA^2_{xy}=E\sum_{i=1}^{n}\sum_{j=1}^{n}(X_i-\bar{X})(Y_i-\bar{Y})(X_j-\bar{X})$$

$$(Y_j-\bar{Y})=\sum_{i=1}^{n}(X_i-\bar{X})^2[b^2(X_i-\bar{X})^2+$$

$$+(1-\frac{1}{n})\sigma^2]+\sum_{i\neq j}(X_i-\bar{X})(X_j-\bar{X})$$

$$[b^2(X_i-\bar{X})(X_j-\bar{X})-\frac{1}{n}\sigma^2]=b^2A^2_{xx}+\sigma^2A_{xx}.$$

Thus

$$EQ=b^2A_{xx}+(n-1)\sigma^2-b^2A_{xx}-\sigma^2=(n-2)\sigma^2.$$

Thus $s^2_{y\cdot x}=\frac{Q}{n-2}$ is a good estimate of the residual variance $\sigma^2$.

From the properties of the normal distribution it follows that $\frac{s^2_{y\cdot x}}{\sigma^2}$ is $X^2(n-2)$-distributed.

We will now investigate the properties of the estimates $a^*$ and $b^*$.

$EY_i=a+b(X_i-\bar{X})$ giving $Ea^*=a$ and

$$EA_{xy}=\sum_{i=1}^{n}(X_i-\bar{X})(a+b(X_i-\bar{X}))=$$

$=bA_{xx}$ giving $Eb^*=b$.

With calculations similar to those above it can be shown that

$$\text{Var } a^*=\frac{\sigma^2}{n}$$

$$\text{Var } b^*=\frac{\sigma^2}{A_{xx}}$$

$$\text{Cov}(a^*, b^*)=0.$$

## III. TESTS OF THE REGRESSION COEFFICIENT

To test if there is a dependence between the weight and the amount of DDT and PCB substances the hypothesis $b=0$ is tested against $b\neq 0$.

We have

$$b^*=\frac{A_{xy}}{A_{xx}}=\frac{\sum_{1}^{n}(X_i-\bar{X})(a+b(X_i-\bar{X})+\varepsilon_i-a-\bar{\varepsilon})}{A_{xx}}=$$

$$=b+\frac{\sum_{1}^{n}(X_i-\bar{X})(\varepsilon_i-\bar{\varepsilon})}{A_{xx}}$$

and thus $b^*$ is normally distributed. Under the hypothesis $b=0$ thus $Eb^*=0$ and $\mathrm{Var}\ b^*=\frac{\sigma^2}{A_{xx}}$

Since $\sigma^2$ has to be estimated with $s^2_{y\cdot x}$ we perform

$$t=\frac{b^*}{\frac{s^2_{y\cdot x}}{A_{xx}}}$$

which is t(n—2)-distributed and thus the hypothesis is rejected on the 5 % level when

$$|t|>t_{0.975}(n-2)$$

where $t_{0.975}(n-2)$ is 97.5:th percentile for the t-distribution with (n—2) degrees of freedom.

This and all other tests are described on the 5 % level. In the tables, however, it is indicated whether the hypothesis is accepted or rejected on the 5 %, 1 % or 0.1 % levels. There the letter p denotes the calculated level on which the hypothesis is rejected.

## IV. CONFIDENCE INTERVAL FOR MEAN VALUES

Consider levels of DDT and PCB substances where the dependence between the weight and the levels is neglected. Let $Y_1, \ldots, Y_n$ be the observed values for a compound in n different pikes, which are assumed to be independent and normally distributed with the common mean value n and variance $\sigma^2$. A 95 % confidence interval for n is then given by

$\bar{Y}\pm t_{0.975}(n-l)s$ where

$$s^2=\frac{1}{n}\frac{1}{n-l}\sum_{1}^{n}(Y_i-\bar{Y})^2$$

For the mercury level of a standard pike (weight 1 kilogram), *i.e.* $X_i=1000$, $EY_i=a+b(1000-\bar{X})$ is valid.

An estimate $\bar{M}$ of $a+b(1000-\bar{X})$ is thus given by $\bar{M}=a^*+b^*(1000-\bar{X})$.

For $\bar{M}$ we have

$E\bar{M}=a+b(1000-\bar{X})$ and

$$\mathrm{Var}\ \bar{M}=\sigma^2\left(\frac{1}{n}+\frac{1000-\bar{X})^2}{A_{xx}}\right).$$

Since $\sigma^2$ is unknown we perform

$$\frac{\bar{M}-a-b(1000-\bar{X})}{s_{y\cdot x}\sqrt{\frac{1}{n}+\frac{(100-\bar{X})^2}{A_{xx}}}}$$ which is t(n—2)-distributed.

Define

$$s^2=s^2_{y\cdot x}\left(\frac{1}{n}+\frac{(1000-\bar{X})^2}{A_{xx}}\right)$$

A 95 % confidence interval for $a+b(1000-\bar{X})$ is thus given by $M\pm t_{0.975}(n-2)s$.

## V. COMPARISONS BETWEEN MEAN VALUES

Firstly the comparisons of the levels of DDT and PCB substances are studied and here the dependence between the weight and the levels is neglected.

Let $Y^{(1)}_1,\ldots,Y^{(1)}_{n_1}$ and $Y^{(2)}_1,\ldots,Y^{(2)}_{n_2}$ be the two different observations which we assume to be independent and normally distributed with mean values $\mu_1$ and $\mu_2$ and variances $\sigma^2_1$ and $\sigma^2_2$ respectively. The hypothesis $\mu_1=\mu_2$ will be tested against $\mu_1\neq\mu_2$. If $\sigma^2_1$ and $\sigma^2_2$ were known we should perform

$$Z=\frac{\bar{Y}^{(1)}-\bar{Y}^{(2)}}{\frac{\sigma^2_1}{n_1}+\frac{\sigma^2_2}{n_2}}$$

and reject the hypothesis when

$|Z|>Z_{0.975}$ where $Z_{0.975}$ is 97.5:th percentile for the normal distribution.

Since $\sigma^2_1$ and $\sigma^2_2$ are known we have to estimate

$\frac{\sigma^2_1}{n_1}$ and $\frac{\sigma^2_2}{n_2}$ respectively.

