GÖTEBORGS UNIVERSITET

INSTITUTE OF FRESHWATER RESEARCH

DROTTNINGHOLM

Report No 49

E R R A T A

Page 74

line 14: „ten square metres" should read „ten metres square"

" 18: „one fish per square metre" should read „one fish per
ten square metres"

Page 145

line 19: „1966" should read „1964b"

" 21: „1966" should read „1964b"

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Contents

Life-cycle and growth of *Asellus aquaticus* (L.)

With special reference to the effects of temperature

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Contents

I. Introduction

Asellus aquaticus (L.) is a common bottom animal in many Swedish lakes. The biology of *Asellus* or parts of it has been treated in many papers e.g. Wesenberg-Lund (1939), Thorup (1963) and Berglund (1968). It constitutes an important source of food for many fish species and in some case it will be the dominant form (Berglund 1968). Thus, the production of *Asellus* in a lake is of great interest. The method which I used of determining *Asellus* productivity was based on my study of its population-dynamics in the field. Such studies have been pursued in Lake Pajep Måskejaure in Swedish Lapland and in Lake Erken in Central Sweden.

Many authors e.g. Wesenberg-Lund (1939), Illies (1952), Le Cren (1958) and Taube and Nauwerck (1968) have pointed out the correlation between temperature and the rate of growth.

From preliminary data from both Pajep Måskejaure and Erken this relationship appeared to be a most important one. Therefore the effect of temperature on the growth of *Asellus aquaticus* was determined under field as well as laboratory conditions.

II. Description of the sampling Areas

Lake Pajep Måskejaure (Fig. 1) is one of many lakes in the Pite River's upper course. It lies 40 kms north of Arjeplog and is surrounded by a forest

consisting predominately of fir. The lake has an area of 5 km², its altitude is 438 m, and its greatest measured depth is 26 m. Both upstream and downstream there is a chain of shorter stretches of stream alternating with other lakes. There are large seasonal variations in the discharge of the Pite River which among other things results in a considerable variation in the water level of the lake (annual amplitude ca 1.3 m). The lowest level of the lake occurs in late winter and the highest about one month after the break-up of ice at the end of May or beginning of June. The lake has a high flushing rate. A rough estimate is that during the summer months the replenishment time of the lake is between two and four days. A permanent thermal stratification did not occur (1964—66) nor was it to be expected. The surface temperature usually reaches its peak at the end of July (highest values for the years 1964—1966 were 12.1°, 12.7°, and 12.7°C, respectively). Ice cover usually extends from November until June. Secchi disc readings depend to a great extent on the quantity of silt in the water flowing into the lake. The highest values were measured on October 30, 1965, (18.3 m) and on May 18, 1966, (16.7 m) and the lowest values on May 27, 1966, (11.2 m) and under ice in April 1967 (10.0 m). pH value range from 6.8 to 7.4, and specific conductivity of surface water (20°C) from 22 to 27 μ mhos and Ca^{++} from 2.2 to 2.8 mg/l.

As a rule the bottom is steep in the southern part of the lake. In the other parts it slopes slowly towards the middle of the lake where the current has scoured a deep trench from the inlet towards the outlet. Near the shore the bottom is normally composed of sand and stone, but as the depth increases, the mud layer becomes thicker. In the eastern part of the lake the bottom is to a large extent covered with stones of different sizes.

The submerged vegetation forms thick cover in limited areas, but can usually be found sparsely at depths between 2 and 14 m. Two species of moss, as well as *Nitella sp.* (cf. opaca) are the most common plants. *Isoëtes, Potamogeton, Myriophyllum,* and *Ranunculus* are also represented by one or more species.

The most common fishes in Lake Pajep Måskejaure are grayling (*Thymallus thymallus* L.), whitefish (*Coregonus sp.*), brown trout (*Salmo trutta* L.), pike (*Esox lucius* L.), and burbot (*Lota lota* L.).

Lake Erken (Fig. 1) is situated some 50 kms east of Uppsala and surrounded by forest and arable land. It has an area of 23 km², its altitude is 11 m and its greatest depth 21 m. The lake has an inlet and an outlet, but they are comparatively small. The ice usually breaks up in April, and the lake is again covered with ice in December. Maximum surface temperature for 1967 was 20.0°C (August 2). For further information about Lake Erken, see NAUWERCK (1963).
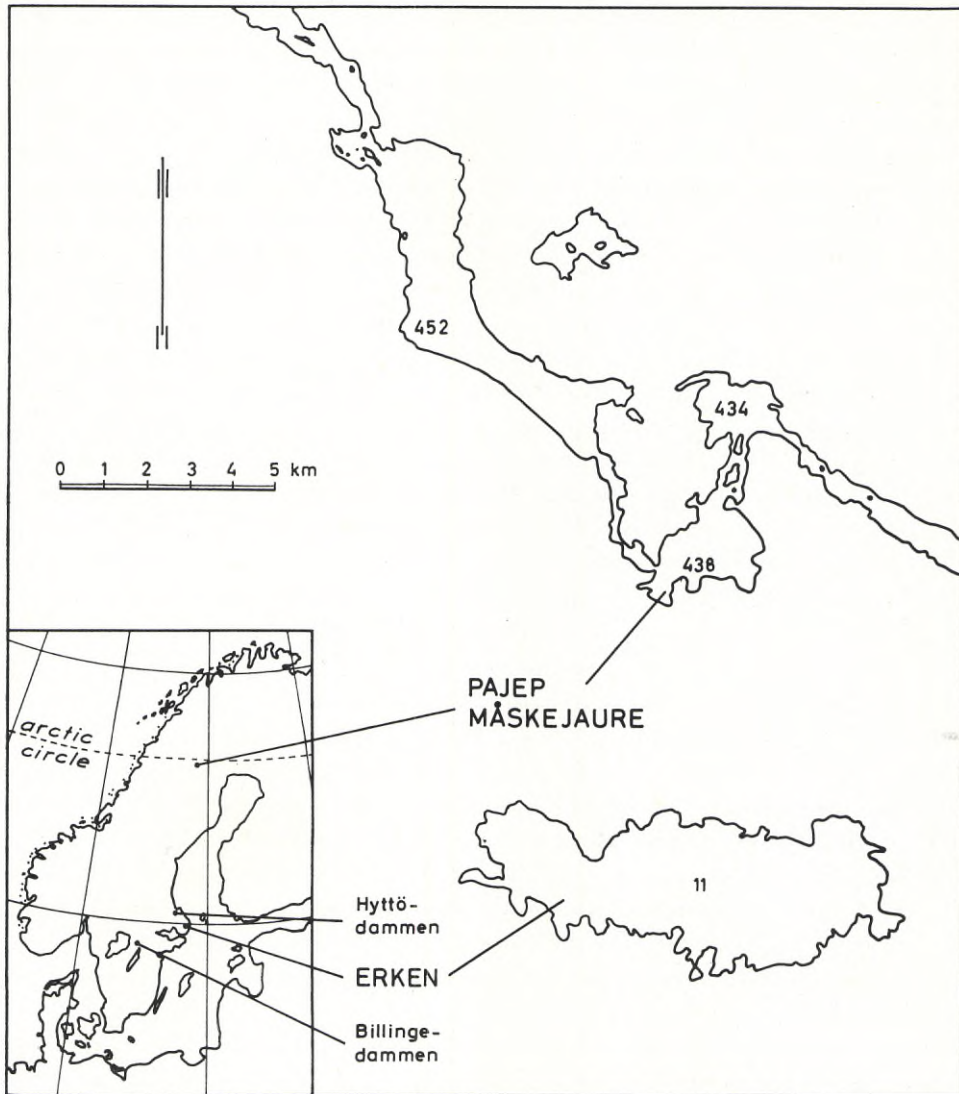
Fig. 1. Map of the lakes of investigation. The figures within the lake boundaries give the altitudes (metres above sea level).

III. Methods

Quantitative samples have been taken by means of EKMAN-BIRGE bottom samplers, with sample areas of 225 cm² (Lake Pajep Måskejaure) and 250 cm² (Lake Erken). The sifting has been carried out in screens with 0.6 mm mesh. However, for the Erken material, 0.2 mm mesh screens were used throughout the whole investigation period in order to avoid missing newly-

hatched *Asellus* young which might slip through the 0.6 mm mesh. This mesh was not available at the time of the Pajep Måskejaure study. The animals were usually picked out alive immediately after the samples were taken and were preserved in a 4 per cent formalin solution. All weights of *Asellus* given are based on preserved material. The lengths of the animals have been measured from the forepart of the head to the utmost part of the rear abdominal segment. During this process the animal was placed on its back under a 10 power stereoscopic microscope. This microscope had a measuring-scale in its eyepiece which made it possible to measure the animals with an exactness of 0.1 mm. In all about 13,000 animals have been measured (from 140 samples). As the animals were measured, they were also sorted into different groups according to size. A scale with an accuracy of ± 0.1 mg was used for the weighing. The preserving fluid was removed using a piece of filter paper under a stereoscopic microscope until there was no visible fluid left, after which the animals were weighed. During the periods June to August 1965, October 1965 and April 1966, 98 bottom samples were taken from different types of bottom and at different depths in Lake Pajep Måskejaure. These samples have been divided into depth zones of 0—1.9 m, 2.0—3.9 m, 4.0—5.9 m, and so on. All depths were measured according to the high water level of early summer.

As the quantitative and qualitative composition of the bottom fauna varies with the nature of the substrate (and the depth of the lakes), three bottom types were recognized — "hard", "soft" and vegetation. Hard bottom refers to gravel or to sand without vegetation and with a mud layer thinner than 1 cm. Soft bottom has no vegetation and a mud layer thicker than 1 cm, and vegetation bottom has live plants or parts of live plants. The Lake Pajep Måskejaure samples from April 1966 to April 1967 were taken from a bottom rich in vegetation with *Isoëtes lacustris* as the predominant plant and the Lake Erken samples from a bottom dominated by *Cladophora aegagropila*.

In a number of samples from Lake Erken both *Asellus* and *Cladophora aegagropila* have been collected together. The *Cladophora* part of the samples was separated from sand and detritus after which it was left to dry in a drying-oven for about 24 hours at a temperature of 105°C. After cooling in a desiccator it was weighed on the scale.

On the whole, two types of vessels have been used during laboratory studies of *Asellus* growth and reproduction at various temperatures. During the studies of the development of eggs and embryos 60 ml plastic vessels were used. They were filled with tap water, which was then aerated, and a tuft of *Cladophora aegagropila* was put into each vessel. In order to prevent evaporation the vessels were covered with lids. Once a week the vessels were cleaned and filled with new water. For the studies of growth at different temperatures the samples were taken with the help of an EKMAN-BIRGE

bottom sampler. A careful sifting was made in order to remove the small mud particles. The samples were then transported to Uppsala where they were put into 10 litre aquaria. The aquaria were exposed to electric light for 12 hours a day, and the water was not aerated. The various populations were maintained in the mud and water from their own lake. However, distilled water that had been aerated was now and then added to compensate for evaporation.

The material for the aquaria investigations was taken from the lakes mentioned above, and from the Fyris River near Uppsala. Females with eggs were taken from Hyttödammen, a pond situated some 70 kms north of Uppsala.

IV. Results and discussion

1. Abundance of *Asellus* in relation to depth and substrate

Asellus aquaticus (L.) is a very common animal in Lake Pajep Måskejaure and is a very important source of food for the fish. The mean value of 98 bottom samples taken during June 1965 to April 1966 shows that 29 per cent of the individuals and 39 per cent of the biomass consisted of *Asellus*.

The nature of the bottom has a great influence on the density and average weight of *Asellus* (Table 1). Hard bottoms usually give the lowest number

Table 1. Occurence of *Asellus aquaticus* in relation to bottom type (in Lake Pajep Måskejaure) October 1965 (2—10 m)

	Generation 1 a			Generation 1 b			Generation 1 a + 1 b		
	Hard bottom	Soft bottom	Vegetation bottom	Hard bottom	Soft bottom	Vegetation bottom	Hard bottom	Soft bottom	Vegetation bottom
Number/m²	317	550	588	122	267	1,255	439	817	1,843
Average weight mg	3.9	4.8	7.3	0.55	0.47	0.69	2.98	3.38	2.79
Biomass g/m²	1.24	2.63	4.28	0.07	0.13	0.87	1.31	2.76	5.15
Biomass of all groups of animals									
(*Chironomidae, Oligochaeta*, etc.) g/m²							2.11	6.10	10.67

April 1966 (2—10 m)

	Generation 1 a			Generation 1 b			Generation 1 a + 1 b		
	Hard bottom	Soft bottom	Vegetation bottom	Hard bottom	Soft bottom	Vegetation bottom	Hard bottom	Soft bottom	Vegetation bottom
Number/m²	44	178	662	111	146	1,092	155	324	1,754
Average weight mg	5.3	6.2	7.5	0.58	0.73	0.69	1.94	3.73	3.27
Biomass g/m²	0.24	1.10	4.99	0.06	0.11	0.75	0.30	1.21	5.74
Biomass of all groups of animals									
(*Chironomidae, Oligochaeta*, etc.) g/m²							0.48	4.43	13.69
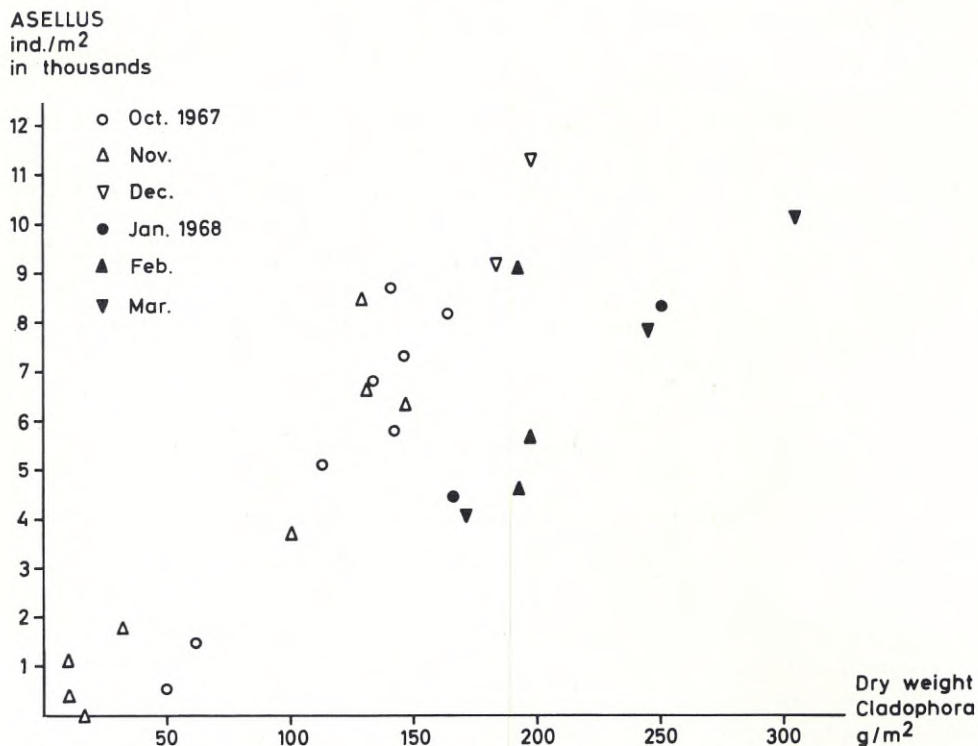
Fig. 2. Relation between number of *Asellus aquaticus* and quantity of *Cladophora aegagro-pila* in Lake Erken samples (October 1967—March 1968).

of individuals and the lowest average weight. Soft bottoms have an inter-mediate position, and vegetation bottoms show the highest values. The fact that vegetation bottom is more favourable to *Asellus* than are the other types seems logical in view of their food preferences — largely vascular plant remains and filamentous algae (WILLIAMS 1962). Moreover, a given area of vegetation bottom offers a larger "living space or interior area" than a corresponding area of hard or soft bottom. The quantity of vegetation also has a very great influence on the number of *Asellus* per m² (Fig. 2), (cf. BERGLUND 1968). From October to December 1967 there is a definite connec-tion between the quantity of *Cladophora* (expressed in dry weight) and the number of *Asellus* per m², with a monthly mean value of 46—53 animals per gram of *Cladophora*. A similar relationship holds from January to March although the mean values are lower (31—33 *Asellus* per gram of *Cladophora*) possibly due to fish predation.

Asellus is found almost everywhere in Lake Pajep Måskejaure, and Fig. 3 shows its occurrence at different depths. Generally speaking, the number of *Asellus* per m² decreases as the depth increases. In the zone nearest to the
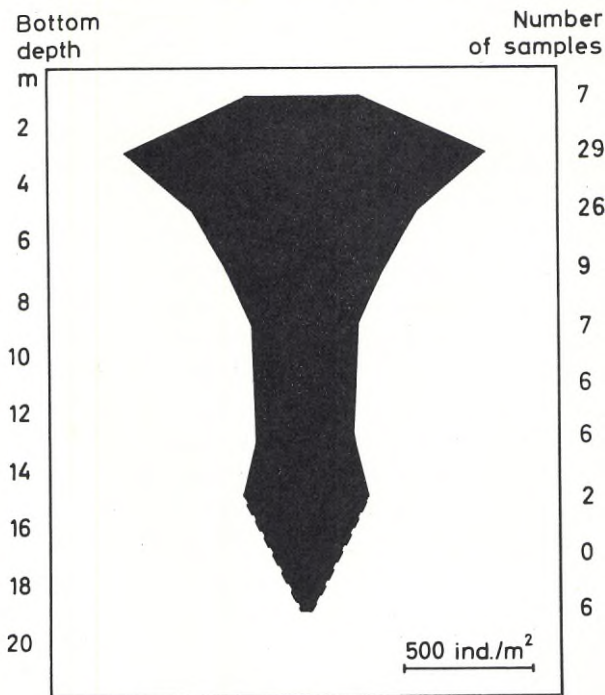
Fig. 3. Average benthic distribution of *Asellus aquaticus* in Lake Pajep Måskejaure (June 1965—April 1966).

surface the number of *Asellus* varies between 0—1,200 individuals per m² with an average of 400 individuals per m² (Fig. 3). The large variation is probably due to extensive environmental fluctuations in this zone. The level of the lake varies by about 1.3 m during a year and is lowest in the winter when there is also an icecover of about 0.7 m. Thus the entire upper zone is affected by desiccation following partial lake drainage and ice scouring. There is an almost complete lack of submerged vegetation in this zone. Presumably this zone must be settled by new *Asellus* every spring. In the second zone (2.0—3.9 m) we find the greatest density of individuals. Two thirds of the samples collected in this zone are taken from vegetation bottom which contributes to the great number of individuals. The mean value of this zone is 1,400 individuals per m², but single values are considerably higher with a maximum value of 7,000 individuals per m². In the third zone (4.0—5.9 m) we find about 900 individuals per m² and in lower zones the number of individuals per m² is still lower. In the deepest zone investigated (18.0—19.9 m) *Asellus* were found in only two out of six samples.