These quantities are estimated with

$$s^2_1=\frac{1}{n_1}\frac{1}{n_1-1}\sum_{1}^{n_1}(Y^{(1)}_i-\bar{Y}^{(1)})^2$$

and

$$s^2_2 = \frac{1}{n_2}\,\frac{1}{n_2-1}\sum_{1}^{n_2}(Y^{(2)}_i-\bar{Y}^{(2)})2 \text{ respectively.}$$

Thus we perform

$$t=\frac{\bar{Y}^{(1)}-\bar{Y}^{(2)}}{\sqrt{s^2_1+s^2_2}}.$$

The following approximation, proposed by COCHRAN (1964) requires only minor calculations and gives sufficient accuracy for our purposes. According to Cochran the hypothesis $\mu_1=\mu_2$ is rejected when

$$|t|>\frac{s^2_1\, t_{0.975}(n_1-1)+s^2_2\, t_{0.975}(n_2-1)}{s^2_1+s^2_2}.$$

Secondly, for the mercury levels where the hypothesis

$$a_1+b_1(1000-\bar{X}^{(1)})=a_2+b_2(1000-\bar{X}^{(2)})$$

is to be tested against the hypothesis

$$a_1+b_1(1000-\bar{X}^{(1)})\neq a_2+b_2(1000-\bar{X}^{(2)}).$$

Thus with the notations used in section IV, we perform

$$t=\frac{\overline{M}_1-\overline{M}_2}{\sqrt{s^2_1+s^2_2}}$$

and reject the hypothesis when

$$|t|>\frac{s^2_1\, t_{0.975}(n_1-2)+s^2_2\, t_{0.975}(n_2-2)}{s^2_1+s^2_2}$$

## VI. CONFIDENCE INTERVALS FOR THE PROPORTIONS OF VARIOUS DDT SUBSTANCES

We are to calculate an approximate confidence interval for the proportion of DDT, 1.11×DDE and 1.11×DDD respectively with respect to sDDT=DDT+1.11×DDE+1.11×DDD.

Consider the ratio $P=\frac{DDx}{sDDT}$

where DDx stands for DDT, 1.11×DDE or 1.11×DDD.

Assume that n pike have been collected. Let $X_i$ and $T_i$ be the amount of DDx and sDDT respectively in the i:th pike. Strictly P is defined by $P=\frac{EX_i}{ET_i}$.

Put $P^*=\frac{\bar{X}}{\bar{T}}$

where $\bar{X}=\frac{1}{n}\sum_{1}^{n}X_i$ and $\bar{T}=\frac{1}{n}\sum_{1}^{n}T_i$.

From the assumptions in section V it follows that $\bar{X}$ and $\bar{T}$ are normal distributed with mean values $E\bar{X}$ and $E\bar{T}$ and variances Var $\bar{X}$ and Var $\bar{T}$ respectively. By a Taylor expansion of $P^*$ we get

$$P^*\approx\frac{E\bar{X}}{E\bar{T}}+\frac{\bar{X}-E\bar{X}}{E\bar{T}}-\frac{E\bar{X}(\bar{T}-E\bar{T}}{(E\bar{T})^2}$$

and thus $P^*$ is approximately normally distributed with mean value

$\frac{E\bar{X}}{E\bar{T}}=P$ and variance

$$\frac{\mathrm{Var}\,\bar{X}}{(E\bar{T})^2}+\frac{(E\bar{X})^2\,\mathrm{Var}\,\bar{T}}{(E\bar{T})^4}-\frac{2\,E\bar{X}\,\mathrm{Cov}(\bar{X},\bar{T})}{(E\bar{T})^3}=$$

$$=\frac{1}{n(E\bar{T})^4}[(E\bar{T})^2\,\mathrm{Var}\,X_i+(E\bar{X})^2\,\mathrm{Var}\,T_i-$$

$$-2E\bar{X}\,E\bar{T}\,\mathrm{Cov}(X_i,T_i)]$$

An estimate of this variance is given by

$$s^2=\frac{1}{n(\bar{T})^4}[(\bar{T})^2\,s^2(X)+(\bar{X})^2\,s^2(T)-\bar{X}\bar{T}\,r(X,T)]$$

where

$$s^2(X)=\frac{1}{n-1}\sum_{1}^{n}(X_i-\bar{X})^2$$

$$s^2(T)=\frac{1}{n-1}\sum_{1}^{n}(T_i-\bar{T})^2$$

$$r(X,T)=\frac{1}{n-1}\sum_{1}^{n}(X_i-\bar{X})(T_i-\bar{T})$$

An approximative 95 % confidence interval for P is thus given by $P^*\pm Z_{0.975}s$. It should, however, be observed that this approximative confidence interval probably is somewhat too short.

## VII. COMPARISONS OF THE PROPORTIONS OF VARIOUS DDT SUBSTANCES

Let $P_1$ and $P_2$ be two ratios from different locations for the same DDT substance. Let $P^*_1$ and $P^*_2$ be defined as in section VI and let $s^2_1$ and $s^2_2$ be the estimates of the respective approximative variances.

Perform

$$Z = \frac{P^*_1 - P^*_2}{\sqrt{s^2_1 + s^2_2}}$$

which is approximatively normally distributed with mean value $P_1 - P_2$ and variance 1.

The hypothesis $P_1 = P_2$ against $P_1 \neq P_2$ is thus rejected when $|Z| > Z_{0.975}$. For this test it is very important to observe that the level of the test is approximative. Firstly, $P^*_1$ and $P^*_2$ are only approximatively normally distributed and secondly, we cannot use the t-distribution instead of the normal distribution in order to compensate for the use of estimated variances instead of true variances. Probably this implies that the true level of this test is lower than the approximative and thus the results should be interpreted with great care.

## VIII. REFERENCE

Cochran, W. G. 1964. Approximate significance levels of the Behrens-Fisher test. *Biometrics* 20: 191.

# On Long-Term Stability of Zooplankton Composition

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## I. INTRODUCTION

While zooplankton studies comprising a period of several years are relatively often reported in the literature, comparisons of samples from different decades and centuries are rarely found. One reason is that most of the investigations of the zooplankton biocenose as such are quite recent, the first planktologists having been mainly interested in taxonomic and morphological problems and, occasionally, in the autecology and geographical distribution of separate species.

Only in a few cases were such comparisons between samples from different decades undertaken, and in most of these cases only one lake was dealt with, *e.g.* Lake Zürichsee by MINDER (1938), Lake Constance by KIEFER (1972, 1973), Lake Erie by GANNON and BEETON (1971), Lake Michigan by GANNON (1972), Lake Washington by EDMONDSON (*e.g.* 1969 and 1972), seven Norwegian lakes by LANGELAND (1972), some North Swedish lakes by HJELM (1972), Lake Hjälmaren and Ekoln (a bay of Lake Mälaren) by GRÖNBERG (1971 a and 1973 a, respectively). In the present paper the author has tried to compile data from as many as possible of the Central Swedish lakes, where good investigations were made during different decades.

Comparisons of this kind, of course, involve a certain inaccuracy (see further the Chapter 3). However, this should not be too discouraging, considering the great need for continuous registration of environmental data and, at the same time, the rare opportunities of following real long-term variation. Here it is, of course, presupposed that the conclusions are drawn in a correct way with regard to all sources of error mentioned below.

## II. MATERIAL

Archived material (previously unpublished), the author's own records, as well as the literature, form the basis of the comparisons. There are three authors of the archived material: W. LILLJEBORG, S. EKMAN and B. BERZINS. EKMAN's and BERZINS' material consists of lists referring to separate samples. Frequencies are often given in EKMAN's lists (*cf.* Tables 1, 3 and 4). They are archived at the Institute of Limnology in Uppsala. LILLJEBORG's material constitutes a special case, as it is compiled from a great number of labels referring to single, isolated specimens picked out for taxonomic purposes. This means that, where a gap for a species occurs in his records, this does not necessarily imply that LILLJEBORG had not encountered that species, but only that no specimen was picked out. This could be considered a reason for excluding LILLJEBORG's records from the comparisons, but as these records constitute the only material preserved from the previous century it can nevertheless be considered to be of great documentary interest and has therefore been included. Besides, considering the special character of this material it seems surprisingly complete, in most cases containing about as many species as in later records. LILLJEBORG's collections (and labels) are archived at the Institute of Zoology in Uppsala.