In Lake Erken there are large numbers of *Asellus* in areas close to the shore and also in a limited area at a depth of 4 to 6 m which is covered with *Cladophora aegagropila*. As many as 10,700 individuals per m² (August 9, 1968) have been found in the former area whereas the maximum value for the latter is 12,400 individuals per m² (September 21, 1967).

2. Annual cycle of *Asellus*

There are two generations of *Asellus* in Lake Pajep Måskejaure (Figs. 4, 5). The two generations can be distinguished from each other on the basis of their length. For April 1966 there was a difference of about 3 mm between the two generation mean lengths of 6.1 mm and 2.7 mm, respectively. The same mean lengths are valid for May. The older generation (generation 1 a) then starts to grow during June and July. Simultaneously with this increasing growth, reproduction takes place after which the animals die. A few specimens may be found in August, but in September there is nothing left of generation 1 a. Generation 1 b, the first specimens of which were hatched at the beginning of August 1965 (1.1—1.2 mm on August 6, 1965), grows until October of the same year and has then reached a mean length of 2.7 mm. In April, May and June, 1966, the mean length is still 2.7 mm. Then there is a rapidly increasing growth during the summer months, concomitant with an obvious reduction of the number of individuals from some 2,000 individuals per m² to about 1,000 individuals per m². This reduction in numbers is probably a combination of fish predation and other mortality. In April 1967 the mean length was 6.7 mm, i.e., a little longer than that of the previous year which was 6.1 mm for the older generation. Generation 2 a, which was being hatched at the beginning of August, 1966, grew during the autumn, and in April 1967 its mean length was 2.9 mm (cf. 2.7 mm for the previous generation at the same age). On August 24, 1967, it had reached a mean length of 5.7 mm (cf. 5.6 mm for generation 1 b at the same date the year before). As can be seen from these comparisons, the variations from one year to another are comparatively small. As a rule the animals hatch at the beginning of August, live for two years and reproduce once during their second year. Usually there is also a reduction of the number of individuals per m² during the whole year, but it is most evident during the part of the year when there is no ice.

For Lake Erken the picture is quite different. Here *Asellus* grows to be one year old. The growth of generation 1 a begins about one month earlier than in Lake Pajep Måskejaure as does reproduction and hatching of the new generation. The new generation, 2 a, starts to hatch at the end of June or beginning of July, and is almost completed one month later. During July both generations can be found, but in August one generation has probably

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Fig. 4. Length distribution of the *Asellus* populations in Lake Pajep Måskejaure and Lake Erken. The animals have been divided into size groups of 0.5—0.9 mm, 1.0—1.4 mm, 1.5—1.9 mm etc.; heights of bars show number of individuals expressed in per cent of monthly totals. Figures within brackets give number of animals measured. Dashed bars represent the per cent of *Asellus* from a single generation (group) and ignoring the other (newer) generation. 1 a, 1 b, and 2 a are designations of the generations, 1 a being the oldest generation and 2 a the youngest.

PAJEP MÅSKEJAURE
1966-1967

ERKEN
1967-1968
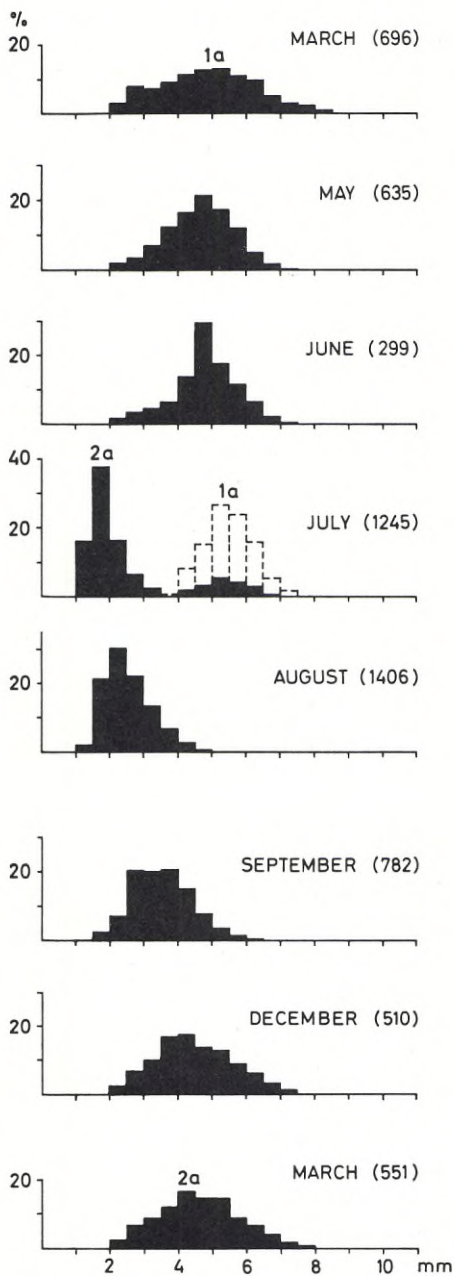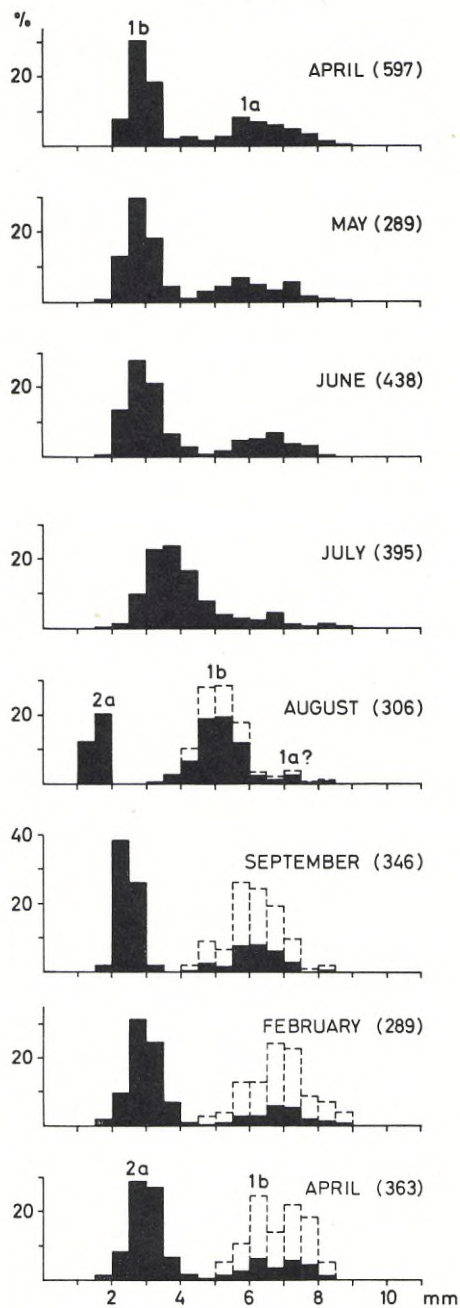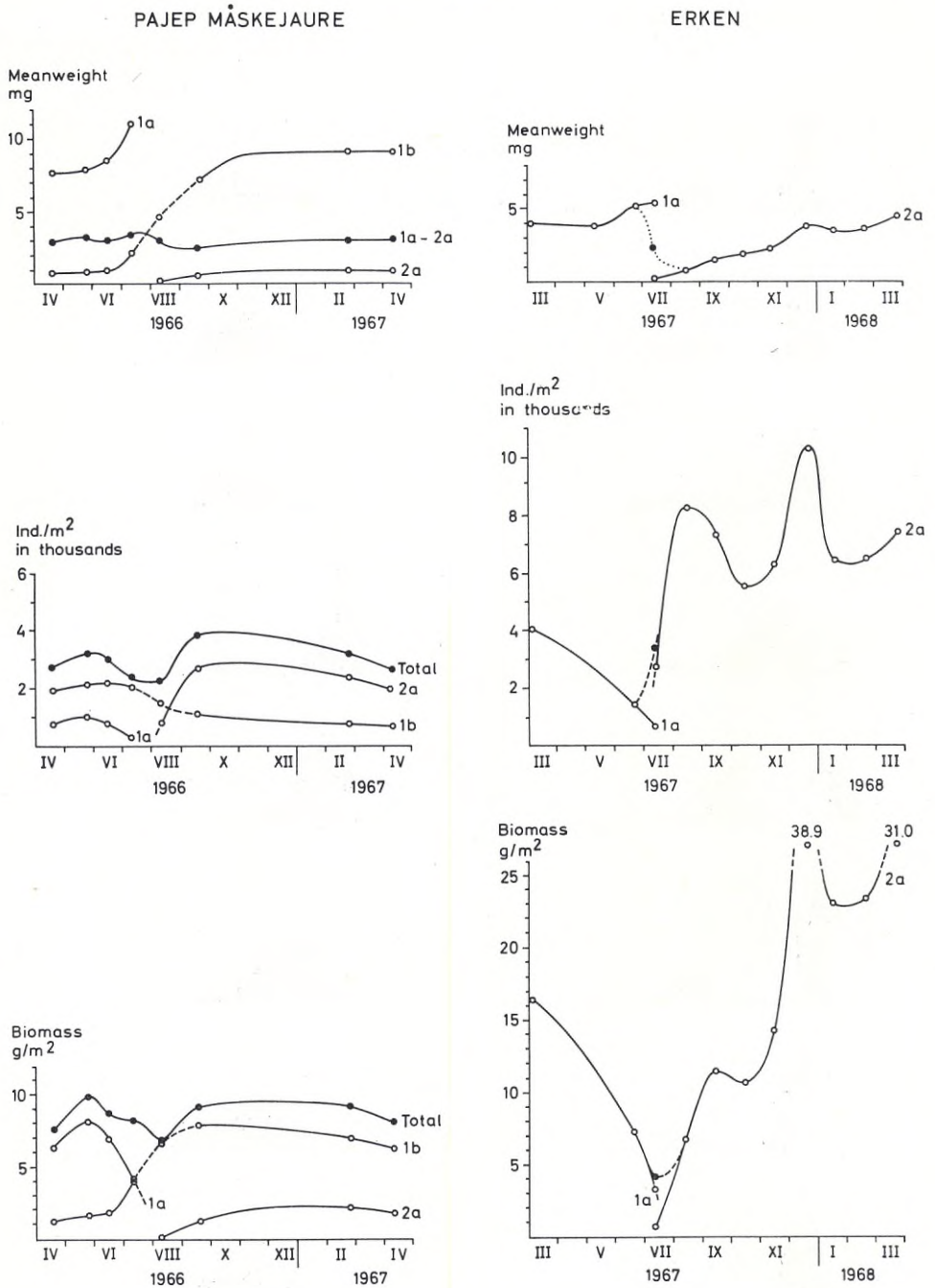
Fig. 5. Annual variation of mean weight, individuals per m², and biomass per m² of *Asellus* in Lake Pajep Måskejaure and Lake Erken. Designations of generations as in Fig. 4. •=mean value and sum of 3 (Lake Pajep Måskejaure) or 2 (Lake Erken) generations.

been succeeded by another. (A few specimens seem to reproduce in their first summer; see Fig. 8 a). Then the new generation continues to grow throughout the autumn. The large fluctuation in numbers and biomass between August and March in Lake Erken (Fig. 5) is chiefly due to the varying quantities of vegetation in the samples.

The changes in the biomass during a year (Fig. 5) present an obvious difference between the two lakes. In Lake Pajep Måskejaure the biomass varies between 7 and 10 grams per m² whereas in Lake Erken the variation is much greater, ranging from 4 to 39 grams per m².

The type of alteration of generations presented by *Asellus* in Lake Erken has been suggested or shown in investigations from other parts of Middle Sweden. In his investigations from Pond Hyttödammen NORLIN (1961—62) says that the mean weight decreases during spring and that the alteration of generations is completed in September. A similar tendency appeared in Pond Billingedammen (BERGLUND, 1968).

In Denmark the *Asellus* type of alteration of generations is quite different. "The attentive observer cannot fail to notice that the big specimens measuring about 20 mm (males) and about 15 mm (females) are almost always to be found in spring. Those found in summer are much smaller. Both sizes of the species are sexually mature. However, the smaller summer specimens, hatched in spring, are most likely young animals which start reproducing during the summer, go on growing during the winter and show a new, distinct sexual period in spring after which they die. There are no clearly delimited breeding periods as reproduction can continue all winter." (WESENBERG-LUND 1939). An attempt to confirm WESENBERG-LUND's observations of the growth conditions and life-cycle of *Asellus* in Denmark was made by THORUP (1963). He investigated a number of Danish springs, but found that the pattern seems to be complex in Denmark. For one thing the hatching covered a long period of time; for another there were probably several generations succeeding each other. Moreover, his material was too limited to allow any definite conclusions.

3. Growth

Growth is to a great extent dependent on temperature and when the temperature has reached a certain minimum value, growth ceases. ILLIES (1952) calls this value "Entwicklungsnullpunkt". When the temperature again rises above this value, the animals start growing. Different groups of animals show different values of their growth, and LE CREN (1958) has proved that the growth of perch (*Perca fluviatilis* L.) is directly proportional to the number of degree-days above 14°C. The same principle will also apply to other groups of animals. TAUBE and NAUWERCK (1967) give some data of the time of development of the first nauplius stage of *Meso-*

cyclops leuckarti. They found that the time of development in hours at the different temperatures was:

25°C	20°C	14°C	8°C
34—39	49—53	83—91	> 290

The dependence of the time of development on temperature can be figured out from these values. These calculations show that the time of development is directly proportional to the number of degree-hours above 6°C. Thus the number of degree-hours at the different temperatures is 694 ± 48; 714 ± 28; 696 ± 32 and about 700, respectively, which corresponds to 27—31 degree-days above 6°C.

In Lake Pajep Måskejaure there was no growth from October 1965 to May 1966 during which time the temperature was 0—3°C. In Lake Erken there was also some retardation of the growth rate during the winter. There is an obvious relation between the mean length of the animals and the number of degree-days above 3°C (Fig. 6 a). This temperature limit is a little difficult to fix but should be within ± 1 degree. The growth rate of *Asellus* per degree-day above 3°C in Lake Pajep Måskejaure is quite different from that in Lake Erken (Fig. 6 a). In the former the length increment is about 4 mm and in the latter about 1.5 mm per 1,000 degree-days above 3°C. These marked differences may result from genetic differences of the populations. The water in the part of Lake Pajep Måskejaure where the samples have been taken is in continuous motion and certainly has a favourable influence on the growth of *Asellus,* as it is rich in nutrients and oxygen and constantly flows over the bottom. Besides *Isoëtes* (rich in periphyton) may be a better bottom substratum than *Cladophora.* Another reason may be differences in fish predation as the fish prefer the biggest specimens of *Asellus* (BERGLUND 1968). During the period November—December Lake Erken produced a comparatively rapid growth in spite of low temperature (Fig. 5). This "increment" (perhaps more apparent than real) may be due to emigration of larger individuals from areas close to the shore as the water is covered with ice. Such emigration has been noticed by BERGLUND (1968). The reason for the growth from February to March cannot be differences in temperature as this remains relatively unchanged from December 12, 1967, to March 23, 1968. The immediate effect of longer days with increasing light intensity might be to initiate the growth processes of the animals (THORUP, 1963). However, further studies are required to reveal the existing connections.

In order to study the dependence of growth on temperature an aquarium experiment was carried out on animals from Lake Pajep Måskejaure for which 10 samples were taken on August 24, 1967. All *Asellus* were immediately picked out of two of these samples (73 *Asellus* of the new-born generation and 42 *Asellus* of the older generation), and the remaining samples

Meanlength
mm
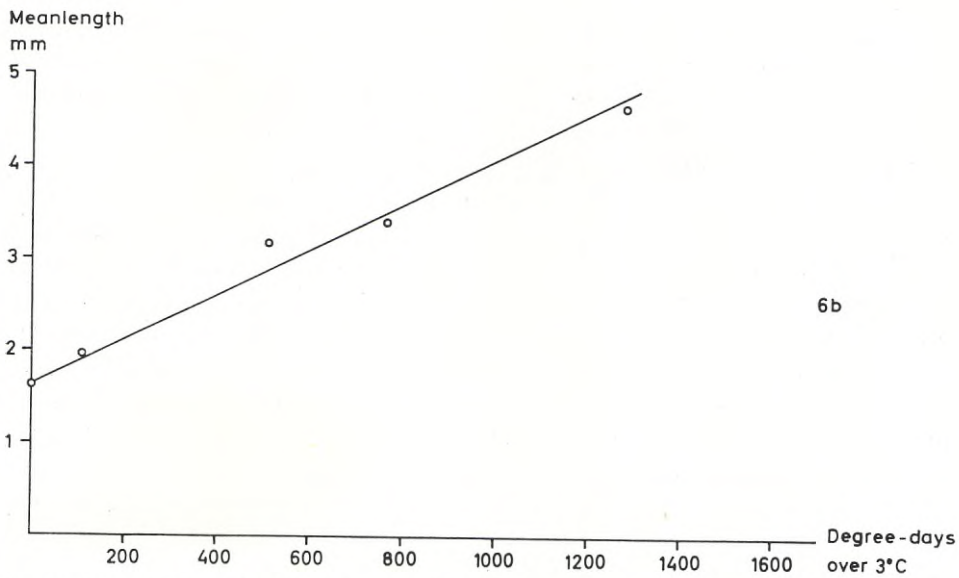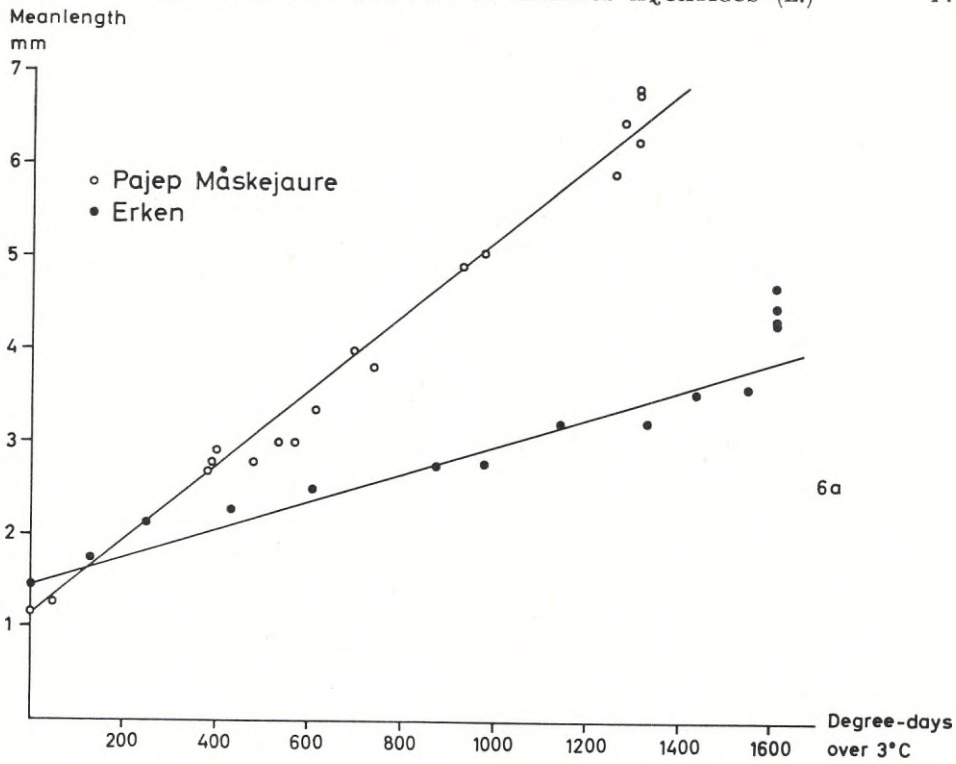
Fig. 6. Relation between *Asellus* growth and number of degree-days above 3°C.
    a) single generations in natural environments
    b) youngest generation (from Lake Pajep Måskejaure) in aquarium experiments.