Most samples determined by the author (partly aided by KERSTIN WALLSTRÖM) derive from lakes in the province of Uppland. Those from 1941—45 were collected by W. RODHE, the others by the author. The samples from 1955 were, like RODHE's, caught with a fine plankton net (mesh size c. 60 μ) and previously investigated for rotifers (see PEJLER 1957), while those from 1973 were collected with a coarse net (mesh size *c.* 250

µ), in order to facilitate comparisons with older records, also based on coarse-net samples (see further next chapter). The samples from Lake Vänern in 1973 were collected in connection with an investigation performed by NLU (National Swedish Environment Protection Board, Limnological Survey). The author's samples were always collected in the central parts of the respective lakes above the greatest depth encountered, a vertical hauling being made through the whole water column.

As very few investigations of rotifer plankton were made in the earlier decades, and as, furthermore, the taxonomy used then is now obsolete, no really meaningful long-term variation could be studied concerning these animals. The cyclopoid copepods, too, had to be excluded, as their taxonomy is partly confused and often only copepodids (and/or nauplii) were found, permitting no determination of species. Only in a few cases were reliable determinations found. The protozoan zooplankton of Swedish lakes is still mainly unknown. Thus, the groups remaining for long-term comparisons are *Cladocera* and *Copepoda Calanoida.*

Only true eulimnoplanktic species are dealt with, littoral species, such as *Polyphemus pediculus,* being excluded. Also excluded are records containing very few species, since this indicates that the sample was very meagre and not representative, perhaps having been collected from the shore. Lists with only generic names had, of course, to be excluded, as well as those with an obsolete or otherwise doubtful taxonomy.

The author applies the same taxonomy as in a previous paper (PEJLER 1965), and the species are arranged in the same sequence. For this reason it was considered unnecessary to mention in this paper the authors of the species, except as regards the following two species not previously discussed by the present author:

1. *Bythotrephes cederstroemi* SCHOEDLER has often been regarded as merely a form (or "variety") of *B. longimanus.* However, it was treated as a separate species by HERBST (1962), and N.-A. NILSSON (1974) has recently, in connection with studies of food choice of coregones in Lake Vänern, found strong biometric evidence for distinguishing the two species; this is further supported by the present author's much more limited material from this lake.

2. *Eurytemora velox* (LILLJ.), called "*Temorella Clausii*" in LILLJEBORG's annotations.

For a description of the lakes (including the geographical situation) the reader is referred to the papers mentioned in the text of the tables and in Chapter 4, concerning the lakes of Uppland also to WILLÉN 1954 and PEJLER 1957.

## III. METHODS AND SOURCES OF ERROR

Samples gathered by different authors and during different periods show, of course, a great variation concerning methods of collection. One important consideration is the mesh size of the net, since the smaller species will be underrepresented (or non-existent) in samples taken with a coarse net, while the larger species will be overrepresented. When fine nets are used the case is reversed.

According to EKMAN's annotations he used MÜLLER gauze no. 4 (mesh size *c.* 300 µ). LILLJEBORG gives no information, but it is probable that coarser nets were employed. The same is true concerning the author's material from 1973, but the samples from 1941—45 (taken by W. RODHE) and 1955 were collected with fine nets (60—65 µ). Fine nets were also used by, *e.g.*, GRÖNBERG (1971 b). Both fine and coarse nets were employed by PEJLER in 1959—61 (1965). In several cases the records are based upon quantitative samples, *e.g.* by RODHE (1941), CARLIN (1943), BERZINS (1958), STJERNA-POOTH (1962), GRÖNBERG (1971 a and 1973 a), and DOTTNE-LINDGREN and PERSSON (1973). These circumstances, of course, were taken into account when conclusions were drawn.

In practically all cases here reported, the samples were evidently taken in central parts of the respective lakes (*e.g.* in the author's previously unpublished material, including Ekman's records). Lilljeborg, however, gives no information on this.

Frequency symbols were used by GRÖNBERG (1971 b), PEJLER (1965) and in the author's present material (including EKMAN's lists). In some cases the material consists of quantitative samples (*cf.* above), in still other cases (especially concerning LILLJEBORG's collections) it is not possible to obtain any idea at all of the frequency. Some of the investigations referred to are very thorough, encompassing many samples, while others consist of single samples. In intensive studies the probability of encountering rare species is, of course, much higher. An extreme example represents the only specimen of *Holopedium gibberum* found in Ekoln by EKMAN (1907), his counted planktic crustaceans totalling many thousands.

Another important consideration is the season. In all material here reported, samples were collected from late summer, in many cases from other seasons as well. In late summer and early autumn the zooplankton is ordinarily most characteristic and representative of the lake (*cf.* PEJLER 1965, p. 410). Determinable stages of most cladocerans and calanoid copepods occur at this time in high numbers. This is not true to the same extent for rotifers and cyclopoid copepods, which are here excluded.

When so many sources of error exist, the question arise as to how this material can be utilized. If, for example, a certain species was not found by LILLJEBORG or EKMAN but in a recent investigation, no conclusion can be drawn at all. If, on the other hand, a species was reported as being fairly common at the beginning of the century, but was not found at all in a very intensive study later on, this circumstance may be interpreted as an indication that the species has permanently decreased in importance. The evidence is still stronger if the same tendency is observed in several lakes which have all undergone the same environmental changes. Above all, if a species is found in a certain lake on several different occasions, this may be considered a sign of continuous occurrence.

## IV. SUPPLEMENTARY NOTES AND COMMENTS ON THE TABLES

The tables contain the main material for comparisons, but it seems justifiable to offer the following additional notes referring to separate lakes.