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were put into four 10 litre vessels, each containing two samples. They were then kept at temperatures of 4.5°, 10°, 13.5° and 20.5°C, respectively. There were 79—120 animals at each of the different temperatures. After 73 days (on November 5) the experiment was terminated. There is in this case too (Fig. 6 b), a connection between growth of the youngest generation (born in August 1967) and number of degree-days. However, the rate of growth here is lower, the value being about 2.5 mm per 1,000 degree-days above 3°C. The aquaria were not aerated which may be one of the reasons for the lower value. The oxygen supply was dependent upon surface diffusion and production by the existing vegetation, and poor oxygen conditions might possibly have influenced both growth and reproduction. There might also be some scarcity of nutrients. The *Asellus* females from Lake Pajep Måskejaure had reached sexual maturity when they are about 5 mm long (Table 3). Remarkably enough, the older generation (44—51 animals) had not reproduced during the aquarium experiments in spite of a mean length of not less than 5.7 mm (4.5°C), 7.3 mm (10°C), 7.0 mm (13.5°C), and 7.2 mm (20.5°C). It is evident that some other factor prevented reproduction. According to BERG-LUND (priv. com.) the water must probably be cooled for some time before reproduction can start again. Light may also be of some importance. The proportionality between temperature and growth mentioned above only applies at temperatures lower than the optimal temperature of each animal species.

A similar experiment with material from Lake Erken was started on January 22, 1968, and concluded 68 days later (March 30). In this case one sample per aquarium was taken (100—170 animals). There was no obvious increase of the average length at any temperature. At the lowest temperature (4.5°C) the average length was 4.4 mm, two couples, and one female with an empty ovisac were found. In the 10°C aquarium the alteration of generations had advanced somewhat further. One female with eggs, three females with embryos, two females with empty ovisacs, and a few young that had recently left their mother were found. Neither couples nor females with eggs nor young were found in the 13.5°C aquarium. An explanation of this cannot be given. In the 20.5°C aquarium the alteration of generation had advanced further than in the others. A new generation with an average length of 1.4 mm had been produced, and there were no females at all with ovisacs. The percentage of big animals had decreased, and the average length of the older generation was only 4.1 mm. The increase in growth that might be expected at that temperature was counteracted by a greater mortality among the fully grown individuals as a result of their reproduction.

4. Reproduction

At the initial stage of the reproductive cycle the male seeks a female, seizes one and carries her under his venter (cf. WESENBERG-LUND 1939).

Mean length of a number of couples from Lake Pajep Måskejaure in 1966 show that the male is usually 0.5—2.5 mm longer than the female. The average length of the males was 7.3 mm and of the females 6.0 mm.

At the beginning of June 1967 a series of investigations of 116 couples of *Asellus* taken from the River Fyris and Lake Erken was started to study the rate of development of eggs at different temperatures. The couples were put into little plastic vessels at 4.5°, 10.0°, 13.5° and 20.5°C. Moults of successive stages proceeded much faster at higher temperatures — after 8.8, 3.3, 2.5, and 2.1 days, respectively, at the temperatures mentioned above. Some time later the males and females parted. The mean time for separation was 9.8, 5.5, 4.7, and 2.5 days, respectively. Shortly afterwards eggs were found in the brood-pouch of the females. According to WESENBERG-LUND (1939) the female is said to moult after the mating, but in most cases the moulting has been observed to take place before the male leaves the female.

The eggs of the females examined had a diameter of 0.3—0.4 mm. This was independent of the size of the female. The number of eggs per female is, however, directly dependent on the size of the brood-pouch of the female which in turn is dependent on the size of the female. When the eggs have hatched and the embryos have started to grow, their number decreases in the limited room of the brood-pouch on the ventral side of thorax. Thus, there are about half as many embryos or youngs as there are eggs (cf. WESENBERG-LUND 1939). These facts are shown in Fig. 7. The females have here been divided into size groups of 3.0—3.9 mm, 4.0—4.9 mm, 5.0—5.9 mm, and so on. When the samples are taken and sifted, the animals are shaken and some of the eggs lost. In order to give more specific information about the number of eggs of the females, the variation within each size group and its mean value have been shown for the Pond Hyttödammen investigation. The number of females examined within each size group from the smallest to the largest specimens is 11, 12, 16, 12 and 2, respectively. As can be seen, the number of eggs may be very large, and a female measuring 9.3 mm in length had the largest number of eggs found, viz. 293. The values for Pond Hyttödammen are generally higher, possibly because the whole sample was preserved immediately without sifting after it had been taken.

The length of time needed for the development from the newly laid egg to the fully developed *Asellus* young (leaving its mother when it has become 1 mm long) varies with the temperature. WESENBERG-LUND (1939) estimates the length of this time at 3—6 weeks, the lower figure referring to summer. Table 2 shows the time of development of two separate populations at their respective temperatures. The figures for the River Fyris material are about 2, 4, and 6 weeks at temperatures of 20.5°, 13.5° and 10°C respectively. As a rule the females from Lake Erken needed a somewhat longer time. When the investigation at the lowest temperature, 4.5°C, started, there were 27 females in all. As the females carry their eggs for a long time at such a low
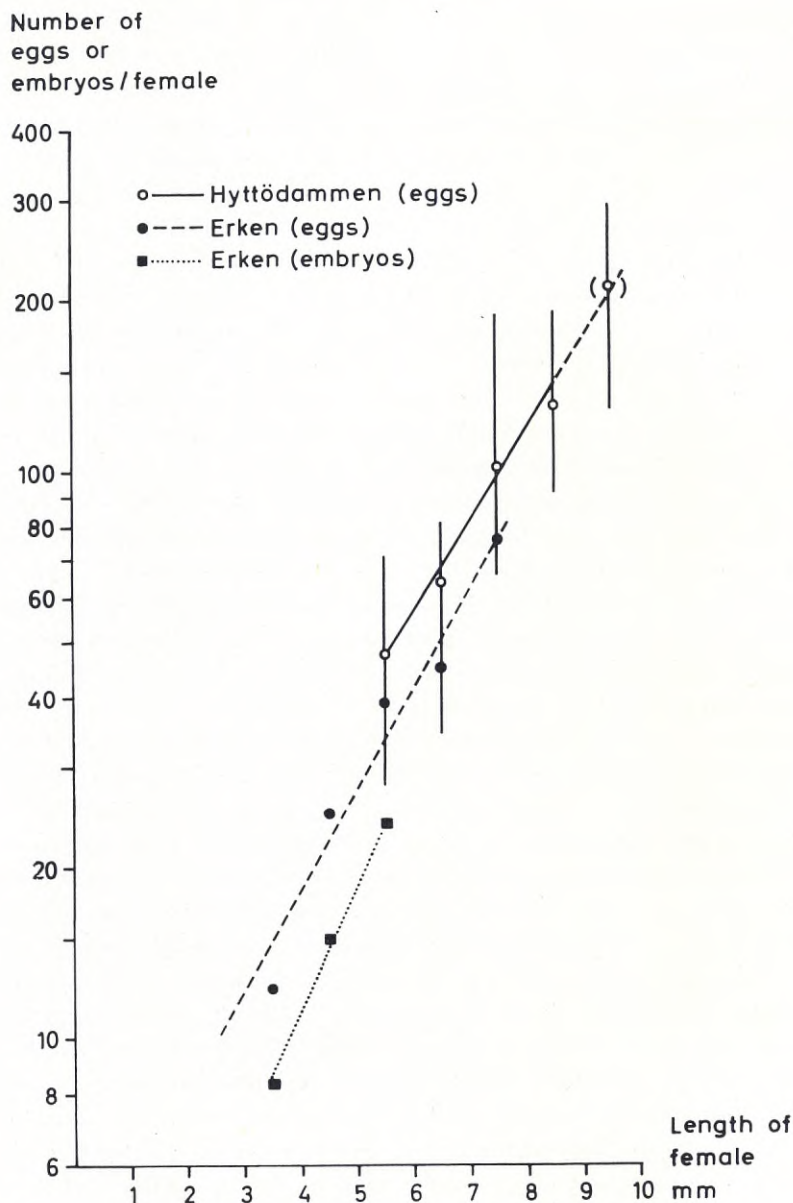
Fig. 7. Relation between length of *Asellus* female and number of eggs and embryos.

temperature, the mortality was very great. Only two of the females released their young after more than 19 to 20 weeks. At that time the young were still not fully developed and died soon afterwards. Three of the other females moulted after 4.5—5.5 months when they also lost their eggs, 4 of them lost their eggs after 3—5.5 months, 17 died after 1—4 months while still carrying

Table 2. Time of development from new-laid egg to fully grown *Asellus* young during aquarium experiments. Time at different temperatures given in days. Mean value within brackets.

	20.5°C	13.5°C	10.0°C	4.5°C
The River Fyris	13—17 (14.6)	25—29 (26.5)	41—43 (42.0)	> 134
Lake Erken	15—17 (16.0)	29—32 (30.6)	44—48 (45.3)	> 148

their eggs, and one of them was still carrying her eggs after 6 months. Of the 27 females there were 13 carrying their eggs for more than 3 months and 9 carrying their eggs for more than 4 months. Obviously temperature is of primary importance for the development of the egg. An attempt has been made to calculate the time of development expressed in "effective degree-days". The results show that the rate of development is directly proportional to the number of degree-days above 4°C, viz. for the Fyris River 4.4°C and for Lake Erken 4.2°C. For the former material 235—241 "effective degree-days" are needed, and for the latter 258—282 degree-days above 4.4°C are needed. Thus, a temperature of 5.4°C would mean that the females would carry their eggs for 8—9 months. These values also seem to be correct for the Lake Pajep Måskejaure observations. The first couples can be found in May and the greatest number per m² between June 10 and June 20. Females with eggs begin to appear at the end of May and are most numerous between July 10 and July 20. The mean temperature of this period is 11.3°C, that is 7°C above the initial value. The length of the growing period was about one month. Thus effective degress × 30 days giving a value of 210 "effective degree-days" for that time.

In Lake Erken at the beginning of August a minimum in number of females with eggs or embryos is followed very soon by a maximum (Fig. 8 a), a similar trend is evident in Lake Pajep Måskejaure. The average length of sexually mature females is also considerably shorter in August and later than it is at the beginning of the season. From Table 3 it can be seen that not only the mean value decreases but also the variation in size. The reason for this may be that the biggest specimens of the (one year) younger generation have now reached their reproduction period. This would mean that few of the animals in Lake Pajep Måskejaure are sexually mature when they are one year old whereas most of them reproduce only in their second year. The animals which hatched at the end of May or beginning of June in Lake Erken were sexually mature at the beginning of August of the same year. Whether or not these females which were sexually mature so early, survive their first reproduction and are able to mate a second time is hard to say.

There are great variations in the time of occurrence of females with eggs depending upon the lake's latitude. In Lake Pajep Måskejaure females with
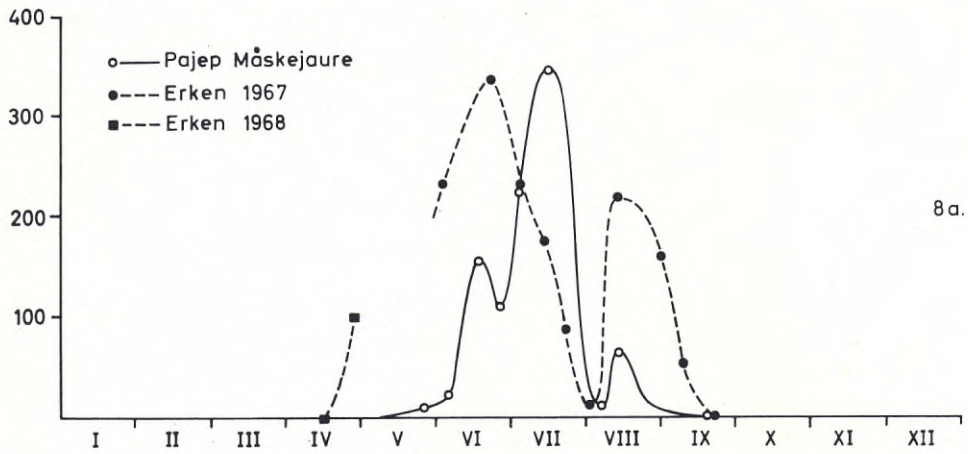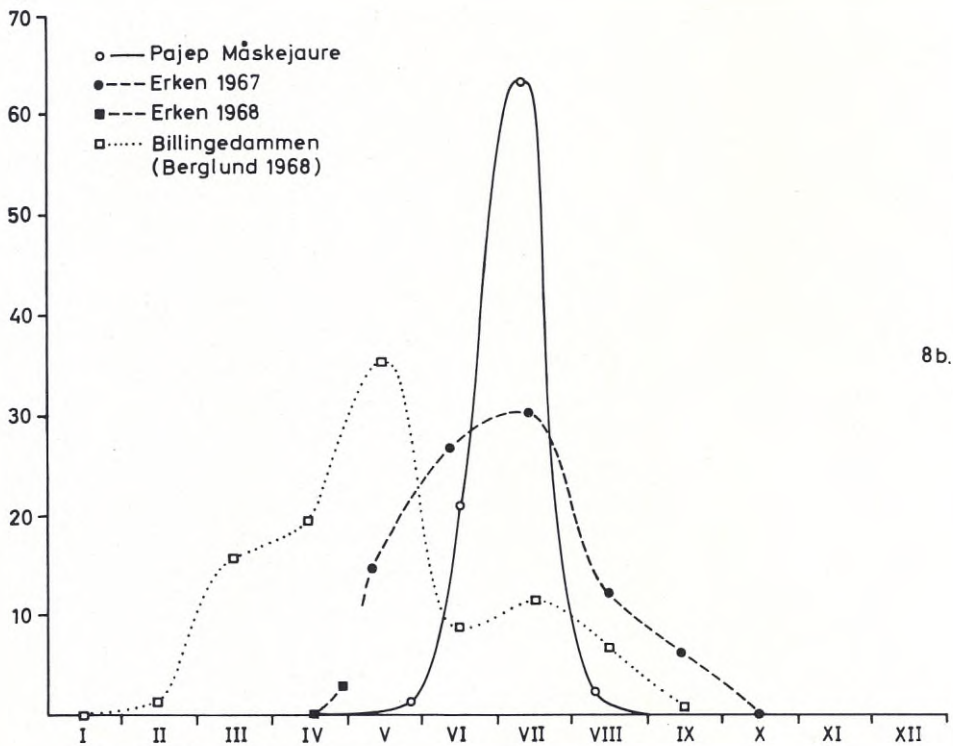
Fig. 8. Occurrence of females with eggs or embryos during the year.
a) in number per m²
b) in per cent of the number of sexually mature animals.

Table 3. Length in mm of females with eggs and embryos during investigation period. Mean value within brackets. n=number of animals examined.

	May	June	July	August	September
Lake Pajep					
Måskejaure	6.7	5.2—7.2 (6.2)	5.0—8.5 (6.5)	4.4—5.5 (5.1)	—
n=	1	21	44	4	
Lake Erken	4.1—6.8 (5.3)	4.2—5.9 (4.9)	4.1—6.4 (5.3)	3.5—4.4 (4.0)	3.5—4.2 (3.8)
n=	81	71	62	13	4

eggs are found during 3 months (May—August), in Lake Erken during 5 months (April—September), in Pond Billingedammen during 7 months (February—September) (BERGLUND 1968), and in Denmark during 12 months (WESENBERG-LUND 1939). For Lake Pajep Måskejaure the number of females with eggs has been expressed as per cent of the older generation (Fig. 8 b). August is an exception as it is difficult to make a distinction between generation 1 a and generation 1 b, so the percentage has been calculated on the two generations put together. In Lake Erken the percentage has been calculated on individuals measuring 3.5 mm (minimum length of sexually mature individuals) or more in length, and in Pond Billingedammen BERGLUND (1968) has calculated the percentage on "sexually mature individuals". Pond Billingedammen reaches its maximum value in May whereas Lake Erken and Lake Pajep Måskejaure reach theirs in July (Fig. 8 b). However, Lake Pajep Måskejaure shows a much higher maximum value than the two other locals. This is due to the shorter time available for the alteration of generations. In all three cases the new generation has taken the place of the old generation several months before the lakes are covered with ice. (In Lake Pajep Måskejaure about 2.5 months, in Lake Erken about 3.5 months, and in Pond Billingedammen about 2.5 months.) This may be fortuitous, but it certainly gives the animals a greater chance of survival if they reach a certain minimum size before winter.

5. Production

An attempt has been made to measure the annual production of *Asellus* in Lake Pajep Måskejaure and in Lake Erken (Tables 4 a and b). On the whole, the method follows that used by KAJAK and RYBAK (1966). The monthly average weights (a) and monthly average numbers (c) have been used. Production (e) has then been worked out by multiplying the increase of growth (b) by the average number of *Asellus* taken on the two successive sampling occasions (d). The annual average biomass represents the mean value of the monthly mean values of one year (thus, the value of the last sampling is not included). In Lake Pajep Måskejaure the annual production

Table 4 a. Production and biomass of *Asellus aquaticus* in Lake Pajep Måske-
jaure 1966—1967. Example of calculation.

Month	Gene-ration	Biomass of one specimen mg a	Increase of biomass of one specimen/ interval mg b	Average number of ind./m² c	Average number of animals of 2 successive intervals ind./m² d	Produc-tion P g/m² e	Average biomass B g/m² f
Apr	1 a	7.84	—	813	—	—	6.37
	1 b	0.69	—	1,946	—	—	1.34
May	1 a	7.73	—0.11	1,055	934	—0.10	8.16
	1 b	0.80	0.11	2,155	2.051	0.23	1.72
Jun	1 a	8.00	0.27	667	861	0.23	5.34
	1 b	0.86	0.06	2,207	2,181	0.13	1.90
Jul	1 a	11.93	3.93	366	516	2.03	4.37
	1 b	1.88	1.02	2,023	2,115	2.16	4.09
Aug	1 a	—	—	—	183	—	—
	1 b	4.46	2.58	1,695	1,859	4.80	7.56
	2 a	0.13	0.13	978	489	0.06	0.13
Sep	1 b	7.00	2.54	1.033	1.414	3.59	7.93
	2 a	0.57	0.44	2.711	1,845	0.81	1.5£
Feb	1 b	9.00	2.00	778	956	1.91	7.00
	2 a	0.91	0.34	2,433	2,572	0.87	2.21
Apr	1 b	9.00	0.00	696	737	0.00	(6.26)
	2 a	0.90	—0.01	1,992	2,213	—0.02	(1.79)

For the whole year   16.70   8.52

$$\frac{P}{B} = 1.96$$

Table 4 b. Production and biomass of *Asellus aquaticus* in Lake Erken
1967—1968. Example of calculation.

Month	Gene-ration	Biomass of one specimen mg a	Increase of biomass of one specimen/ interval mg b	Average number of ind./m² c	Average number of animals of 2 successive intervals ind./m² d	Produc-tion P g/m² e	Average biomass B g/m² f
Mar	1 a	4.14	—	3,980	—	—	16.47
Jun	1 a	5.22	1.08	1,376	2,678	2.89	7.19
Jul	1 a	5.49	0.27	632	1,004	0.27	3.33
	2 a	0.25	0.25	2,707	1,354	0.34	0.76
Aug	1 a	—	—	—	316	—	—
	2 a	0.81	0.56	8,162	5,435	3.04	6.67
Sep	2 a	1.37	0.56	7,277	7,720	4.32	11.41
Oct	2 a	1.93	0.56	5,490	6,384	3.58	10.59
Nov	2 a	2.28	0.35	6,250	5,870	2.05	14.22
Dec	2 a	3.82	1.54	10,200	8,225	12.67	38.93
Jan	2 a	3.60	—0.22	6,380	8,290	—1.82	22.98
Feb	2 a	3.63	0.03	6,435	6,408	0.19	23.36
Mar	2 a	4.22	0.59	7,345	6,890	4.07	(30.95)

For the whole year   31.60   15.59

$$\frac{P}{B} = 2.03$$

is 16.70 grams per m² and the annual average biomass 8.52 grams per m² whereas values for Lake Erken are about twice as large (31.60 and 15.59 grams per m², respectively). Thus the ratio of production to biomass $\left(\frac{P}{B}\right)$ of the two lakes is much the same. Thus Lake Pajep Måskejaure had a $\frac{P}{B}$ value of 1.96 and Lake Erken 2.03. If this ratio also applies to other lakes, a calculation of their production of *Asellus* can be made based on the yearly average biomass. KAJAK's and RYBAK's work (1966) gives a $\frac{P}{B}$ ratio of 2—6 for the profundal fauna and for species with a long development-cycle. As for animal species with several generations a year the ratio rises to 12—15 for the sublittoral zone.

V. Summary

Studies on the life-cycle and growth of *Asellus aquaticus* (L.) have been carried out in Lake Pajep Måskejaure in Northern Sweden and in Lake Erken in Central Sweden.

1. *Asellus* prefers bottoms rich in vegetation, and the number of individuals per m² depends chiefly on the density of vegetation.

2. In Lake Pajep Måskejaure *Asellus* can be found at all depths investigated, but can be found in the greatest numbers in the depth zone of 2.0—3.9 m.

3. In Lake Pajep Måskejaure the animals hatch in August and live for two years during which time they reproduce once in their second year. In Lake Erken the animals start hatching in July, live for one year and reproduce only once.

4. The growth of *Asellus* is dependent on temperature and is nearly directly proportional to the temperature above 3°C.

5. The number of eggs and embryos depends on the size of the female.

6. During aquarium experiments the rate of development from newlylaid eggs to fully grown *Asellus* young was directly proportional to the number of degree-days above 4°C.

7. The breeding period in northern lakes is shorter than that for similar lakes further south.

8. The mean annual *Asellus* biomass in Lake Pajep Måskejaure is 8.5 grams per m² and of Lake Erken 15.6 grams per m². Corresponding values of the mean annual production of *Asellus* are 16.7 and 31.6 grams per m², respectively. The ratio of production to biomass is the same in both cases, *viz.*, 2.

VI. Acknowledgements

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Tag shedding, growth and differential mortality in a marking experiment with trout and char

By Å. Fagerström, K.-J. Gustafson and T. Lindström

I. Introduction

A marking experiment was carried out as part of a population study in Lake Långbjörsjön, a small high mountain lake in Jämtland with a length of one kilometre, a width of about one hundred metres and a maximum depth of 11 metres. The fish population consists exclusively of char, and trout, (*Salvelinus alpinus* L. and *Salmo trutta* L.), and for many years prior to the experiment mainly fly fishing was practised. The annual yield will be described in a following report (MS). In the present paper only the methodological aspects of the marking experiment will be treated, such as tag shedding, increased mortality or reduced growth due to the tagging. Some relevant results from the neighbouring Lake Dörstjärn will also be included. The brook running from this lake, which is even smaller than L., ultimately becomes the main inlet to Lake Långbjörsjön (cf. Gustafson *et al.* 1969).

II. The marking experiment

The fish were captured for marking, with gear described on p. 29 and p. 34 at the beginning of the fishing season, *i.e.* early in July. During this period very few fish were killed; recaptured marked fish were released again. Fin-cut fish from 1959 recaptured in 1960 and 1961 were, however, put back only after they had been tagged. Apart from fly fishing by a small group of visitors at a neigbouring resort, our own netting, seining and hook fishing, constituted the whole of the fishing during the rest of the fishing season.

As reported in a preliminary paper (*Svenskt Fiske* 1962) there was some evidence of shedding of tags early in the experiment. In 1962 seine-caught trout were tagged in Lake Dörstjärn (Fig. 1); the adipose fin was also cut from these trout. Their total length varied between 16 and 39 cm. The fishing in Lake Dörstjärn was deliberatedly reduced during this year. A check of recapture was made according to Table 1.

During period II all fish caught, 198 trout, were checked. The mean length of fin-cut and tagged fish was above the mean length of the whole catch. The same seine was used as in 1962, and trout of a later year class had
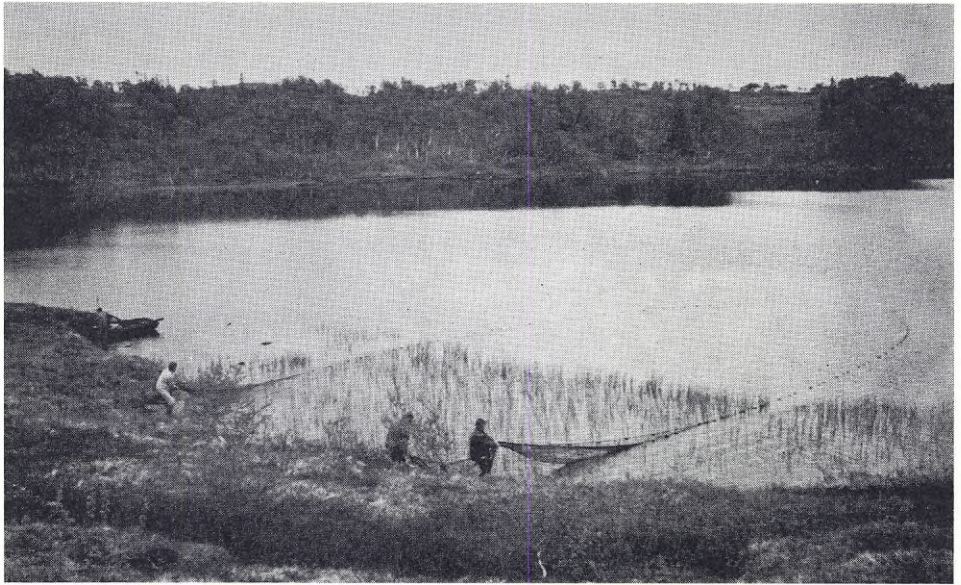
Fig. 1. Seining in Lake Dörstjärn for trout. The seine was floating and a seine haul covered more than half the distance between opposite shores.

entered the vulnerable population while the tagged ones had increased in length.

A slightly higher rate of recapture of trout still carrying tags was obtained in Lake Långbjörsjön, as is shown in Table 2. Differences in fishing pressure, lake vegetation, etc. may account for differences in rate of recapture. In both lakes the actual tags were of the type described by CARLIN (1955) but anchored in the fish with one thread or wire through the back, between the dorsal fin and the backbone and a loose loop carrying the tag. Some of the tags in 1960 were attached with stainless steel wires, the rest were attached with threads of spun nylon, as also in 1961, Lake Långbjörsjön. Some series contain fish which were only fin-cut (pelvic and/or adipose fin), and recaptures of these were obtained only from our own catches, forming at least half the total catches denoted in Table 2.

A comparison between the two columns on the right of Table 2 shows a higher rate of recapture for fin-cut fish than for tagged fish from the 1961 series and the left-hand column in the table shows recapture of fin-cut fish during more years than recapture of tagged fish. A correction with respect to the number caught in different years does not invalidate this comparison. In Lake Långbjörsjön there were observed during 1960—62 16 trout and 16 char with damage in the back or with a split dorsal fin; in some cases recaptured fish had the thread or wire belonging to the tag in a very super-

Table 1. Recaptures from 203 trout in Lake Dörstjärn. The trout were tagged (spun nylon thread) and the adipose fin cut in 1962 between July 11 and 13.

Period	Completeness of check	Number without tags but fin-cut	Number with tags
I July 14, 1962—June 15, 1963	Only recapture of tagged fish reported	—	1
II June 16—July 5, 1963	Every caught fish checked		
a. Fly fishing		15	7
b. Seining, only July 5		29	6
III July 6, 1963—June 13, 1964	Only recapture of tagged fish reported	—	4
IV June 14—July 12, 1964	Every caught fish checked	10	0

(One detached tag reported without further specification).

ficial position (Fig. 2), and sometimes it was actually torn off during recapture.

The rate of recapture does not vary only with the shedding of tags. The following schedule comprises the factors discussed in the present paper in the order in which they are treated.

Rate of exploitation of tagged fish	—	Small fish/large fish
Shedding of tags	—	,,
Tags increasing the natural mortality rate	—	,,
Tagged fish behaving differently from untagged	—	,,

III. Rate of exploitation of tagged fish

If tagged fish are undersized in relation to the gear generally in use in a lake, the reduction by natural mortality of the tagged lot up to the prevalent "age at first capture" in the lake (HOLT 1960) will be greater the smaller they were at the time of tagging. Besides, the age and length at first capture, typical of the tagged ones only, will be less in comparison with untagged fish if the tags of small fish are easily entangled in certain types of gear. In the present experiments tagging is made with a seine with 65 mm inner perimeter of the meshes in the bag, 32 stretched meshes per 120 centimetres, an otter with flies or small spoons, gill nets with 2.50 mm knot-to-knot measure and fly fishing with hooks nos 10—12 etc. (see tables of length distribution, following paper in MS). The tagged fish are fully vulnerable to the fishing gear used for recapture, gill nets with mesh sizes 1.67, 2.14 and 2.50 mm and fly fishing during the year of tagging.

Nevertheless, the presence of a tag may increase the probability of capture. If this increase is important it would be plausible to assume that tags with

Table 2. Recaptures from tagging and fin-cutting experiments in Lake Lång-björsjön. The tagging and fin-cutting were carried out at the commencement of each fishing season, i.e. at the beginning of July. Recaptures include a small number recaptured more than once, 13 tagged trout and 4 tagged char — recaptures during the tagging period were released again.

	1959	1960	1961	1961
	Fin-cut	Tagged	Tagged	Fin-cut
A. Trout				
Tagged or fin-cut	177	162	153	24
Catch:				
Number caught or checked, year of marking	508	416	781	
Ditto, first year after marking	416	781	267	
Ditto, second „ „ „	781	267	255	
Ditto, third „ „ „	267	255	147	
Ditto, fourth „ „ „	255	147	250	
Recaptures:				
(a) Recaptures during marking, year of marking	7	15	25	6
(b) Own recaptures later on „ „ „	28	21	39	7
(c) Other „ „ „ „ „ „	—	6	12	—
(a) Ditto, first year after marking	23	20	—	
(b) Ditto, „ „ „ „	17	7	12	3
(c) Ditto, „ „ „ „	—	3	1	—
(a) Ditto, second „ „ „	10	—	—	—
(b) Ditto, „ „ „ „	12	0	2	0
(c) Ditto, „ „ „ „	—	0	0	—
(a) Ditto, third „ „ „	—	—	—	—
(b) Ditto, „ „ „ „	2	0	0	0
(c) Ditto, „ „ „ „	—	0	0	—
(a) Ditto, fourth „ „ „	—	—	—	—
(b) Ditto, „ „ „ „	1	0	0	0
(c) Ditto, „ „ „ „	—	0	0	—
Totals	100	72	91	16
Per cent recaptures	56	52		67
B. Char				
Tagged or fin-cut	70	55	130	21
Catch:				
Number caught or checked, year of marking	488	379	643	
Ditto, first year after marking	379	643	256	
Ditto, second „ „ „	643	256	151	
Ditto, third „ „ „	256	151	182	
Recaptures:				
(a) Recaptures during marking, year of marking	1	0	10	2
(b) Own recaptures later on „ „ „	13	6	44	9
(c) Other „ „ „ „ „ „	—	1	1	—
(a) Ditto, first year after marking	3	1	—	—
(b) Ditto, „ „ „ „	3	5	1	4
(c) Ditto, „ „ „ „	—	2	1	—
(a) Ditto, second „ „ „	6	—	—	—
(b) Ditto, „ „ „ „	11	0	0	0
(c) Ditto, „ „ „ „	—	0	0	—
(a) Ditto, third „ „ „	—	—	—	—
(b) Ditto, „ „ „ „	2	0	0	0
(c) Ditto, „ „ „ „	—	0	0	—
Totals	39	15	57	15
Per cent recaptured	56	39		71
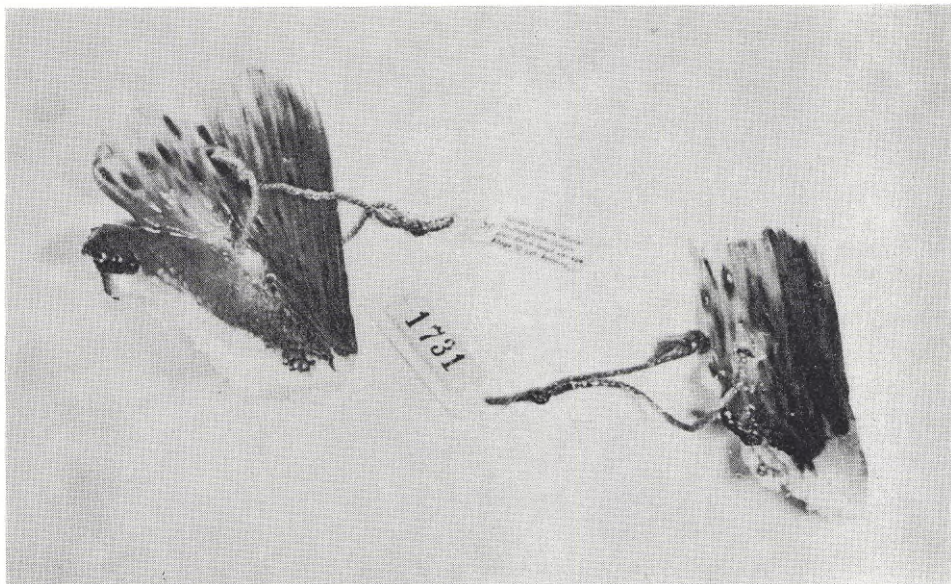
Fig. 2. Dorsal fin and adjacent tissue of two trout tagged on July 11 and 13 in 1962 and recaptured July 2 and 3 in 1963. The tags were originally attached with the nylon thread penetrating the back of the trout well below the dorsal fin.

stainless steel wires would increase the risk of capture in gill nets during the summer of the tagging year more than would tags with nylon thread, Table 3, column 1960.

The trout data confirm the assumption to some degree, but the char data do not. Chi² for heterogeneity, the trout data=0.4—1.1, p=0.5—0.3. The figures in brackets indicate the values after discarding the 16 smallest trout from the nylon thread group.

IV. Shedding of tags

Material illustrating shedding of tags is presented in chapter II and it would be reasonable to assume that different tag types would give different rates of tag loss. However, the effect of length at tagging is such that tagging series containing many small fishes generally have a low rate of recapture, so the effect of length should be taken into account.

The higher rate of recapture in the stainless steel wire group in 1961 becomes still more pronounced (only 7 recaptures of nylon thread type) if the 16 smallest trout are discarded from Table 3, the nylon thread group and there remains an equal proportion of small and large trout in each of the two tag-type groups (figures in brackets). Most of the 1961 recaptures were caught with hook or seine (18 out of 30 trout).

Table 3. Recapture of fish tagged in 1960 with different tags, Lake Långbjör-
sjön. Figures in brackets are explained in the text.

Chi² for hetero-geneity, 1961 recaptures of trout, 14.0	Tagged:				Recaptures:	
	Mean length	Number	≲ 24 cm	> 24 cm	Gill nets 1960	All recaptures 1961
Trout, stainless steel	24.8	63	38	25	10	22
„ nylon thread	23.2 (24.2)	99	66 (50)	33	9 (9)	8 (7)
Char, stainless steel	22.2	18	17	1	1	8
” nylon thread	21.8	37	33	4	5	0

A shedding of tags after the first summer explains why nylon thread tags
give such a low rate of recapture in the second year. According to Table 1,
period II a and b, the shedding of nylon thread tags in Lake Dörstjärn seems
to have been in progress during the early summer, one year after the tagging.
In the year 1961 char and trout were tagged in Lake Långbjörsjön with
green and white cardboard in the tags, and in a third group the adipose fin
was cut (in that year and the next, pelvic fins were thoroughly examined
under the microscope to avoid confusing fish fin-cut in 1961 with those from
1959 with regenerated pelvics). The different tag types and the fin-cutting
were distributed as correctly as possible over the period July 3—7 and over
the gear in use, etc., every fifth fish being fin-cut (Table 4).

In spite of the efforts made, the fin-cut fish thus proved to have a slightly
lower mean length. Cutting the adipose fin constitutes only a slight inter-
ference, but small fish may have been under greater stress during the capture.
The importance of this source of error is probably small. The outcome of the
experiment is likely to illustrate the complex action of higher probability of
capture for tagged fish than for fin-cut fish in gill nets and the incipient loss
of tags during the autumn, but it does not point to a serious tag loss during
the first summer.

Recaptures from the 1959 series of fin-cut fish during the second, third
and fourth years after the tagging year imply that tags with stainless steel
wires are also lost, as they are almost only obtained in the year of tagging
and the following year, Table 2.