### *Lake Erken (Table 1).*

A very intensive investigation of the zooplankton and its relation to phytoplankton was made in 1957 by NAUWERCK (1963). He found the same species as those found by the present author, with one exception: *Eurytemora lacustris* was noted instead of *E. velox,* determined by EKMAN in 1898, by the present author in samples from 1941, 1954, 1955 and 1973 (see Table 1) and furthermore, according to diaries at the field

Table 1. *Zooplankton records from Lake Erken not previously reported. The material from 1898 was determined by* EKMAN, *the rest by the present author.*

| | 1898 July 7 | 1941 July 11 | 1941 Oct. 27 | 1954 June 7 | 1954 July 8 | 1954 July 15 | 1954 Sep. 12 | 1954 Oct. 19 | 1954 Nov. 16 | 1954 Dec. 14 | 1955 Jan. 25 | 1973 July 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Diaphanosoma brachyurum* | ++ | ++ | – | + | +++ | + | ++ | – | – | – | – | ++ |
| *Daphnia galeata* | +++ | +++ | ++ | + | ++ | + | ++ | ++ | ++ | +++ | ++ | +++ |
| *Daphnia cristata* | – | – | – | – | – | – | + | – | – | – | – | + |
| *Daphnia cucullata* | – | – | + | – | – | – | + | ++ | + | – | – | + |
| *Ceriodaphnia quadrangula* | ++ | + | + | + | + | ++ | – | ++ | + | – | – | ++ |
| *Bosmina coregoni* s.l. | +++ | +++ | ++ | ++ | +++ | ++ | +++ | +++ | +++ | +++ | + | ++ |
| *Bosmina longirostris* | – | – | – | – | – | – | – | – | – | – | – | + |
| *Chydorus sphaericus* | – | – | – | – | – | – | + | ++ | ++ | + | + | + |
| *Bythotrephes longimanus* | + | + | – | – | – | – | – | – | – | – | – | + |
| *Leptodora kindti* | ++ | + | + | + | + | + | + | – | – | – | – | + |
| *Eudiaptomus graciloides* | +++ | +++ | ++ | ++ | ++ | ++ | ++ | +++ | + | ++ | + | +++ |
| *Eurytemora velox* | ++ | + | + | – | – | – | – | – | – | – | – | + |

Table 2. *Zooplankton records from Lake Trehörningen, Uppland. The material from 1969 is determined by* Grönberg *(1971 b), the other by the present author.* Lilljeborg's *collections contain specimens of* D i a p h a n o s o m a  b r a c h y u r u m *and* B o s m i n a  c o r e g o n i *from 1879.*

| | 1955 July 7 | 1955 Aug. 22 | 1969 May 13 | 1969 Aug. 25 | 1973 Aug. 17 |
|---|---|---|---|---|---|
| *Diaphanosoma brachyurum* | ++ | ++ | – | + | +++ |
| *Daphnia cristata* | ++ | ++ | – | – | – |
| *Daphnia cucullata* | ++ | ++ | – | + | ++ |
| *Ceriodaphnia quadrangula* | – | + | – | – | – |
| *Bosmina coregoni* s.l. | ++ | ++ | + | + | +++ |
| *Bosmina longirostris* | – | – | – | + | – |
| *Chydorus sphaericus* | + | ++ | – | + | + |
| *Leptodora kindti* | – | – | – | – | ++ |
| *Eudiaptomus graciloides* | +++ | +++ | + | ++ | +++ |

laboratory at Norr Malma, in 1949 by B. Svenonius, in 1951 by H. Löffler, in 1955 and 1956 by L. Karlgren. H. Löffler lists the following planktic cladocerans and calanoid copepods from July 19, 1951: *Diaphanosoma brachyurum, Daphnia galeata, Ceriodaphnia quadrangula, Bosmina coregoni, Chydorus sphaericus, Bythotrephes longimanus, Leptodora kindti, Eudiaptomus graciloides* and *Eurytemora velox.* The composition is in close agreement with that reported in Table 1.

Dottne-Lindgren (1973) focused her interest upon the horizontal variation and not on taxonomic problems. Owing to this, she adopted Nauwerck's determination of *Eurytemora* and did not distinguish between different species of *Daphnia* and *Bosmina.* However, she found one species observed neither earlier nor later (*e.g.* not in the thorough investigation by Nauwerck), *viz. Limnosida frontosa.*

During the investigation on July 7, 1973, one sample was collected from the surface down to the greatest depth, and one sample close to the shore. *Bosmina longirostris* was found only in the littoral plankton, where the semiplanktic cladoceran *Polyphemus pediculus* (not previously reported from the lake) was also rather common. In the sample from the deepest part of the lake, the parasitic copepod *Ergasilus* sp. occurred in fairly large numbers.

*Lake Vendelsjön* (*Table 3*).

Two winter samples are included, though the number of species is very small. However, they should have some documentary interest as showing the specific zooplankton during conditions poor in oxygen (*cf.* the meagre rotifer plankton in the same samples, reported in Pejler 1957, Table 3). In addition to the species included in Table 3, *Polyphemus pediculus* and cyclopids were found on April 26, 1954.

Table 3. *Zooplankton records from Lake Vendelsjön, Uppland. 1876—1887:* Lilljeborg's *collections (no frequencies noted); 1898:* Ekman's *manuscript; 1969:* Grönberg *(1971 b); 1953—1955 and 1973: the present author's determinations.*

| | 1876–1887 | 1898 July 1 | 1898 Aug. 8 | 1898 Sep. 10 | 1953 Dec. 14 | 1954 April 26 | 1954 June 11 | 1955 July 20 | 1969 May 13 | 1969 Sep. 2 | 1973 Aug. 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Diaphanosoma brachyurum* | + | + | ++ | + | – | – | – | + | – | ++ | +++ |
| *Daphnia longispina* s.str. | + | – | – | – | – | – | – | – | – | – | – |
| *Daphnia cucullata* | + | +++ | ++ | +++ | – | – | – | + | – | – | +++ |
| *Daphnia cristata* | + | – | – | – | – | – | – | – | – | – | – |
| *Ceriodaphnia quadrangula* | + | + | +++ | +++ | – | – | + | + | – | ++ | +++ |
| *Bosmina coregoni* s.l. | + | +++ | – | +++ | – | + | – | – | – | – | ++ |
| *Bosmina longirostris* | – | + | – | – | + | – | +++ | + | + | – | – |
| *Chydorus sphaericus* | – | + | – | – | + | + | + | – | + | + | – |
| *Leptodora kindti* | – | ++ | + | – | – | – | – | – | – | – | ++ |
| *Eudiaptomus graciloides* | – | +++ | + | + | – | – | – | – | – | – | +++ |
| *Eudiaptomus* sp. | – | – | – | – | – | – | – | – | + | + | – |
| *Eurytemora velox* | + | ++ | +++ | +++ | – | – | – | – | – | + | – |
| *Eurytemora* sp. | – | – | – | – | – | – | – | – | – | – | + |

Table 4. *Zooplankton records from Lake Björklinge-Långsjön, Uppland. 1898:* EKMAN *(manuscript); 1969:* GRÖNBERG *(1971 b); 1955 and 1973: the present author's determinations.*

| | 1898 July 23 | 1955 June 22 | 1955 Aug. 14 | 1969 May 13 | 1969 Sep. 2 | 1973 Aug. 16 |
|---|---|---|---|---|---|---|
| *Diaphanosoma brachyurum* | ++ | + | ++ | – | – | ++ |
| *Daphnia galeata* | ++ | ++ | + | – | + | + |
| *Daphnia cucullata* | – | + | ++ | – | ++ | +++ |
| *Bosmina coregoni* s.l. | + | – | ++ | – | + | ++ |
| *Leptodora kindti* | + | + | – | + | – | + |
| *Eudiaptomus graciloides* | +++ | +++ | +++ | + | +++ | +++ |
| *Eurytemora velox* | ++ | + | – | – | – | – |

*Lake Valloxen (Table 6).*

From a vertical sample collected on April 12, 1897, EKMAN reports only *Eudiaptomus graciloides* and "*Cyclops miniatus f. vicinus*". A vertical sample from August 12, 1955 (fine net) was also remarkably poor in crustaceans, containing only *Daphnia cucullata, Bosmina longirostris* and cyclopids.