V. Increased natural mortality or divergent behaviour of small tagged fish

The trout recaptured with tags in Lake Dörstjärn had a mean length at
tagging of 27.6 cm (18 specimens) and the mean length for all tagged trout
was 26.3 cm at tagging (203 specimens). 70 trout measuring 24 cm or less at

Table 4. Recapture during 1961 of fish tagged or fin-cut July 3—7 (part of the tagging season). Nylon thread tags.

	Number tagged	Mean length at start, cm	Recapture in our own fishing			
			Otter July 11—25	Gill nets Aug. 9—11	Gill nets Oct. 5—7	Total
Trout, white cardboard tags	48	24.0	1	12	2	15
,, green ,, ,,	48	23.9	6	4	4	14
,, fin-cut	24	23.4	2	3	3	8
Char, white cardboard tags	42	23.7	0	13	3	16
,, green ,, ,,	42	23.5	0	12	5	17
,, fin-cut	21	22.4	1	3	4	8

tagging gave 5 recaptures and 133 trout over 24 cm gave 13 recaptures. In Lake Långbjörsjön less than one third of tagged trout that were 20 cm or smaller at tagging were recaptured, whereas half the total number of trout larger than this were recaptured. Recaptures of char and trout were distributed according to Table 5.

In Lake Långbjörsjön, as in Lake Dörstjärn, the length at tagging of fish subsequently recaptured deviates from the mean length of all tagged fish, and this is illustrated for the first few months in Table 6.

A better idea of the effect is gained if one singles out the recaptures during the first months of the trout that were 20 cm or less at tagging, Table 7.

The low rate of recapture for small fish is thus noticeable already during the first summer. The material does not actually disprove the hypothesis that the tag shedding is more pronounced for small fish during this period, but the hypothesis is not very plausible — the tag thread works its way out through the back and the dorsal fin of the fish when shedding.

If any group should avoid the littoral region fished with gill nets in the summer (p. 70 GUSTAFSON *et alii,* 1969) it should be the small char but certainly not small trout. Suspended, floating gill nets were set just below the surface in the middle part of the lake in August 1961, mesh sizes 2.50,

Table 5. Recaptures of tagged fish in Lake Långbjörsjön, distribution according to length at tagging.

Length at tagging	Char		Trout	
	Number tagged	Recaptures, per cent	Number tagged	Recaptures, per cent
16—20	23	13	59	29
21—25	139	36	168	49
26—30	15	80	73	53
31—35	0	—	16	44

Table 6. Lake Långbjörsjön, mean length at tagging of fish recaptured during the tagging period and later in the summer of the tagging year. Some fish that were recaptured twice are included.

| | Recaptures: | | All tagged: |
	Number	Mean length at tagging	Mean length at tagging
Trout 1960, stainl. steel tags	19	24.4	24.8
„ 1960, nylon thread tags	23	23.6	23.2
„ 1961, „ „ „	64	24.1	23.6
Char 1960, stainl. steel tags	1	(23.6)	22.2
„ 1960, nylon thread tags	6	24.1	21.8
„ 1961, „ „ „	43	23.8	23.2

2.14 and 1.67 cm knot-to-knot, three nights. Only nine trout were caught in all, and of the 64 char 11 were tagged (1961) and their mean length at tagging was 23.8 cm (cf. Table 6).

The gear used when tagging the fish may have some relation to the rate of recapture of different size categories. The tagging started with seining and continued with fly otter trolling and fly on rod and line in 1960. Mean length for seine- and fly-caught fish at tagging differed by only a few millimetres. Recaptures during the tagging period were of course influenced by the order of tagging. During gill netting later in the summer, the fishing was distributed fairly regularly over the ten sections of the lake and the recaptures were distributed according to Table 8.

In 1961 the tagging started with otter trolling with small spoons, fly on rod and line and single gill nets (mostly 2.50 mm knot-to-knot) and continued with seining and fly otter trolling. During gill netting later in the summer the nets were regularly distributed over the ten sections of the lake. The recaptures are presented in Table 9.

A plausible hypothesis would seem to be that the seine-caught fish had suffered less from the first capture than had other fish. Now the table shows

Table 7. Comparison between recaptures of small tagged trout and all tagged trout. Recaptures during the tagging period are included in the summer of the tagging year. Some fish were recaptured twice.

| Chi^2 for heterogeneity=0.9—2.2, p=0.3—0.1 | Number tagged | Recaptures | | |
		Summer of tagging year	Autumn of tagging year	Following years
Trout 1960, $\leq$ 20 cm	31	5 (16 %)	—	4 (13 %)
„ 1961, $\leq$ 20 cm	27	7 (30 %)	1 (4 %)	1 (4 %)
„ 1960, all tagged fish	162	42 (26 %)	—	30 (19 %)
„ 1961, „ „ „	153	66 (43 %)	10 (7 %)	15 (10 %)

(Only three char of the small category were recaptured.)

Table 8. Recaptures during gill netting in 1960 of fish tagged with different types of gear at the beginning of the summer.

	Trout tagged with:		Char tagged with:	
	seine	fly	seine	fly
Number tagged	71	91	35	20
Number recaptured	8	11	2	4

that spoon otter trolling and single gill nets at first capture gave a tagged lot with highest rate of recapture, and those groups had also the highest length at tagging.

Among the possible factors gear selectivity should be important. Judging from the length distribution of tagged fish, the gear starts retaining fish of about 15 centimetres total length and the effectiveness of the gear increases in the length interval 15—20 centimetres. This is shown by the action of the seine in Lake Dörstjärn during the tagging period 1962. The hauls covered somewhat more than half the lake surface on July 11 and this was repeated on July 12 and 13 (cf. Fig. 1). Recaptures were obtained from the already tagged trout during all three days. From the trout tagged on the first two days 9 were ≤20 centimetres in total length and gave 1 recapture, 31 were more than 20 but less than 24.5 centimetres and gave 19 recaptures, and 98 were 24.5 centimetres or more and gave 66 recaptures. Certainly, a small proportion of fish below 20 centimetres total length was tagged in Lake Långbjörsjön too. This is compensated for by the use of gill nets with 1.67 centimetres mesh size among the gill nets of the summer fishing, but it is difficult to determine whether this is a full compensation. If it is, the material indicates increased natural mortality or divergent behaviour of small tagged fish.

Char might be suspected to suffer more from tag shedding, the increased mortality of tagged fish, etc., as the percentage of fin-cut char recaptured

Table 9. Recaptures during gill netting in 1961. Fish tagged in 1961 or tagged fish from 1960 checked during the tagging period at the beginning of summer 1961.

	Trout tagged with					Char tagged with				
	Fly otter	Fly, rod & line	Seine	Spoon otter	Gill nets	Fly otter	Fly, rod & line	Seine	Spoon otter	Gill nets
Number tagged	19	31	44	28	44	9	6	26	35	55
Number recaptured	2	2	6	6	7	1	0	2	6	14
Mean length at tagging ..	22.6	23.4	23.8	24.4	24.6	21.9	21.9	21.9	22.8	24.3
Number ≤ 20 cm at tagging	4	5	11	4	3	2	2	5	4	0

does not deviate from that for trout in Table 2, the bottom lines, whereas the recapture of tagged char is lower than that of trout. The mean length for tagged char is less than that of tagged trout (Table 6) and there is also a different trend in the ratio fish length — percentage recaptures (Table 5). This will be further discussed in the following paper, 1969.

VI. Growth of tagged fish

The growth of tagged trout has been noted from observation of length at tagging and length at recapture and has also been "back-calculated" from scales, and the results will be compared.

Ten of the trout from Dörstjärn in Table 10 were aged (3+ to 6+ years). A corresponding group of untagged fish shows a mean growth of about 4 cm/year according to growth analysis. The tagged trout from Lake Lång-björsjön were mostly 4+ to 5+ years old at tagging and a corresponding group of untagged trout from that lake shows a mean growth of 3 1/2—4 1/2 cm/year.

Back-calculated growth is presented in Table 11. The correlation fish length to scale radius length is the one generally used at the laboratory for trout. The graph is reproduced in plastic form and made movable round the body length axis intercept with the prolongation of the graph (to compensate for scales with sub- or supernormal total radius length). Fin-cut fish from Lake Långbjörsjön are included in Table 11, as also are fish from Lake Dörstjärn which were originally both tagged and fin-cut (adipose fin) but had since lost their tags.

Some data in Table 11 illustrate the growth of normal fish from the beginning of one year up to the tagging period when captures are made at the beginning of July[1] (columns 4—6). This gives an indication of the extent of normal growth the tagged fish experienced up to the tagging at the beginning of July, which means that their growth tabulated in columns 1—3 is composed of one part normal growth and one part "post-tagging growth". Thus the calendar-year growth in Table 11 cannot be directly compared with the July/July yearly growth in Table 10.

Observations of the length of recaptured tagged fish are made sometimes two to three hours after recapture. Studies of the length of living fish and its reduction in the dead fish during the hours following capture in Lake Långbjörsjön have shown a length reduction of about 1 % of the total length during this interval (FAGERSTRÖM 1967). The difference between length at

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[1] Strictly, it is the scale growth that is observed in back-calculation. One of the assumptions underlying this back-calculating technique is that scale growth and fish length growth keep pace also within the season. This is not always true, as shown by DE BONT 1967, but the error does not affect the conclusions.

Table 10. Growth of recaptured, tagged trout according to direct length observations. Number=n, mean length at *tagging*=l̄, mean increase=d̄. Classification according to length at tagging.

| Interval between tagging and recapture | Trout from Lake Långbjörsjön | | | | | | Trout from Lake Dörstjärn | | | | | |
| | Length ≤ 24 cm | | | Length > 24 cm | | | Length ≤ 24 cm | | | Length > 24 cm | | |
	n	l̄	d̄	n	l̄	d̄	n	l̄	d̄	n	l̄	d̄
Up to 7 days, x̄=2 days	14	21.6	0	14	26.6	0	—	—	—	—	—	—
8 days to ≃ one month, x̄=25 days	33	20.9	0.7	29	26.7	0.4	—	—	—	—	—	—
About three months	4	21.3	2.2	6	27.5	1.0	—	—	—	—	—	—
One year, x̄=374 days in Lake L.	12	21.3	3.2	22	26.6	2.1	5	21.9	3.0	10	29.4	0.9
Two years=736 days	1	(20.8)	(5.2)	1	(25.3)	(4.7)	—	—	—	—	—	—

tagging and length at recapture in Table 10 and Table 12 is thus artificially decreased.

The outermost zone on a scale from the beginning of the summer may represent the bad growth of the previous year or the incipient growth of the present year. Decisions must be made with the guidance of an extensive knowledge of scales from the district, sampled at different times during the growing season. Individuals with bad (as tagged fish) or good growth may be assigned to the wrong year class and age group.

To sum up, the growth of tagged fish is reduced, and reduced by more than the amount illustrated in columns 1—3 in Table 11. On the other hand, the growth shown in Tables 10 and 12 is a little too low, and adding one per cent of the total length to d̄ in the line "One year" gives perhaps the best estimate of the reduced growth. Fin-cut fish does not suffer such a high growth reduction as does tagged fish (Table 11, Lake Långbjörsjön), and consequently the tagged fish from Lake Dörstjärn which have lost their tags but have nevertheless been included with the tagged fish in Table 11 may have led to an underestimation of the growth-reducing effect of tagging.

VII. Discussion

As stated in the comprehensive papers on marking, by RICKER 1958, VIBERT & LAGLER 1961, ICNAF 1964, CHADWICK 1966 and STOTT 1968, there exists a large variety of marking methods, giving different results in different environments. The results obtained in the present experiment should mainly be of value for the study of fish populations in the lakes treated in the following papers and for other similar experiments and lakes.

The serious shedding of tags found in the experiment is not susceptible of general application. The CARLIN tags have given good results, *e.g.* in compar-

Table 11. Back-calculated growth of trout according to scale analysis. Number=n, mean length at *recapture* or capture (the normal trout) $=\bar{l}$, mean increase during the period of the column$=\bar{d}$.

	Growth during year of tagging, one complete year			Growth during following year up to recapture (or capture) in July			Growth during complete year following the year of tagging		
	n	$\bar{l}$	$\bar{d}$	n	$\bar{l}$	$\bar{d}$	n	$\bar{l}$	$\bar{d}$
Lake Dörstjärn:									
Tagged and fin-cut in 1962, age 3+ at tagging	15	24.5	4.6	15	24.5	1.3	—	—	—
Normal trout, same age and year class	24	23.9	4.9	24	23.9	1.2	—	—	—
Tagged and fin-cut in 1962, age 4+ at tagging	16	27.6	4.0	16	27.6	1.2	7	—	3.1[1]
Normal trout, same age and year class	6	26.0	4.6	6	26.0	0.9	6	—	3.4[1]
Lake Långbjörsjön:									
Fin-cut 1959, age 3+ and 4+	9	24.6	4.7	9	24.6	2.2	—	—	—
Normal trout, same age and year classes	42	23.7	4.7	42	23.7	2.4	—	—	—
Tagged 1960 and 1961, age 4+ at tagging	5	25.7	3.0	5	25.7	2.0	—	—	—
Normal trout, same age and year classes	39	27.3	4.4	—	—	—	—	—	—
Tagged 1960, and 1961, age 5+ at tagging	9	27.6	2.7	9	27.6	1.7	—	—	—
Normal trout, same age and year classes	18	30.4	3.4	—	—	—	—	—	—

[1] Growth during the next year up to capture at beginning of July about one cm.

ative tagging tests (CARLIN 1967). The standard material for the wire is stainless steel and the standard method is to attach the tags firmly with two wires penetrating the flesh below the dorsal fin. In the present case the tags are attached with one wire of stainless steel or one thread of nylon and a fairly loose loop and the tag trailing behind the dorsal fin, p. 28. The matter is complicated inasmuch as the deviating technique used in Lake Långbjörsjön (nylon thread, loose loop) has also been used for big char in Lakes Borga-

Table 12. Growth of tagged char recaptured from Lake Långbjörsjön according to direct length observations. Number=n, mean length at *tagging* $=\bar{l}$, mean increase$=\bar{d}$. Classification according to length at tagging.

Interval between tagging and recapture	Length $\leq$ 24 cm			Length $>$ 24 cm		
	n	$\bar{l}$	$\bar{d}$	n	$\bar{l}$	$\bar{d}$
Up to 7 days	4	23.5	0.1	1	25.0	(0.2)
8 days to $\approx$ one month, $\bar{x}$=31 days	24	22.5	0.0	15	25.7	—0.3
About three months	2	22.9	—0.2	6	25.8	—0.2
One year	9	21.5	0.1	1	24.2	(0.0)

sjön and Överuman with recaptures of 57 and > 48 %, totals over several years to be compared with Table 2, 39 %, in the present study (mimeographed papers in Swedish, RUNNSTRÖM 1963, LINDSTRÖM 1966).

The problem of distinguishing between shedding of tags in connection with variation in rate of exploitation and, on the other hand, excess natural mortality or differing behaviour of tagged fish is a difficult one. Evidence of actual shedding of tags has been presented (p. 27—29); this shedding seems to start between three months and a year after the tagging, and affects tags with nylon thread more seriously, perhaps because they tend to "work" more in the fish flesh than do the stiff stainless steel wires. This hypothesis should also apply to a comparison between firmly and loosely attached tags giving high losses for loosely attached ones, as in the present experiment.

The shedding may very well be dependent on size, as *e.g.* 20 out of 398 big char (≥ 25 cm) tagged in 1956 were recaptured with tags in Lake Överuman in the years 1959—64, but there is a low rate of recapture of small char and trout in Lake Långbjörsjön that cannot be attributed to tag shedding only, as the low rate of recapture is evident already during the first summer. It is attributed to gear selectivity, excess natural mortality of small tagged fish or divergent behaviour of small tagged fish.

The high number of fin-cut fish recaptured, extending over several years, in spite of a lower chance of recognition, is due to the absence of the possibility of shedding, but low mortality and normal behaviour could also affect the result.

It was possible to obtain this information about the action of tagging thanks to the fact that the marked fish were immediately vulnerable to the gear in use and to the fact that the lake is small and much of the yield was taken in the experimental fishing. In the larger Lake Överuman there was also a low rate of recapture in the case of small char (below 25 centimetres total length) though otherwise there was a high rate of recapture. This situation could not be so thoroughly investigated, as in Lake Överuman the fish was not aged and the exploitation was not so well known. The high recapture of char in Lake Borgasjön reported above occurred in an experiment where all tagged char were big ones, fished on the spawning grounds. Thus the information from these three lakes where the tags were attached with one nylon thread (Lake L. also steel wire) in a loose loop can be quite logically interpreted as the result of tag shedding (in all sizes?) and gear selectivity, excess mortality or divergent behaviour of small fish.