*Ekoln, a bay of Lake Mälaren (Table 7).*

Apart from LILLJEBORG's material Table 7 constitutes a compilation from GRÖNBERG 1973 a, where a fuller account is to be found.

Table 6. *Zooplankton records from Lake Valloxen, Uppland. 1864—1887:* LILLJEBORG's *collections (no frequencies noted); 1969:* GRÖNBERG *(1971 b); 1942, 1945 and 1973: the present author's determinations.*

| | 1864—1887 | 1942 Oct. 14 | 1945 June 12 | 1945 Nov. 19 | 1969 May 12 | 1969 Aug. 25 | 1973 Aug. 26 |
|---|---|---|---|---|---|---|---|
| *Diaphanosoma brachyurum* | + | – | + | – | – | + | +++ |
| *Daphnia galeata* | + | – | – | – | – | – | – |
| *Daphnia cucullata* | + | ++ | + | ++ | – | + | +++ |
| *Ceriodaphnia quadrangula* | + | – | – | – | – | – | – |
| *Bosmina coregoni* s.l. | + | + | +++ | + | – | + | +++ |
| *Bosmina longirostris* | – | – | ++ | – | + | – | – |
| *Chydorus sphaericus* | – | + | + | + | + | + | +++ |
| *Leptodora kindti* | + | – | – | – | – | – | + |
| *Eudiaptomus graciloides* | + | +++ | +++ | +++ | + | + | +++ |

*Lake Hjälmaren (Table 8)*

For a more detailed comparison the reader is referred to GRÖNBERG 1971 a.

*Lake Vänern (Table 12).*

LILLJEBORG gives no information as to how his samples were taken, but in the case of all other authors it is clear that the samples were collected in central parts over great depths, in some

Table 5. *Zooplankton records from Lake Siggeforasjön, Uppland (the present author's determinations).*

| | 1941 April 14 | 1941 May 26 | 1941 Aug. 23 | 1941 Oct. 29 | 1945 May 29 | 1945 June 28 | 1945 Aug. 6 | 1945 Sep. 11 | 1945 Nov. 5 | 1955 July 12 | 1973 Aug. 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Limnosida frontosa* | – | – | – | – | – | – | – | – | – | – | ++ |
| *Diaphanosoma brachyurum* | – | – | ++ | – | + | ++ | ++ | + | – | ++ | ++ |
| *Holopedium gibberum* | – | ++ | – | – | + | + | – | – | – | – | – |
| *Daphnia galeata* | – | – | – | – | – | – | – | – | – | – | ++ |
| *Daphnia cristata* | ++ | + | +++ | +++ | ++ | +++ | ++ | ++ | +++ | +++ | +++ |
| *Ceriodaphnia quadrangula* | – | – | + | – | – | – | + | – | – | + | + |
| *Bosmina coregoni* s.l. | – | + | + | +++ | + | + | + | ++ | +++ | + | +++ |
| *Bosmina longirostris* | ++ | + | – | ++ | ++ | – | – | – | + | + | – |
| *Chydorus sphaericus* | – | + | – | – | – | – | – | – | – | – | – |
| *Leptodora kindti* | – | – | + | – | – | + | + | – | – | – | + |
| *Eudiaptomus gracilis* | +++ | + | +++ | + | + | +++ | +++ | +++ | +++ | +++ | +++ |
| *Heterocope appendiculata* | – | – | – | – | – | – | – | – | – | – | + |

Table 7. *Zooplankton records from Ekoln, a bay of Lake Mälaren. 1860—1888:* LILLJEBORG'S *material; 1906—1907:* EKMAN *(1907); 1947—1956:* THOMASSON'S *manuscript; 1965—1966 and 1967—1969:* GRÖNBERG *(1973 a).*

| | 1860-1888 | 1906-1907 | 1947-1956 | 1965-1966 | 1967-1969 |
|---|---|---|---|---|---|
| *Limnosida frontosa* | + | + | – | – | + |
| *Diaphanosoma brachyurum* | + | + | + | + | + |
| *Holopedium gibberum* | – | + | – | – | – |
| *Daphnia galeata* | + | + | + | + | + |
| *Daphnia cristata* | + | + | + | + | + |
| *Daphnia cucullata* | + | + | + | + | + |
| *Ceriodaphnia quadrangula* | + | – | – | – | + |
| *Bosmina coregoni* s.l. | + | + | + | + | + |
| *Bosmina longirostris* | + | – | – | + | + |
| *Chydorus sphaericus* | + | + | + | + | + |
| *Bythotrephes longimanus* | + | + | – | – | + |
| *Leptodora kindti* | + | + | + | + | + |
| *Limnocalanus macrurus* | + | + | + | + | + |
| *Eudiaptomus gracilis* | + | + | + | + | + |
| *Eudiaptomus graciloides* | + | – | + | – | – |
| *Eurytemora velox* | + | + | – | – | – |
| *Heterocope appendiculata* | + | + | – | + | + |

Table 8. *Zooplankton records from Lake Hjälmaren. 1914—1915:* ALM *(1916 and 1917); 1960:* PEJLER *(1965); 1965—1967 and 1969:* GRÖNBERG *(1971 a).*

| | 1914-1915 | 1960 | 1965-1967 | 1969 |
|---|---|---|---|---|
| *Limnosida frontosa* | – | – | + | + |
| *Diaphanosoma brachyurum* | + | + | + | + |
| *Holopedium gibberum* | + | – | – | – |
| *Daphnia galeata* | – | – | + | + |
| *Daphnia cristata* | + | + | + | + |
| *Daphnia cucullata* | + | + | + | + |
| *Ceriodaphnia quadrangula* | + | + | + | + |
| *Bosmina coregoni* s.l. | + | + | + | + |
| *Chydorus sphaericus* | + | + | + | + |
| *Bythotrephes longimanus* | + | – | + | – |
| *Leptodora kindti* | + | + | + | + |
| *Eudiaptomus gracilis* | + | + | + | + |
| *Heterocope appendiculata* | + | – | + | + |

cases in the littoral region as well. EKMAN used an ordinary plankton net (about 300 μ mesh size), VALLIN a closing net according to NANSEN (collected by O. NORDQVIST; mesh size not mentioned), STJERNA-POOTH a water-sampler (quantitative samples), GRÖNBERG an ordinary plankton net (mesh size 60 μ), PEJLER (in 1965) plankton nets, with mesh sizes of 60 μ and 250 μ respectively, and PEJLER (in 1973) a CLARKE-BUMPUS sampler, with a mesh size of 160 μ. The samples from 1973 were collected at the NLU (The Swedish Environment Protection Board Limnological Survey) stations 6 (depth 70 m), 9 (45 m) and 19 (70 m), the situation of which appears

Table 9. *Zooplankton records from some smaller lakes in the province of Närke. 1890:* LILLJEBORG'S *material; 1907:* EKMAN'S *manuscript; 1932:* BERZINS' *manuscript; 1959—1960:* PEJLER *(1965); 1969:* GRÖNBERG *(1971 b).*

| Lake | Storbjörken | | | Multen | | Toften | | Teen | | | Logsjön | | | Vibysjön | | | Östra Laxsjön | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1907 | 1932 | 1969 | 1907 | 1969 | 1907 | 1969 | 1890 | 1907 | 1969 | 1907 | 1959–1960 | 1969 | 1907 | 1959–1960 | 1969 | 1959 | 1969 |
| *Limnosida frontosa* | + | + | + | – | – | – | + | + | + | – | – | – | – | – | – | – | + | + |
| *Diaphanosoma brachyurum* | + | + | – | – | – | + | – | – | – | – | + | + | + | + | + | – | + | – |
| *Holopedium gibberum* | – | – | – | + | – | – | – | + | + | – | – | – | – | – | – | – | + | – |
| *Daphnia galeata* | – | – | + | – | + | – | + | + | – | + | – | – | – | – | – | – | + | – |
| *Daphnia cristata* | – | + | + | – | – | – | + | + | – | + | – | – | – | – | – | – | + | + |
| *Daphnia cucullata* | – | – | – | – | – | – | – | – | – | + | – | + | + | – | + | – | – | – |
| *Daphnia* sp. | – | – | – | + | – | + | – | – | + | – | + | – | – | + | – | – | – | – |
| *Ceriodaphnia quadrangula* | – | + | – | – | + | – | – | – | – | – | – | + | + | – | + | – | – | – |
| *Bosmina coregoni* s.l. | + | + | – | + | + | – | + | + | – | + | – | – | – | – | – | – | + | + |
| *Bosmina longirostris* | – | – | + | – | – | – | – | + | – | – | – | – | + | – | + | + | – | – |
| *Bosmina* sp. | – | – | – | – | – | + | – | – | – | – | + | – | – | – | – | – | – | – |
| *Chydorus sphaericus* | – | – | – | – | + | – | + | – | – | + | – | + | + | – | – | + | – | – |
| *Bythotrephes longimanus* | + | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | + | – |
| *Leptodora kindti* | + | – | – | – | – | + | – | – | – | + | – | + | + | + | + | – | + | – |
| *Eudiaptomus gracilis* | + | + | + | + | – | + | + | – | + | + | + | + | + | + | + | – | + | + |
| *Heterocope appendiculata* | + | – | – | + | – | + | + | – | + | – | – | – | – | – | – | – | + | + |

Table 10. *Zooplankton records from some lakes in eastern Central Sweden. 1890—1893:* LILLJEBORG's *material; 1912—1915:* EKMAN's *manuscript; 1935—1940:* CARLIN *(1943); 1959—1960* PEJLER *(1965); 1969:* GRÖNBERG *(1971 b).*

| | Lejon-dals-sjön | | Öljaren | | Glan | | Sommen | |
|---|---|---|---|---|---|---|---|---|
| | 1890–1893 | 1969 | 1960 | 1969 | 1912 | 1935–1940 | 1913–1915 | 1959–1960 |
| *Limnosida frontosa* | – | – | + | + | + | – | + | + |
| *Diaphanosoma brachyurum* | – | + | + | – | – | + | + | – |
| *Holopedium gibberum* | – | – | – | – | – | – | + | – |
| *Daphnia longispina* s.l. | – | – | – | – | – | + | – | – |
| *Daphnia galeata* | + | + | – | + | – | – | + | + |
| *Daphnia cristata* | + | – | – | – | – | – | – | + |
| *Daphnia cucullata* | + | + | + | + | – | – | – | – |
| *Ceriodaphnia quadrangula* | – | + | – | – | – | + | – | + |
| *Bosmina coregoni* s.l. | + | + | + | – | + | + | + | + |
| *Bosmina longirostris* | – | – | – | – | – | + | – | – |
| *Chydorus sphaericus* | – | – | + | – | – | + | – | + |
| *Bythotrephes longimanus* | + | – | – | – | – | + | + | – |
| *Leptodora kindti* | – | + | + | + | + | + | + | + |
| *Limnocalanus macrurus* | – | – | – | – | – | + | + | + |
| *Eudiaptomus gracilis* | – | – | + | + | + | + | + | + |
| *Eudiaptomus graciloides* | + | + | – | – | – | + | – | – |
| *Eurytemora lacustris* | – | – | – | – | + | + | + | – |
| *Heterocope appendiculata* | – | – | – | – | – | – | + | + |

from Fig. 1 in GRÖNBERG 1973 b. The composition of the zooplankton was altogether the same at the three stations, though these were situated in quite different parts of the large lake. No littoral station was investigated, which is the probable reason why *Daphnia cucullata, Ceriodaphnia quadrangula* and *Chydorus sphaericus* were missing. These species were found by GRÖNBERG (in 1969), who investigated 28 stations of varying character, some of them close to the shore. The latter is true for STJERNA-POOTH, too, who found two of the above-mentioned species in 1959. *Bythotrephes cederstroemi* was found by the present author as well in Vänern on July 21, 1960 (PEJLER 1965), though he then regarded

Table 11. *Zooplankton records from Lake Vättern. 1910—1914:* EKMAN *(manuscript); 1922—1939:* STÅLBERG *(1939); 1959—1960:* PEJLER *(1965); 1962:* BERZINS *(manuscript); 1969:* DOTTNE-LINDGREN *and* PERSSON *(1973).*

| | 1910–1914 | 1922–1939 | 1959–1960 | 1962 | 1969 |
|---|---|---|---|---|---|
| *Limnosida frontosa* | – | + | + | – | + |
| *Diaphanosoma brachyurum* | – | + | + | + | + |
| *Holopedium gibberum* | + | + | + | + | + |
| *Daphnia galeata* | + | – | + | + | + |
| *Daphnia cristata* | – | + | + | + | + |
| *Ceriodaphnia quadrangula* | – | – | – | – | + |
| *Bosmina coregoni* s.l. | + | + | + | + | + |
| *Bosmina longirostris* | – | – | – | + | – |
| *Chydorus sphaericus* | – | + | + | + | + |
| *Bythotrephes longimanus* | + | + | – | – | + |
| *Leptodora kindti* | – | + | + | + | + |
| *Limnocalanus macrurus* | + | + | + | + | + |
| *Eudiaptomus gracilis* | + | + | + | + | + |
| *Eurytemora lacustris* | – | + | + | + | + |
| *Heterocope appendiculata* | + | + | + | + | + |