There is a reduction in the growth rate of tagged trout in all size categories due to tagging that could be confined within certain limits and it lowers the regression line of length increment on total length, (a regression that of course always points downwards with increasing total length, Fig. 3—5). This problem has been surveyed by TESCH (1968). As already stated the recapture of small trout is low. Fig. 3 indicates that some of the small tagged
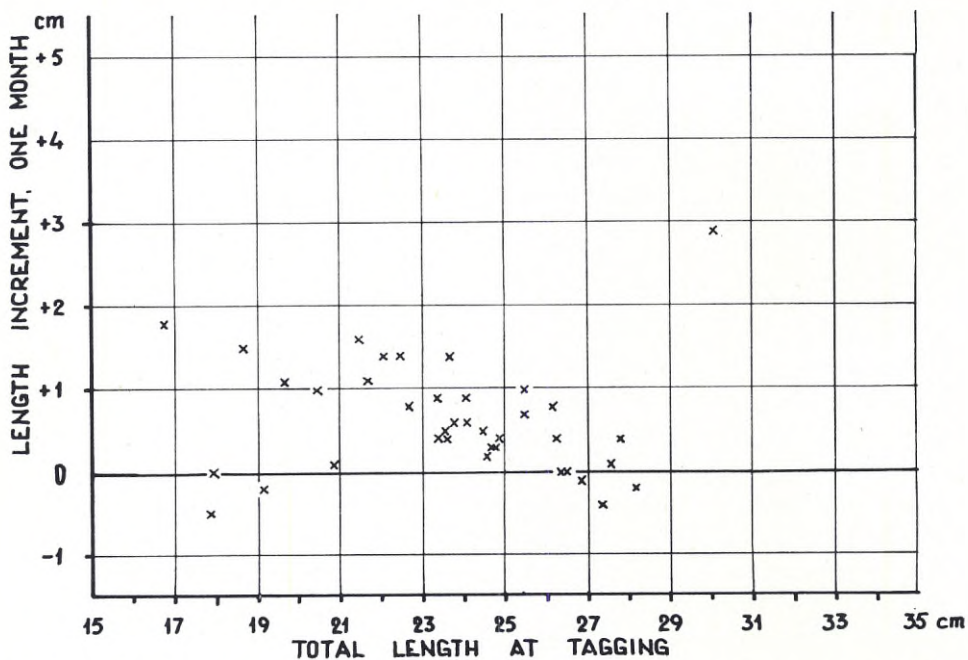
Fig. 3. Growth of tagged trout during the first month after tagging in Lake Långbjörsjön. Four small trout have ceased to grow. The correction discussed on page 37 is not applied to the data in Fig. 3—5.
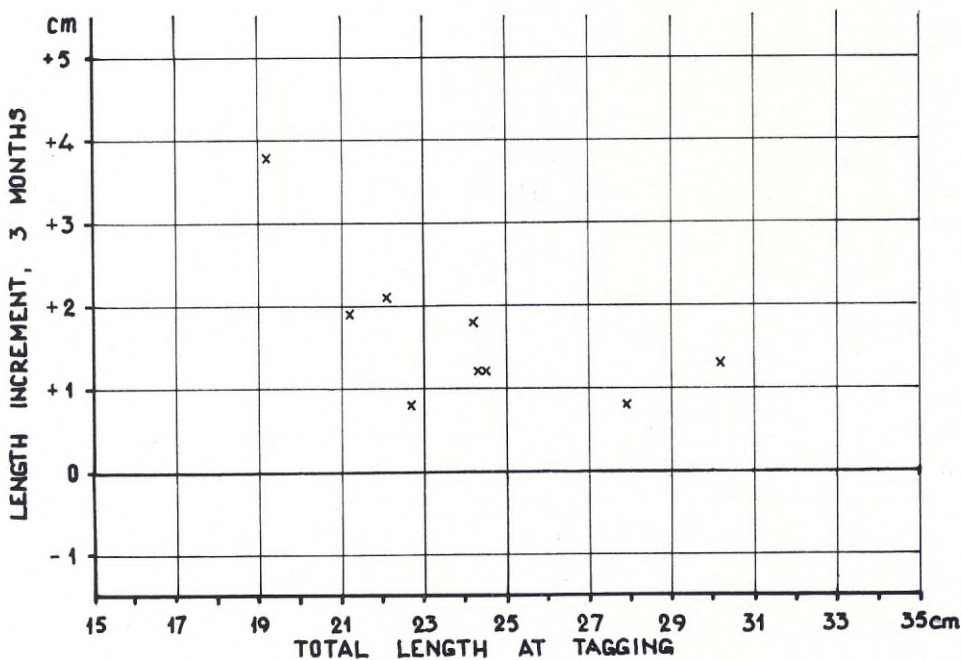
Fig. 4. Growth of tagged trout during about three months after the tagging in Lake Långbjörsjön.
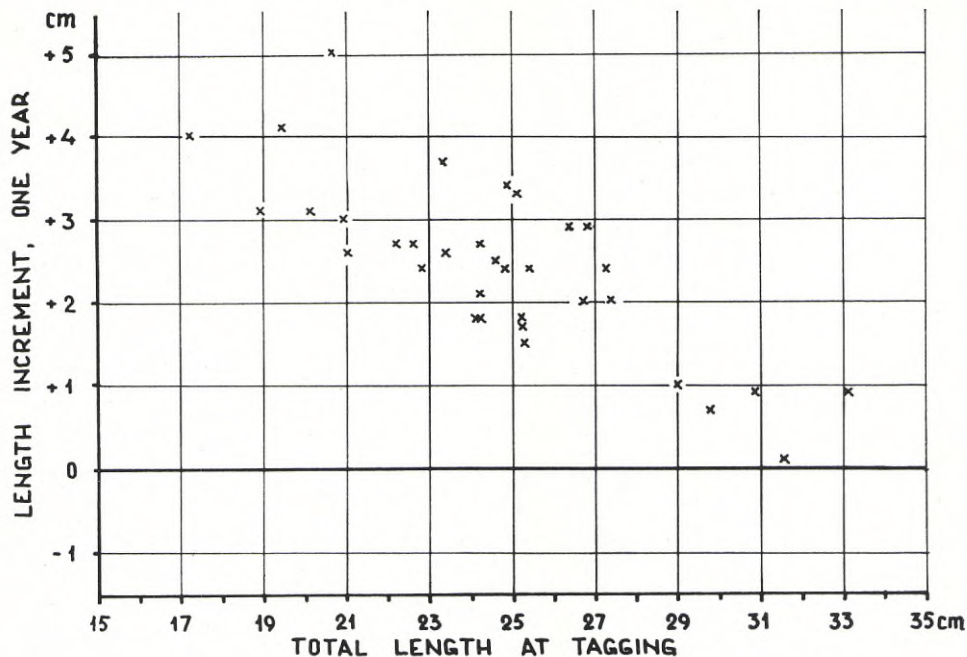
Fig. 5. Growth of tagged trout during one year following the tagging in Lake Långbjörsjön. All the small trout have grown fairly well.

trout show good growth (a) whereas others show no growth at all or even a decrease after one month (b). If the reason for the low recapture during the experiment had been a change in behaviour of the small trout after tagging, the variation in growth after one year should perhaps have been more evident (Fig. 5), some fish corresponding to group (b) being much retarded in growth as a consequence of the restricted activity during the summer after tagging. Now all small trout recaptured after one year had grown fairly well, about 7/8ths of the growth one would have expected of an untagged trout in the length group 21 cm or less, and the mortality in group (b) may be high.

All trout of medium size show an increase in length one month after tagging. The recapture of these trout was also higher during the experiment. The growth of big trout was very small both after one month and after one year from the time of tagging.

Fin-cut trout show no reduction in growth, a fact which at any rate does not disprove a higher mortality of tagged fish, cf. BRYNILDSON *et alii* 1967.

VIII. Summary

There is good evidence of extensive tag shedding in the two small lakes studied in the Swedish high mountain. Variation with fish length is not indicated by this material but it might very well exist. The actual degree

of shedding is highly dependent on the tagging technique used and the environment in this particular experiment.

The shedding of nylon-thread tags probably started somewhere between three months and one year after the tagging. Tags with stainless steel are also shed but not at the same rate.

There is a low recapture rate for the fish that were small at tagging and this is evident already the first summer after tagging. It cannot be attributed to selective fishing as far as trout is concerned. From what can be inferred about the procedure of tag shedding, this shedding can hardly be responsible for the low recapture of small fish during the first summer, so this low recapture rate is attributed to gear selectivity, higher natural mortality or divergent behaviour of small fish.

The problem concerning the low rate of recapture of small fish is thus largely unsolved, but the harmlessness of the gear catching the fish at tagging is at any rate of little importance compared with the gear selectivity or the effect of tagging on small fish during actual tagging and the time that follows.

The growth of tagged fish is retarded and the rate of retardation is estimated. It is inferred that some small trout die soon after tagging while others recover, and that mainly gear selectivity and increased natural mortality remain as explanations for the low recapture of small fish during the first summer.

IX. Acknowledgements

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Isoenzyme Polymorphism in *Mysis relicta* Lovén

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I. Introduction

The significance of gene-frequency estimations for identification of populations is well understood, since the analytical methods concerned do not suffer from the disadvantage of unknown environmental influence. Numerous examples where protein polymorphisms have been used in laboratory experiments or in investigations of wild stocks of animals have been published in the last few years (e.g. BRAEND 1964, NAEVDAL 1965, MØLLER 1966, RASMUSON, RASMUSON and NILSON 1967, KOEHN and RASMUSSEN 1967). The most frequently occurring type of polymorphism is the one-locus two-allele type producing three different electrophoretic phenotypes. In some cases, however, up to six alleles have been reported, usually in the transferrin group. With an increasing number of allelic genes involved, the probability of frequency heterogeneity also increases.

In crustaceans no isoenzyme polymorphism have been previously reported. The present paper describes a supposed five-allele co-dominant autosomal esterase polymorphism in a small crustacean, *Mysis relicta* Lovén.

II. Material and methods

Samples from two lakes were obtained, viz. Lake Mälaren and Lake Skiren. The latter, situated some 150 kilometres south-west of Stockholm is rather small. The Mysis of Lake Mälaren has its normal breeding period in winter (FÜRST 1965) but in Lake Skiren there are in addition to winter-spawning *Mysis,* also summer-breeding shrimps. Since a number of Swedish lakes have been found to contain *Mysis*-populations displaying two breeding periods, a case of sibling species may be involved and the study was initiated to elucidate this problem.

A total of 430 specimens, both young and adult, were analysed. Whole-animal homogenates were prepared in a glass homogenator, cooled in an ice-water mixture. Prior to homogenating, the animals were buffered in equal amounts of body and Ashton buffer (gel-buffer, ASHTON and BRADEN
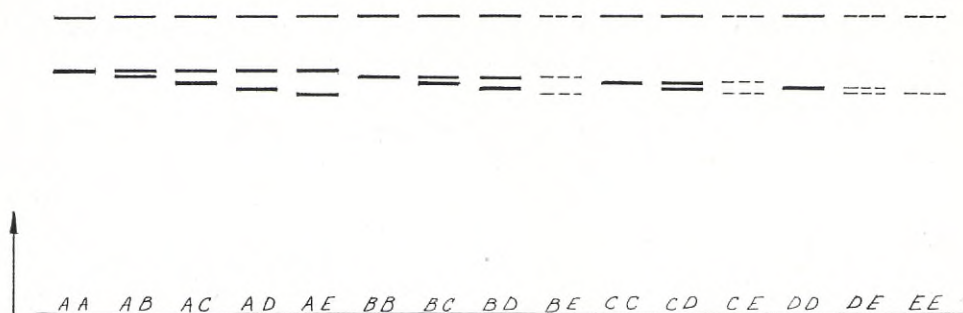
Fig. 1. The fifteen possible esterase patterns. Allelic combinations are indicated by capital letters. The four not obtained in the samples investigated are indicated by short dashes. The arrow indicates the direction of anodic migration.

1961). The homogenate-buffer mixture was transferred to the tubes of the micro-centrifuge belonging to the Beckman Spinco Analytical System, and was centrifuged for 10 minutes. The supernatant thus received was then used for electrophoretic analysis. The electrophoretic procedure employed has been described elsewhere (NYMAN 1967). The gel slices were incubated in a buffer of mono- and dibasic sodium phosphate of pH 7.0 for 15 minutes, after which α-naphthyl acetate (enzyme substrate), diluted in distilled water and acetone, and Fast Red TR salt (dye-coupler) were added. After 30 minutes the esterase zones had reached maximum intensity.

III. Results and discussion

The study was initiated partly in order to find a way of separating two sibling species within a lake. Unfortunately, this aim could not be achieved owing to the fact that the number of individuals tested was too small, mainly because of the high number of allelic genes involved in the polymorphism. The two supposed sibling species were separated and analysed independently, but since no significant heterogeneity could be detected the results presented in the table from Lake Skiren are pooled data.

When whole-animal homogenates of *Mysis relicta* Lovén are subjected to horizontal zone-electrophoresis on starch gel and dyed for esterases, two zones of esterase activity appear, one being indicated by a single band and the other, with a slower anodic migration, being polymorphic.

The different patterns obtained in this zone may evidently be explained by adopting a theory that five allelic codominant genes (A, B, C, D and E) are responsible for the variation. Since only one allele is obtained from each parent, fifteen possible combinations may occur (Fig. 1). The very low frequency of the E-allele, only one heterozygote having been found (AE)

Table 1. Gene frequencies and phenotype distribution calculated according to the HARDY-WEINBERG law.

Lake Mälaren (200 specimens)

Allelic combination	AA	AB	AC	AD	AE	BB	BC	BD	BE	CC	CD	CE	DD	DE	EE
Numbers observed	15	14	21	1	1	32	48	6	—	52	5	—	5	—	—
Numbers expected	5.5	22	29.5	0	1	22	59	7.5	0	39.5	10.5	0.5	0.5	0	0

Lake Skiren (230 specimens)

Allelic combination	AA	AB	AC	AD	AE	BB	BC	BD	BE	CC	CD	CE	DD	DD	DE	EE
Numbers observed	4	6	7	—	—	36	39	7	—	115	10	—	6	6	—	—
Numbers expected	0.5	6	14.5	1.5	0	17	77	8	—	88.5	18	—	1	1	—	—

Gene frequencies   Lake Mälaren $f(A)=0.17$ Lake Skiren $f(A)=0.05$
$f(B)=0.33$ $f(B)=0.27$
$f(C)=0.44$ $f(C)=0.62$
$f(D)=0.06$ $f(D)=0.06$
$f(E)=0.00$ $f(E)=0.00$

in 430 specimens, explains why four of the theoretically possible combinations are absent in these samples. As can be seen in Table 1, there is a remarkable excess of homozygote combinations and thus a parallel deficit of heterozygotes in both populations investigated, when gene frequencies and phenotype distribution are calculated according to the Hardy-Weinberg law. This state of affairs is schematically presented in Fig. 2, where homozygotes are shown in black and heterozygotes in white. The only deviation from this rule of homozygote excess is found in the AE heterozygote — i.e. the only combination found which included an E-allele.

In these populations of *Mysis relicta* we are evidently either confronted by a selection favouring homozygote combinations, a very rare state of things, or we are facing samples representing mixtures of two or more populations, which differ with respect to their gene frequencies. The last assumption seems unlikely however, since both supposed sibling species from Lake Skiren not only had the same gene frequencies but also the same excess of homozygotes. Further investigations should clarify this problem. Similar esterase polymorphisms in vertebrates almost invariably show genetic equilibrium, with a very good accordance between observed and expected frequencies. Since it can be concluded from the different gene frequencies of two of the alleles involved (A and C) that these samples are taken from
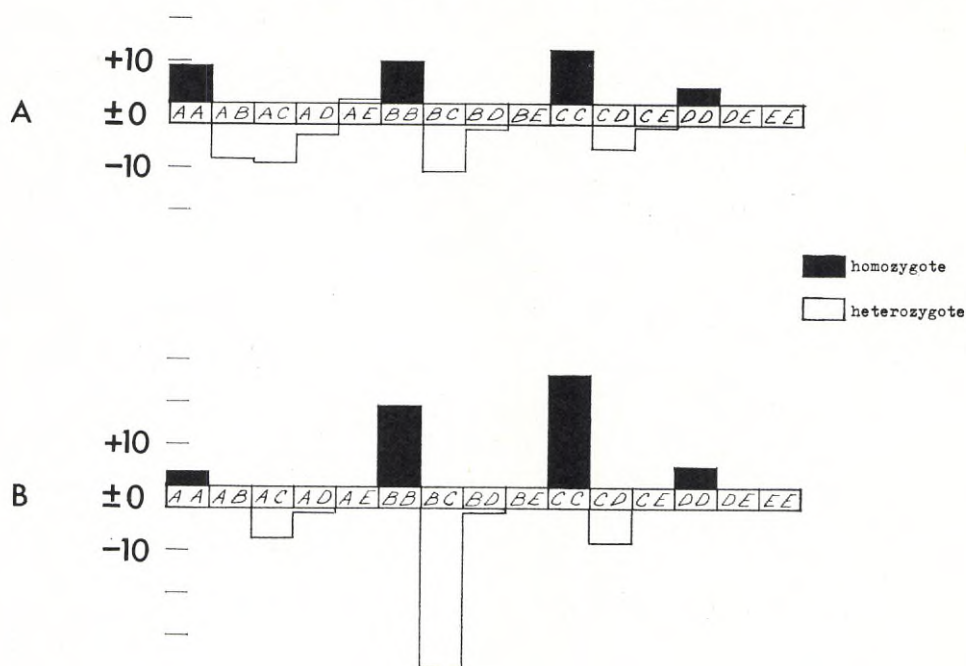
Fig. 2. Diagram showing the excess of homozygote combinations. Scale on left indicates number of individuals deviating from the expected values at ± 0.
A) Lake Mälaren, B) Lake Skiren.

different gene pools, but with the same kind of homozygote excess in both lakes examined, this polymorphism may be a good tool for population studies on *Mysis relicta,* which is an animal of great importance as a food object for many salmonids and for this reason is transplanted into new lakes.

IV. Summary

An esterase polymorphism in *Mysis relicta* LOVÉN is described. Five allelic genes appear to build up the genetic background, with fifteen possible phenotype combinations. A marked excess of homozygotes is found in both populations, but nevertheless may they be separated by means of the different gene frequencies of two of the alleles.

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The bottom fauna of Lake Vättern, central Sweden, and some effects of eutrophication

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I. Introduction

Lake Vättern is one of the large lakes in central Sweden which during the last few years have been the object of intensified physicochemical and biological investigations. The study of the bottom animal community, which constitutes part of this programme, gives information about the specific bioactivity of the various lakes and indications for the rate of progress of eutrophication in the various basins (Grimås 1968).

The special features of Lake Vättern, including the shape of the basin, the extensive seiche systems in the water mass, and the open and exposed shore line, contribute to producing a relatively rapid transport of organogenic material to the profundal zone and a dominance of minerogenic sediments in the littoral. A local and high input of organogenic material thus renders a definite reaction of the littoral animal community of value for the establishment of regional variations within the lake. A comparison with earlier, quantitative investigations (Ekman 1915) indicates a small but definite elevation of the general level of productivity.

II. Some data on the lake

The lake surface area is 1,900 sq. km, the volume 73 billion cub. metres, the maximum depth 128 metres and the main depth about 40 metres. Owing to the limited drainage area, 6,360 sq. km, the discharge of water during the year is low. The lake can be characterized as oligotrophic, with a high oxygen content in the whole water mass during the year and a comparatively low content of phosphorus and nitrogen (total-P 10—15 µg/l, total-N about 470 µg/l in surface water). The specific conductivity ($H_{20} \cdot 10^6$) varies around 95 and the $KMnO_4$ consumption around 12 mg/l (Ahl 1968). With regard to transparency, thermal stratification, and bottom vegetation, the transition zone between littoral and profundal can be estimated as lying at around 30—40 metres depth.

The sedimentation rate has been calculated to be 2.2—3 mm *per annum* in the deepest parts of the basin (Norrman 1968).

Apart from locally eutrophicated areas the composition of the benthic algae vegetation indicates oligotrophic conditions, and only small changes seem to have occurred during the last 30 years (STÅLBERG 1939, STJERNA-POOTH 1968).

In many respects the present condition of the lake can be attributed to human activities. The population shows an increase from about 60,000 to 130,000 individuals in communities along the shore lines since 1920 (data by County Administration, Jönköping), and this must be considered to be one of the important factors affecting the rate of eutrophication during the last decades. At any rate in a rough manner the human contribution to this process can be estimated (AHL 1968). Investigation in feeder streams and estimates of the capacity of the catchment area indicate an input of phosphorus into the lake which has increased from about 60 tons to 200 tons *per annum* during the last 50 years, the increase mainly emanating from the communities. The actual input of nitrogen by feeders is about 1,300 tons *per anuum,* of which 400 tons derives from communities within the drainage area. For comparison, the addition of nitrogen by precipitation over the lake surface is estimated at about 600 tons *per annum.*

The input of organic material, excluding that emanating from industries and communities, is about 50,000 tons *per annum,* estimated as permanganate consumption. It is of special interest that about 50 per cent of this matter is derived from the southernmost inlets, a circumstance which to some extent explains the benthic situation in southern Vättern. The environmental conditions in this area are accentuated by the fact that about 130 tons of the annual loading of phosphorus can be attributed to the southern inlets.

III. Methods and material

The benthic animal community has been studied in four sections of the lake (Fig. 1). Sections 1, 2, and 4 are located in the main basin of the lake, where section 4 extends over the outlet area. Section 3 covers an isolated basin in the northernmost area of the lake. The quantitative collecting was carried out in August 1966, May 1967, August 1967 and 1968 with a bottom sampler, type EKMAN-BIRGE. The samples were sifted with a 0.6 mm gauge and the weighing was carried out to a limit of $\pm$ 0.1 mg on material preserved in alcohol. The quantitative material comprises 9,964 animals resulting from 180 samplings.

IV. Results and discussion

1. General features

The average biomass and abundance of bottom animals in the various depth zones are given in Table 1 and Fig. 2. The small discrepancy between
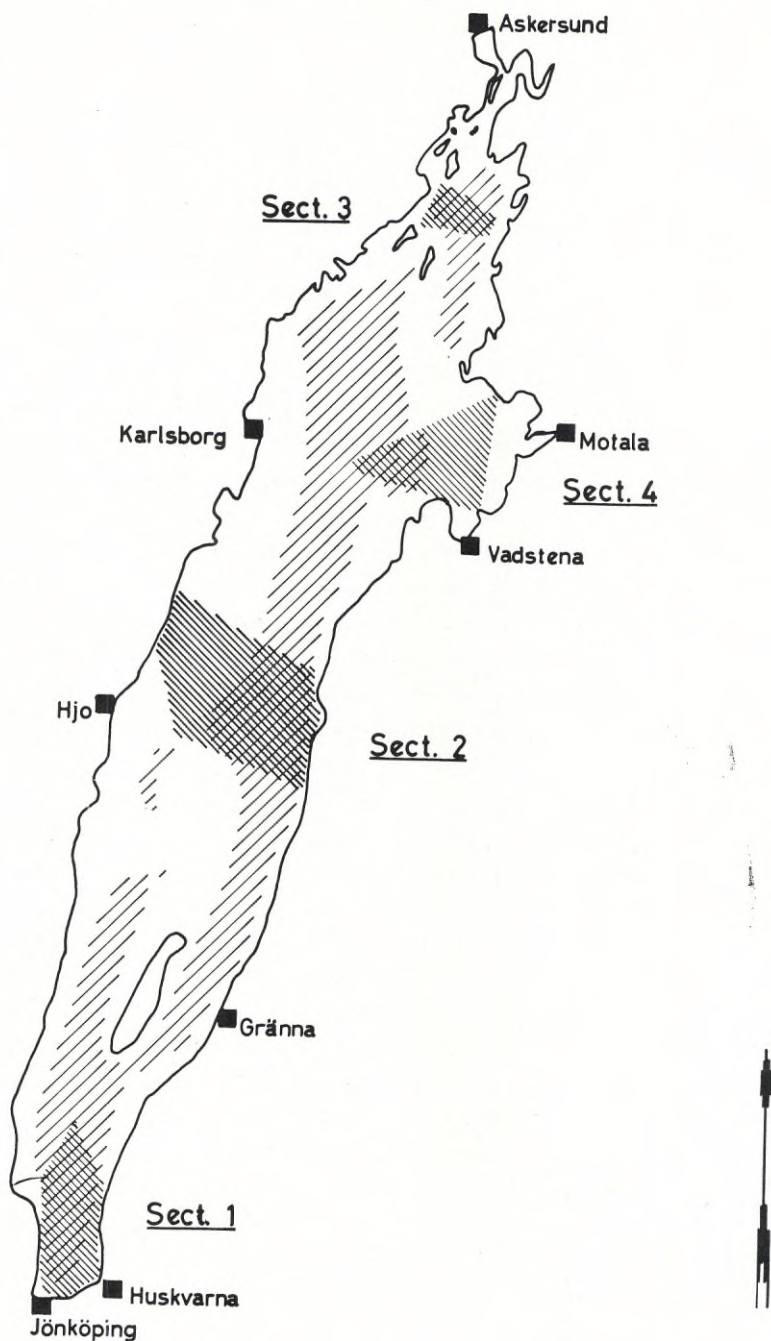
Fig. 1. Lake Vättern. The investigated sections. Sparsely lined areas represent bottoms below 40 metres depth.

Table 1. Lake Vättern. The distribution of bottom animals in the various sections and depth zones in biomass, g/m², and abundance, ind/m².

Depth, m.	Biomass in section				Abundance in section			
	1	2	3	4	1	2	3	4
0— 20	6.6	1.1	1.1	1.9	3,201	766	1,126	2,188
20— 40	3.9	0.6	2.0	3.1	2,003	634	1,099	3,208
40— 60	5.2	7.8	9.6	3.9	2,349	2,677	3,352	2,131
60— 80	—	—	1.9	—	—	—	616	—
80—100	6.3	9.1	1.1	—	3,425	3,132	587	—

the bathymetric distribution in weight and number is due to the qualitative composition of the fauna in the various regions.

A noticeable feature is the increase in quantities as the profundal zone is approached. This is in contrast to the general situation in many otherwise comparable lakes. This distribution of animals in Lake Vättern can be regarded as an effect of the distribution of organogenic material in the sediments with low concentrations in the littoral region. This pattern is best defined at section 2 in the central part, which can be regarded as representative for most areas of the lake. The discrepancy between the stations from this pattern includes the profundal as well as the littoral zone.

A feature found in the main basin is a high abundance of big crustaceans in the profundal combined with high amounts of oligochaetes in the deepest regions (Fig. 3). In the depth zone 40—100 m, big crustaceans represented 61 % and oligochaetes 18 % of the total benthic population. In this respect the situation in 1966—67 corresponds to the conditions in the lake in the years 1911—14 (EKMAN 1915), in each case probably an effect of the relatively high and rapid transport of organogenic material to the deep portions of the lake. In both instances the big crustaceans are represented mainly by the marine-glacial relict forms, particularly *Pontoporeia affinis* LINDSTR. In 1966—68 *Pallasea quadrispinosa* SARS, *Mesidothea entomon* L., *Gammaracanthus lacustris* SARS, and *Mysis relicta* LOV., represent together only 3 % of the total number. Besides the relicts, isolated specimens of *Asellus aquaticus* L. and *Gammarus pulex* L. are found.

Similar for all stations is the maximum density of insects, dominated by chironomids in the littoral zone. The main group *Orthocladiinae* amounts to 40 % of the total number of individuals, *Tanytarsini* to 24 %, *Chironomini* to 22 %, and *Tanypodinae* to 14 %. The dominance of orthocladiines and the non-occurrence of *Chironomus* species underline the oligotrophic character of the lake. Among the chironomid larvae may be mentioned *Procladius* ssp., *Thienemannimyia laeta* MEIG., *Ablabesmyia* sp., *Monodiamesa bathyphila* KIEFF., *Prodiamesa ekmani* BRUND., *Heterotrissocladius*

SECTION   I   II   III   IV

BIOMASS

ABUNDANCE
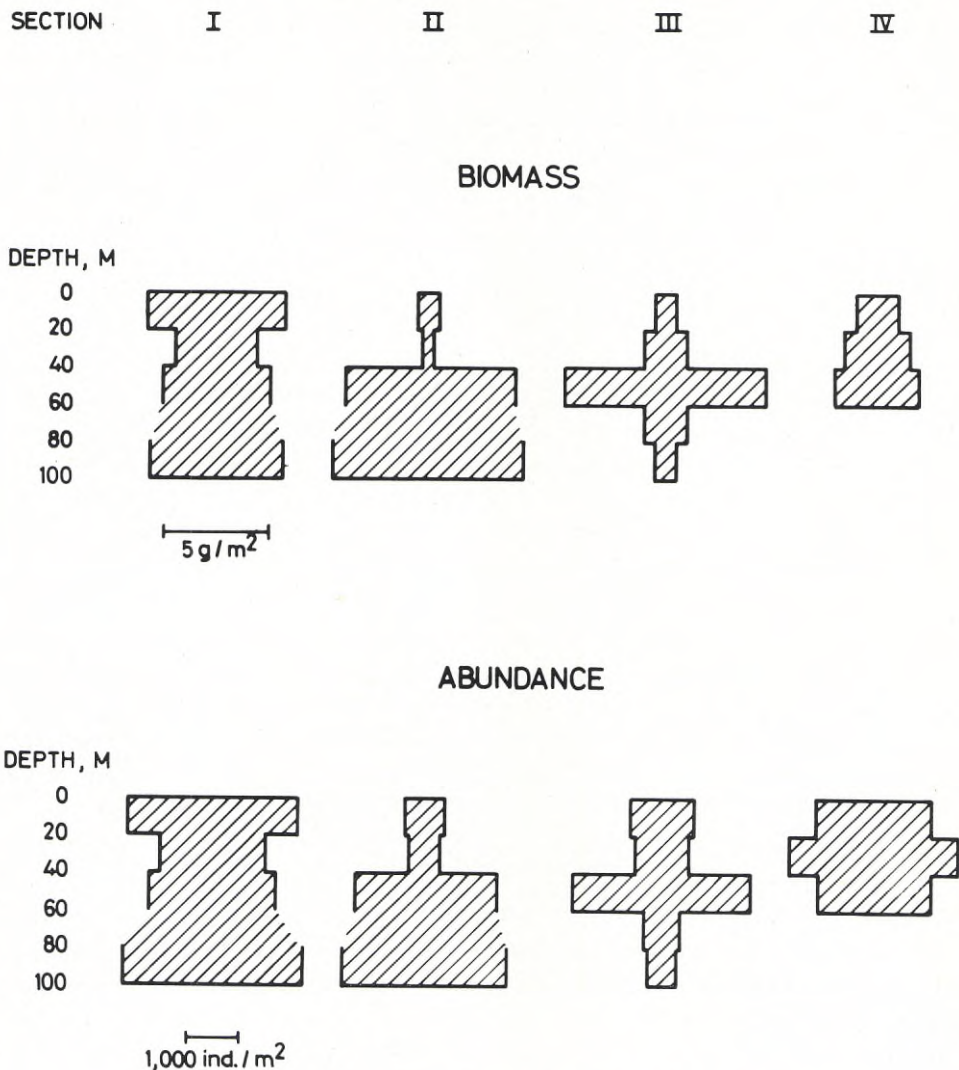
Fig. 2. Lake Vättern. Bathymetrical distribution of bottom animals.

subpilosus EDW., *H. marcidus* EDW., *Protanypus morio* ZETT., *Einfeldia* sp., *Paracladopelma obscura* BRUND., *Stichtochironomus* sp., *Demicryptochironomus vulneratus* ZETT., *Cryptochironomus* s. str., *Polypedilum nubeculosum*-group, *Tanytarsus* s. str., *Microspectra* ssp., *Stempellinella minor* EDW. The predominant chironomid in the profundal is *H. subpilosus,* which is a stenothermal species, typical of cool waters, *e.g.* the high mountain lakes in Sweden (BRUNDIN 1949).

As an average for the whole lake the small crustaceans are relatively

Table 2. Lake Vättern. The quality of the benthic fauna as a percentage of the total abundance of bottom animals.

Animal groups	Autumn 1966	Spring 1967	Autumn 1967
Big crustaceans	28.1	34.3	26.4
Insects	7.9	19.9	9.8
Oligochaetes	20.1	24.4	24.5
Sphaeriids	14.3	16.7	24.0
Small crustaceans	27.5	3.4	14.4
Remainder	2.1	1.3	0.9

evenly distributed over all depths. The copepods constitute 75 %, the ostracods 15 %, and the cladoceres 10 % of the material. Among the cladoceres. *Eurycercus lamellatus* MÜLL. is particularly numerous (77 %), followed by *Sida crystallina* MÜLL (16 %). Isolated speciments of *Iliocryptus acutifrons* SARS, *Acroperus harpae* BAIRD., *Alona affinis* LEYD., *Leptodora kindtii* FOCKE, and *Holopedium gibberum* ZADD. are found.

On average the sphaeriid clams, mainly *Pisidae,* are abundant in the lower littoral and upper profundal. Among the remaining groups of animals the highest frequency in the samples is shown by the turbellarians, followed by nematodes, hydracarines, gastropods, and hirudineans. Among the nematodes small specimens are excluded, since an appreciable percentage of them can be expected to be lost in the sifting.

2. Comparison autumn-spring

Table 2 shows the situation in sections 1+4 in August 1966 and May and August 1967. Concerning the qualitative composition of the fauna the facultative water organisms with a long period of development show a comparatively small variability in the percentage share of the total number, e.g. big crustaceans, oligochaetes, and spaeriids. Great variations occur in the small crustaceans, which develop rich populations during autumn, and which are few in number during spring. By contrast the share of insects is high in spring, when most larvae still remain in the bottoms, and is low in autumn after the emergence period, and partly owing to the loss of newly hatched and small-sized larvae in the process of sifting. In any case the correlation abundance/biomass of the macro-fauna is largely affected by the population dynamics of these two main groups.

3. Regional variations

Littoral. The regional divergences are reflected within the littoral zone, where each section investigated has its own character.
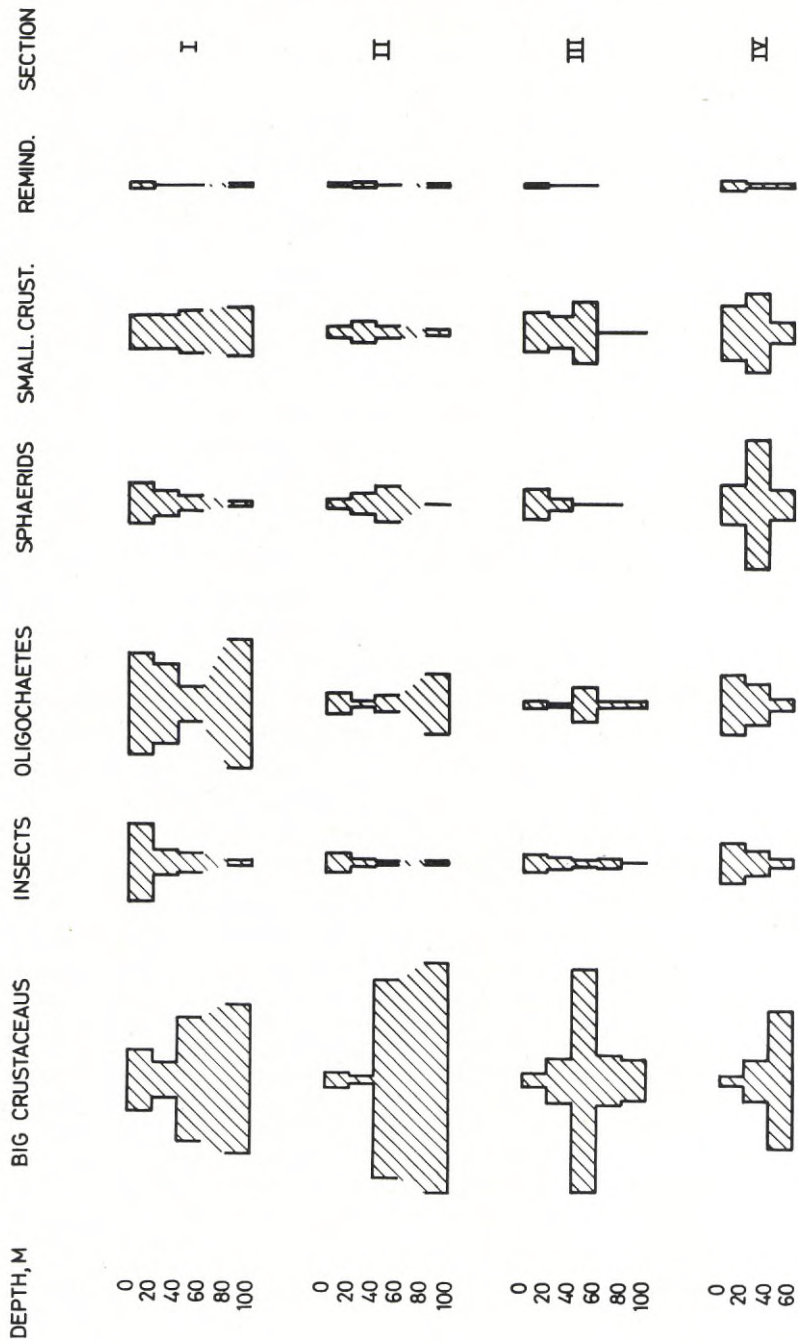
Fig. 3. Lake Vättern. Bathymetrical distribution of the various animal groups in the various sections.

Section 2, in the middle of the lake, can be regarded as representative for the main condition in the lake. The sediments are poor in organogenic compounds. The benthic community is built up by chironomids, sphaeriids, oligochetes, and small crustaceans, no group of which predominates. The biomass very seldom exceeds 1 g/m² and the abundance varies between 400— 1,000 ind/m². This low concentration can partly be explained by the exposure of the bottoms and the predominance of sand in the bottom surface.

The littoral of the northernmost section, *section 3,* reminds one in many ways of the situation in section 2. The abundance and biomass are somewhat higher, but is still low (1,000 ind/m² and 1—2 g/m²), a circumstance which might reasonably be ascribed to the exposure of the bottoms and the predominance of sand. A feature worth noticing is the increasing quantity of big crustaceans in the deeper areas of the littoral and the concentration of small crustaceans in the whole littoral.

In *section 4,* the outlet region, similar conditions prevail as regards the sediments which are dominated by sand. The abundance and biomass of animals are, however, three times as high as in section 2, viz. 1,000—4,500 ind/m² and 2—3 g/m², respectively. All animal groups appear in higher concentrations. Of special interest are pisids and small crustaceans which constitute about 56 % of the total fauna and can be regarded as characteristic for the section. It seems permissible to assume that the prerequisite conditions for the "rain" of organic matter over the bottoms are more favourable in section 4 than in section 2. As an example if may be mentioned that the average total volume of phytoplankton in the surface water is about 10 % higher in section 4 than in section 2 during comparable periods of sampling (GRÖNBERG 1968). Thus no correlation seems to exist between the concentration of filtering animals and the occurrence of fine sediments in the bottom surface. If seems more likely that there is a correlation to the amount of suspended matter which is transported along the bottoms and is available to the organisms without being deposited. A similar correlation between the dominance of different amphipod species in Skagerak and the transport of detritus on or near the bottom surface has been suggested by ENEQUIST (1950).

It also seems possible that the region is to some extent affected by pollution, especially in the inner part, where the oligochaetes of the upper littoral are more numerous.