Table 12. *Zooplankton records from Lake Vänern. 1882—1892:* LILLJEBORG's *material; 1906:* EKMAN *(manuscript); 1921:* VALLIN *(1971); 1959:* STJERNA-POOTH *(1962); 1960:* PEJLER *(1965); 1969:* GRÖNBERG *(1973 b); 1973: the present author's determinations.*

| | 1882–1892 | 1906 | 1921 | 1959 | 1960 | 1969 | 1973 |
|---|---|---|---|---|---|---|---|
| *Limnosida frontosa* | – | + | – | + | + | + | + |
| *Diaphanosoma brachyurum* | + | – | – | – | – | + | + |
| *Holopedium gibberum* | + | + | + | – | – | – | – |
| *Daphnia galeata* | + | – | – | – | – | + | – |
| *Daphnia cristata* | + | + | + | + | + | + | + |
| *Daphnia cucullata* | – | – | – | + | – | + | – |
| *Ceriodaphnia quadrangula* | + | – | – | – | – | + | – |
| *Bosmina coregoni* s.l. | + | + | + | + | + | + | + |
| *Chydorus sphaericus* | – | – | – | + | – | + | – |
| *Bythotrephes longimanus* | + | + | + | – | + | – | + |
| *Bythotrephes cederstroemi* | – | – | – | – | + | – | + |
| *Leptodora kindti* | + | + | + | + | + | + | + |
| *Limnocalanus macrurus* | + | + | + | + | + | + | + |
| *Eudiaptomus gracilis* | + | + | + | + | + | + | + |
| *Eudiaptomus graciloides* | – | – | – | + | – | + | + |
| *Eurytemora lacustris* | + | + | + | + | + | + | + |
| *Eurytemora velox* | + | – | – | – | – | – | – |
| *Heterocope appendiculata* | – | – | + | – | + | + | – |

Table 13. *Zooplankton records from Lake Skärshultsjön. 1938:* Rodhe *(1941); 1950:* Berzins *(1958); 1945 and 1960:* Berzins *(manuscript).*

| | 1938 | 1945 | 1950 | 1960 |
|---|---|---|---|---|
| *Limnosida frontosa* | – | – | + | + |
| *Diaphanosoma brachyurum* | + | + | + | + |
| *Holopedium gibberum* | + | + | + | + |
| *Daphnia cristata* | + | – | + | + |
| *Ceriodaphnia quadrangula* | + | + | + | + |
| *Bosmina coregoni* s.l. | + | + | + | + |
| *Bosmina longirostris* | – | + | + | + |
| *Chydorus sphaericus* | – | – | – | + |
| *Leptodora kindti* | + | – | + | + |
| *Eudiaptomus gracilis* | + | – | + | + |
| *Heterocope appendiculata* | + | – | + | + |

it as merely a form of *B. longimanus*, in view of the transitions to the latter species reported in the literature. For the same reason it is possible that some of the earlier authors, too, had obtained *B. cederstroemi* in their material but reported it as *B. longimanus*.

## V. DISCUSSION

Some of the species discussed are "ubiquitous", occurring in all types of lakes that exist in Central Sweden and seemingly being independent of dispersal barriers. These species, of which *Bosmina coregoni* and *Leptodora kindti* may be mentioned as examples, cannot be expected to furnish much material for the discussion presented here. Instead, the interest should be focused partly on zooplankters known to indicate oligotrophy, eutrophy or some other special environmental conditions and partly on those which have a more or less restricted distribution.

If we concentrate on the real long-run changes, the exposition made in Table 14 may be elucidative. The year 1921 was chosen as a borderline because of the material available from the large lakes, Vänern and Vättern. In the former lake there is a wide gap between the investigations from 1921 and those from 1959, whereas in the latter Stålberg's samples were taken over a long period, extending from 1922 to 1939. In some of Ekman's lists the forms of *Daphnia* and

Table 14. *Number of lakes where a species was found only up to 1921 or only after 1921, respectively.*

| | Only up to 1921 | Only after 1921 |
|---|---|---|
| *Limnosida frontosa* | 2 | 3 |
| *Diaphanosoma brachyurum* | 2 | 3 |
| *Holopedium gibberum* | 6 | – |
| *Daphnia longispina* s.str. | 1 | – |
| *Daphnia galeata* | 1 | 2 |
| *Daphnia cristata* | 2 | 4 |
| *Daphnia cucullata* | – | 3 |
| *Ceriodaphnia quadrangula* | 1 | 8 |
| *Bosmina coregoni* s.l. | – | – |
| *Bosmina longirostris* | 1 | 6 |
| *Chydorus sphaericus* | – | 11 |
| *Bythotrephes longimanus* | 3 | 1 |
| *Bythotrephes cederstroemi* | – | 1 |
| *Leptodora kindti* | 2 | 4 |
| *Limnocalanus macrurus* | – | 1 |
| *Eudiaptomus gracilis* | 1 | – |
| *Eudiaptomus graciloides* | – | 2 |
| *Eurytemora lacustris* | 1 | 1 |
| *Eurytemora velox* | 2 | – |
| *Heterocope appendiculata* | 3 | – |

*Bosmina* were not determined as species, and for this reason the lakes in question were not included in the comparison as far as these genera are concerned.

The investigations carried out after 1921 are, as a rule, both more frequent and more intensive than those undertaken before, and it is therefore quite natural to find, on average, higher numbers in the right-hand column of Table 14 than in the left-hand one. In spite of this, however, for some species the number is higher in the left-hand column. This is especially obvious in the case of *Holopedium gibberum,* as some of the lakes in question have been investigated very thoroughly during the last few decades without this species being found (*e.g.* Ekoln, Lake Hjälmaren and Lake Vänern). This really indicates that nowadays *Holopedium* plays a very inconspicuous role. It is true that the species was of subordinate importance in Ekoln in Ekman's time as well, as that author reports only one specimen (which was actually alive at the sampling according to Ekman, verbal information to the present author). In Lakes Hjälmaren and Vänern, however, *Holopedium* was fairly common when the earlier investigations were made.

*Holopedium gibberum* is regarded as an indicator of oligotrophic conditions (see, *e.g.*, PEJLER 1965, p. 472—473). On the other hand, the only crustacean described as an indicator of eutrophy by PEJLER (1965, *e.g.* at p. 503), *viz. Daphnia cucullata,* was found in some lakes only after 1921, but in none only before that year. The results are in close agreement with the predominating tendency towards eutrophication found in many lowland lakes of Central Sweden (*cf.*, *e.g.*, WILLÉN 1972).

In discussing the changes in the composition of zooplankton caused by eutrophication of North American lakes, BROOKS (1969) calls attention to *Bosmina longirostris* and *Chydorus sphaericus,* both of which species greatly increase in number in connection with this process. According to BROOK's own material (see *op.cit.*, Fig. 1), *Ceriodaphnia* could have been mentioned as well. The mere occurrence of these forms does not indicate eutrophy, since they live in the littoral zone of many types of lakes. A great abundance in the pelagic zone, however, will be observed only in connection with a high trophic degree. From this point of view the figures reported in Table 14 are quite natural. It might appear contradictory that *Ceriodaphnia quadrangula* as well as *Bosmina longirostris* were found in one lake only before 1922 (Lake Valloxen and Lake Teen, respectively), but in both these cases the earlier samples were collected by LILLJEBORG, who does not mention anything about his localities. Probably, in this case it is a question of littoral samples.

According to BROOKS (*op.cit.*, p. 251—252), several studies indicate that *Bosmina coregoni* disappears as a lake is enriched and is replaced by *B. longirostris.* This does not conform with the label "euryœcious" given to the former species at the beginning of this chapter. However, this term is relative, like all other similar concepts, and actually *B. coregoni* was missing in the samples from the six most eutrophic lakes according to the scale used in PEJLER 1965, Table 4. One of these lakes is Vibysjön, which is included also in Table 9 of the present paper. Here it seems as if *B. longirostris* has replaced *B. coregoni* in the pelagic zone.

It could be argued that the forms found only before 1922 are relatively large, and thus more easily caught with the coarse nets used at that time, while the later collections were often made with fine nets and should, for this reason, contain, on average, more smaller species (*cf.* p. 108). In fact, species indicating oligotrophy are usually larger than those indicating eutrophy (*cf.* NILSSON and PEJLER 1973, p. 69, where the reasons for this condition are discussed). However, this source of error does not seem to be too great, considering that the largest species of them all, *viz. Leptodora kindti,* was found only after 1921 in four of the lakes, only before 1921 in two of them. Furthermore, *Bosmina coregoni* s.l. was encountered in earlier and later samples to about the same extent, though it is among the smallest species. Finally, it should be kept in mind that many of the later investigations include coarse-net samples, *e.g.* all collections made by the present author from 1959 onwards.

Concerning the species of *Eudiaptomus* and *Eurytemora,* dispersal barriers seem to play an important role. For the former genus this was pointed out by GRÖNBERG (1971 b, p. 6 and Fig. 18) and PEJLER (1965, p. 490—491). As to the latter genus, EKMAN (1907) considers *E. lacustris* to be a relict of the Ancylus Lake, *E. velox* a relict of the Littorina Sea, thereby explaining the differences in their present distribution. According to Table 14 the species discussed were encountered, in some lakes, either only before or only after 1921. However, these cases are rather few in number, and a closer look at them shows that they are in most cases probably explainable by the circumstance that investigations were less intensive during the period when the species in question is missing. However, a question mark should be put against the two cases when *Eurytemora velox* was found only in early samples, as the lakes in question, Ekoln and Vänern, have since then been the object of very intensive studies.

In some cases human interference can cause very profound changes in the whole ecosystem of a lake. An example of this is the restoration of Lake Trummen in South Sweden, where the effects on plankton were followed up (see ANDERSSON *et al.* 1973). In lakes where the water level has been drastically lowered, the true limnoplankters have more or less disappeared. Thus, in Lake Tåkern no diaptomid has been found during the last few decades (see PEJLER 1965, p. 443 and Table 4), though EKMAN reports *D. gracilis* from the beginning of this century. Similarly, LILLJEBORG reports *Daphnia cucullata* from 1882 in Lake Hornborgasjön, though BERZINS, in samples from 1967 and 1971, found only species typical of the littoral zone, such as *Daphnia longispina* s. str., *Ceriodaphnia quadrangula* and *Chydorus sphaericus*.

The main impression from the material here presented is, however, a high degree of similarity between older and newer samples. This demonstrates a remarkable stability of the zooplankton composition, in contradiction to the hypotheses sometimes put forward of plankton as an extraordinarily labile biocenose. The title of the present paper has been chosen precisely in order to stress this point. The changes actually found seem to be of a short-term rather than a long-term nature.

The only tendency that has been traced in connection with these comparisons is one towards increasing eutrophy. Though this tendency is not very strong, it should not be overlooked, since the first effect of an enrichment ought not to be a change in zooplankton composition but an increase in the individual number of all or most species (*cf.* BROOKS 1969, p. 246 ff., and DOTTNE-LINDGREN and PERSSON 1973, p. 10). However, the abundance cannot be discussed in this context, as quantitative data are often lacking, especially in the older investigations.

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# A Note on the Aggressive Behaviour of Adult Male Sea Trout Towards "Precocious" Males During Spawning

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In the River Jörlandaån, a small sea-trout stream in the south-western part of Sweden, "precocious" males are frequently observed together with spawning adult sea trout (*Salmo trutta* L.). In November 1974, out of 220 electro-fished juvenile sea trout, 100—200 mm in length, 39 were precocious males. Of these precocious males, 15 had a characteristic wound in the form of two parallel cuts in the muscle, 5—6 mm apart (Fig. 1). The injuries were unlike those caused by the hunting heron, and were not found in any of 25 adult sea trout examined for the purpose.

During observations of the spawning in the field, one adult male sea trout, *c.* 400 mm in length, was seen to catch a precocious male, *c.* 150 mm long, and hold it between its jaws for about half a minute.

On examining the teeth of a number of sea trout, the sharp, hook-like papillae on the tongue were found to be arranged in two parallel rows, which in adult trout were 5—6 mm apart. (Fig. 2). Since the aggressive behaviour of male trout is known to be directed mainly towards other males, this behaviour was accepted as the cause of the observed injuries. The tongue of the adult male trout must have worked like a file upon the precocious male secured between its jaws.

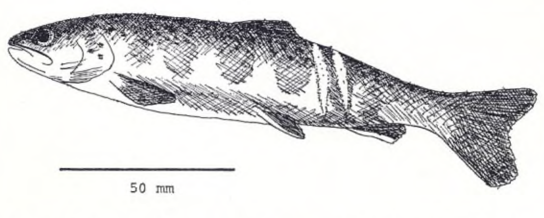


Fig. 1. Injuries of a spawning precocious male, 151 mm in length.

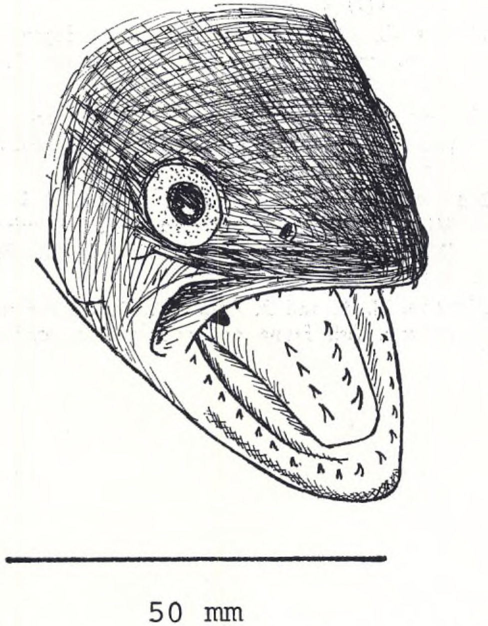


Fig. 2. Head of a sea trout, 400 mm in length, showing the sharp papillae on the tongue, the probable cause of the injuries shown in Fig. 1.

The spawning behaviour of brown trout has been thoroughly studied by JONES and BALL (1954). The above-observed effect of the aggressive behaviour of the adult male trout, however, has not been reported earlier. The injuries are likely to affect the mortality of the precocious males. This invites speculation as to whether this behaviour may have a selective purpose.

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