The highest average biomass, 4—6 g/m², is found in the littoral of *section 1,* in the southern part of the lake. Especially remarkable is the rich fauna in the depth zone 0—20 metres and the high abundance of oligochaetes (35 % of the total number of animals) in the whole littoral 0—40 metres. Since the oligochaetes belong to the animal groups depending on deposited organic material in the sediments, their number indicates a large

and continuous supply of detritus to the bottoms of section 1, while in other respects the environmental conditions do not differ essentially from those of the remaining sections. Other special characteristics in the composition of the fauna point in the same direction, *e.g.* the relatively high concentrations of big crustaceans and insects. The special features of section 1 indicate the effect of a local eutrophication.

Profundal. The fauna of the upper profundal is that with the most uniform composition in the lake. The predominating animal is *Pontoporeia affinis,* which constitutes 65 % of the total number of animals. The conformity between the sections is best defined in the main basin. In the isolated northern area the biomass and abundance are somewhat higher. A comparison of the sections with one another shows that the quantity of the profundal fauna is higher in sections with a poor littoral fauna. It seems possible that this fact, together with the special situation in the deep areas of the northern basin, contributes towards a concentration of animals in the upper profundal of section 3.

In the deeper portions of the profundal there is a definite difference between the northern and the main basin. The occurrence of hydrogen sulphide was established during the samplings, and the results show a decrease of animals not paralleled in any other parts of the lake. The most obvious conclusion is that this is due to pollution and oxygen deficiency in the deeper profundal of the isolated basin. There are, however, reasons for believing that the situation is more complex than a surplus of organogenic matters combined with isolation. One argument for such an opinion is the poorly developed oligochaete fauna in section 3, which diverges from the situation in other parts of Lake Vättern as well as from the situation in heavy polluted waters.

Summing up, the regional variations points to a process of eutrophication in the lake which is easy to establish in the littoral of the southernmost and the profundal of the northernmost area of the lake, is capable of being shown in the outlet region, and is hard to establish in the main, central region of the lake. The definite reactions of the benthic communities in the peripheric parts are to a certain extent an effect of pollution.

The conclusions based on the benthic investigations confirm the observations made on the regional variation in water quality (AHL 1968). In spite of the considerable mixing of water within the layers of the lake, there is discernible in surface waters in the southernmost and northernmost parts a tendency towards an increased consumption of $KMnO_4$ and a decreased oxygen content. The conditions in the southern area are explicable by the regional input of organic matter to the lake from the inlets. This explanation is not applicable to the northern area. It seems possible that the conditions in this area are affected by industrial effluents.

4. Indications of a changed level of productivity

Besides the regional variations there exist indications of a long term process increasing the general level of productivity. On the basis of the biological characteristics of the bottoms, the main part of the lake has changed during the last 50 years within the range of oligotrophic conditions. Only in the northernmost, small and isolated basin do the results indicate effects of oxygen deficiency in the sediments.

The direct interpretation of earlier results (EKMAN, 1915) for purposes of comparison is complicated by several circumstances. EKMAN's total material consists of about 90 sampling stations. Of these stations only 15 are defined with regard to location and depth in the lake. Differences in sampling technique may also be assumed. As an example may be mentioned the estimates of the quantity of bottom animals in correlation to the sieving technique. In the comparison those animals have been excluded which might be expected to show great losses in sieving e.g. nematodes, the smallest oligochaetes and cladoceres and the hydrozoans.

For many reasons the conditions in the profundal region are of interest in the calculations of the biological changes in the lake. Section 3 is excluded in this comparison, since no stations in EKMAN's material cover this area. No major changes have occurred in the qualitative composition of the profundal fauna during the last 50 years. In both cases the amphipods, mainly *Pontoporeia,* dominate and oligochaetes come next in order of abundance. The actual situation thus to a great extend reminds one of the benthic situation at the offshore stations of Lake Michigan and Lake Huron (ROBERTSON and ALLEY 1966, SCHUYTEMA and POWERS 1966, AYERS and CHANDLER 1967), where the share of *Pontoporeia* varies around 60 per cent of the total fauna .

The indications of a changed situation in the profundal of Lake Vättern are a small increase in the total abundance of animals and a small change in the balance between the main groups of animals.

A splitting of the benthos into ecological groups results in an increase of animals living immediately above or within the contact zone of water and sediments, *e.g.* a 50 % increase of big crustaceans and sphaeriids and a 100 % increase of small crustaceans. Especially noticeable is the increase of big crustaceans in the central portions of the lake. The insect populations seem to be of about the same sizes and the oligochaetes within the sediments show a decrease of about 30 %.

In EKMAN's material, only in 2 out of 10 samples from the profundal zone are the oligochaetes represented by more than 500 ind/m². One of these samples, with 1,380 ind/m², comes from the central basin and the other, with 6,180 ind/m², from the southern part of the lake. The significance of the last-mentioned sample for the mean value of oligochaetes in the total pro-

Table 3. Lake Vättern. The average abundance of bottom animals in 1911—
14 (EKMAN) and 1966—68 (GRIMÅS).

	big crust.	chir.	oligoch.	sphaer.	small crust.	re-maind.	total
1911—14							
15 defined stations	676	260	1,020	89	388	42	2,475
Total material	611	129	570	91	200	58	1,659
1966—68	1,001	199	506	301	321	45	2,373

fundal is high. There are therefore reasons for modifying the impression of
a decreased abundance of oligochaetes in the lake profundal in general.
This assumption is confirmed by the values for average abundance and
percentage share of oligochaetes in the total material of EKMAN, which shows
no essential differences from the values today (Table 3).

The increase of animals above or within the upper surface layer of the
sediments is of importance for the understanding of the altered conditions
in the lake. Investigations have shown that this fraction of the bottom fauna
is the most sensitive to changes, induced by several kinds of interference. In
Lake Mälaren the first sign of pollution effects is a decrease of the big
crustaceans, including *Pontoporeia*. The big crustaceans are gradually
eliminated towards the peripherical basins and are replaced, first by a chiro-
nomid fauna and later by an oligochaete fauna as a result of the action of
pollution upon the vital chemical processes in the sediment surface (GRIMÅS
1967). Mechanical disturbance in the bottom surface induced by the im-
poundment of a lake has the same negative effect on the big crustaceans
and the sphaeriids. The populations of small crustaceans on the other
hand, react positively to an impoundment in the short run because of a
temporary increase in the availability of food and the fact that they are not
directly dependent on a stable sediment surface (GRIMÅS 1965).

The positive effect on the abundance of these animal groups, crustaceans
and sphaeriids, in the profundal of Lake Vättern argues in favour of an
increased supply of organic substances suitable as food for animals living in
the bottom surface without changing the environmental conditions to the
advantage of pollution-tolerant forms like oligochaetes and *Chironomus*
larvae. The following up of these tendencies in the littoral region of the lake
by comparison with earlier material must necessarily involve very broad
generalizations. EKMAN's material contains only five defined and comparable
sampling stations. However, the mutual relationship between the sections
corresponds to that prevailing in the present material. The abundance of
animals is highest in the southern part of the lake, next follows the outlet
region, and the lowest quantity is found in the central part of the lake. The
qualitative characteristics, too, of the various sections seem broadly to corres-

pond. Oligochaetes and insects dominate the southern region in both sets of material, which demonstrates the significance of the input of organic matter from the southernmost catchment area. A high abundance of small crustaceans in the outlet section seems also to be common to both sets of results. Animals showing an increase in all littoral regions are big crustaceans and especially the sphaeriids, which predominate in large areas of the outlet region and which on average have increased about six times in the littoral zone.

The comparison with the total material of EKMAN must necessarily be based on average values for the whole lake, owing to the lack of information on the exact location of all his sampling stations. Even such a comparison however, points to an increase of bottom animals in the lake during the last 50 years, which can be estimated at about 45 % (Table 3). The main part of this increase is induced by sphaeriids and crustaceans. The increase of insects is more uncertain, owing to the seasonal variation, and the oligochaete populations are of about the same sizes.

Summing up, the changed situation in Lake Vättern includes an increased quantity of bottom animals in all depth zones and a changed balance between the main animal groups in favour of species in the contact zone water/ sediment. The benthic communities indicate an increased sedimentation of organic matter over the lake bottoms, which in southern Vättern can to a great extent be ascribed to the input of allochtonous material from the inlets. The conditions in the central basin and the outlet area point to a small but definite elevation of the general level of productivity in the lake.

The conclusions are confirmed by, amongst other circumstances, the observations on the decreased transparency in the lake, especially during the last 30 years (AHL 1968). The course of the transparency curve during the last 80 years corresponds very nearly to the phosphorus curve and provides reasons for believing that there is an increased supply of autochtonous products in the water mass.

The correlation between the detrital organic material in the sediments and the biomass of the benthos is evident from the results (cf. POWERS and ROBERTSON 1965). It also seems evident that the increased quantity of bottom animals during the last 50 years, as a long-term effect, can be ascribed to the increased supply or organic material in the lake as a whole.

The development in the large Swedish lakes is broadly reminiscent of the conditions in the St. Lawrence Great Lakes, where the general increase of benthic organisms, it has been suggested, results from the increased organic content in the sediments (BEETON 1965, ROBERTSON and ALLEY 1966). The investigation results from the two groups of large lakes, however, clearly show different stages of eutrophication. In Lake Michigan the actual increase of bottom organisms involves in the main *Pontoporeia* and oligochaetes and

only to a minor extent the sphaeriids. In Lake Vättern there are no signs of a continuous, general concentration of oligochaetes but there is clear evidence of an increased sphaeriid population. It seems reasonable to assume that the enlargement of this special fraction of the benthos, *i.e.* filtrating animals, reflects an early phase in the eutrophication of lakes, caused by an increased "rain" of organic products from the pelagic zone. The accompanying increase of the crustacean fraction underlines a development within the range of oligotrophic or early eutrophic conditions.

In a later phase of eutrophication the increased supply of organic material in the sediments favours such animal groups as depend on deposited organic materials, a stage which seems to have been reached in Lake Erie (DAVIS 1966, BRINKHURST, HAMILTON and HERRINGTON 1968). The last stages include the predominance of a small number of species which are specialized for temporarily severe conditions, *e.g.* regarding the oxygen content in the environment, and a high abundance and biomass owing to the surplus of food. Such conditions are reported from *interalia* peripheral basins of Lake Mälaren in central Sweden (GRIMÅS 1967).

V. Acknowledgements

VI. Summary

Lake Vättern is one of the large lakes of central Sweden. The surface area is 1,900 sq. km and the maximum depth 128 metres.

It is suggested that the distribution of bottom animals is an effect of the distribution of organic content in the sediments with low values in the littoral. Maximum biomass and abundance occur in the profundal zone and the predominating species is *Pontoporeia affinis*. Regional deviations from this pattern including the littoral as well as the profundal zone are described.

A comparison with earlier investigations in 1911—14 shows an increased abundance of bottom animals in the lake and a changed balance between the main ecological groups in favour of those living in the contact zone water/ sediment. The increase of filtering animals such as sphaeriids reflects, it is suggested, an early phase of eutrophication as a result of an increased sedimentation of organic products over the bottoms. The increased abundance of big crustaceans and the unchanged average quantity of animals depending on a high supply of deposited organogenic compounds in the sediments, *e.g.* oligochaetes, indicate a changed level of productivity within the range of oligotrophic conditions.

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Distribution of trout and char within a small Swedish high mountain lake

By Karl-Jakob Gustafson, Thorolf Lindström and Åke Fagerström

I. Introduction

The plasticity in habitat choice and other ecological features may be considerable in certain fish species, and among the environmental factors responsible for the result in any particular case the interaction with other fish species is very important (Nilsson 1967). In the present population study in a small high mountain lake in Jämtland (Fig. 1), char and trout (*Salvelinus alpinus* L. and *Salmo trutta* L.) live as the only fish species in such a small space that the segregation in different habitats within the lake must continually be put to the test, frequent encounters between individuals of different species being ensured by this circumstance.

Lake Långbjörsjön is one kilometre long and about one hundred metres broad; the maximum depth is 11 metres and the vegetation consists *inter alia* of *Carex* reeds in all bays. The *Carex* reeds and the underwater vegetation are particularly abundant in the inlet bay (A) and in the bay J, Fig. 2. In the surroundings isolated spinneys of birch and spruce are interspersed between small mires sloping towards the lake. For many years prior to the experiment fly fishing had mainly been practised. During the experiment marking with different types of gear took place for three years in July at the beginning of the fishing season, and apart from the ordinary fly fishing the lake was fished with netting and other types of gear giving catches that were checked by the investigators and formed an important part of the yield during nine years (cf. following paper, in MS).

II. The action of gear on populations of marked fish

As stated in the preceding paper, Fagerström *et alii*, 1969, Table 2, fin-cut char is recaptured at the same rate as fin-cut trout, whereas the rate for tagged char is lower than that for trout. The data are slightly rearranged in Table 1 and can corroborate the information in chapter III. The marking period and "other fishing" show in most cases low proportions of char, whether fin-cut or tagged. In net fishing the trout/char proportions are about the same as the proportions at tagging or are even high enough to reduce
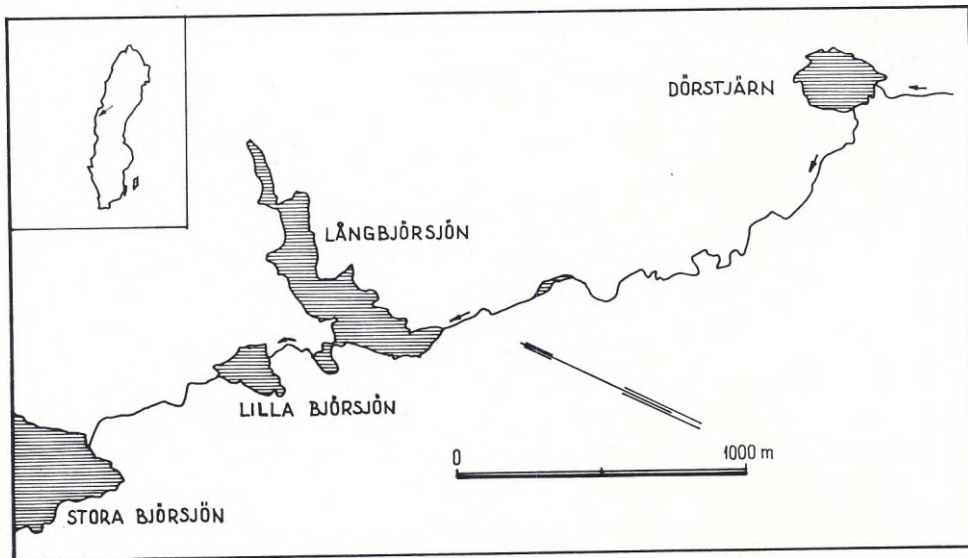
Fig. 1. A map of the surroundings of Lake Långbjörsjön, Jämtland. The lakes on the map belong to the River Indalsälven system.

the underrepresentation of char produced by the marking period and "other fishing". The fishing during the marking period and "other fishing" was largely performed with artificial flies; it was very selective and favoured trout. The resulting totals within a particular year will thus depend on the amount of fly fishing and the amount of net fishing. The fin-cut fish from 1959 are recaptured the second year after the marking year and can be identified as marked. The proportion of char is then high; most of the char catch during the marking period in this year (1961) was obtained with nets. To explain the result for the 1959 marking series an absence of tag shedding and an absence of increased mortality for fin-cut fish should be taken into account (preceding paper p. 35); but the overall picture is dominated by the gear effect.

III. The action of the gear in different habitats

The catch depends on the behaviour of the fish and the fisherman, as well as on his gear and on the environment where it is used. As it is not possible in the present case to isolate these various factors and assess their relative importance, a true index of density cannot be obtained and the relative indices of density will not be comparable between habitat types. The study of the ratio char number/trout number is also rendered difficult. All this does not, however, mean that the catch data are not useful.

Table 1. Distribution of recaptures of trout and char by gear used, number of years after marking and marking methods. A dash, —, indicates no fishing.

	1959 Fin-cut Trout Char	Pro-por-tion	1960 Tagged Trout Char	Pro-por-tion	1961 Tagged Trout Char	Pro-por-tion	1961 Fin-cut Trout Char	Pro-por-tion
Number marked	177 70	— 2.5/1	162 55	— 3/1	153 130	— 1.2/1	24 21	1.1/1
Recaptures:								
Year of marking								
Marking period ...	7 1		15 0		25 10		6 2	
Littoral netting,								
summer	28 13		19 6		22 23		3 2	
Netting, autumn ..	— —		— —		10 10		3 5	
Other fishing	0 0		8 1		19 12		1 2	
	35 14		42 7		76 55		13 11	
First year after marking								
Marking period ...	23 3		20 1		— —		— —	
Littoral netting,								
summer	11 3		2 4		— —		— —	
Netting, autumn ..	— —		4 1		— —		— —	
Other fishing	6 0		4 2	{Seine {Hooks	5 1 8 1		1 3 2 1	
	40 6		30 8		13 2		3 4	
Second year after marking								
Marking period ...	10 6		— —		— —		— —	
Littoral netting,								
summer	4 8		— —		1 0		0 0	
Netting, autumn ..	7 1		— —		— —		— —	
Other fishing	1 2		0 0		1 0		0 0	
	22 17		0 0		2 0		0 0	
Grand totals	97 37		72 15		91 57		16 15	

In Lake Långbjörsjön the different habitat types are situated quite close to another and are of small extent, as the width of the lake is only around one hundred metres.

The gill nets were set on the bottom in gangs of two nets — in a few cases three nets — and extended from the shore of the lake towards the middle. Each net covers 27—30 metres when laid out straight, but in practice the distance covered is somewhat less. The nets do not fish the surface outside the innermost region, since they are set on the bottom, and they do not fish the bottom in the very middle of the lake as they do not reach far enough.

Floating gill nets were used in 1961 only and then were set in the surface in the middle part of the lake. The depth of a net was 3 metres, its length 60 metres when straight. For technical reasons they did not always fish well. The maximum depth in the lake is 11 metres.

The seine — 65 mm inner perimeter of the meshes in the bag, 32 stretched meshes per 120 cm — was generally used floating and covered more than

Table 2. Number of trout and char caught in otter fishing over the whole clear-water surface in July. Fish that were tagged and put back in the lake are also included.

	Flies			Small spoons		
	Trout	Char	% trout	Trout	Char	% trout
1959	158	65	71	—	—	—
1960	67	19	78	—	—	—
1961	41	15	73	41	45	48
1962	49	9	84	8	19	30
Total	315	108	74	49	64	43

half the distance between opposite shores, but it is presumed to fish better when close to the shore where the gear follows the bottom too. The catch of pelagically occurring fish in the middle of the lake is then highly dependent on the behaviour of the fish. The seining was concentrated in certain sections, depending on the nature of the bottom close to the shore.

The rod and line fishing took the form exclusively of fly fishing from the shores. The hooks used were number 10 (the new system), though occasionally number 12 was employed.

For otter fishing there were used double hooks, numbers 8—10, with artificial flies or the smallest obtainable spoons (light spin spoons) with a spoon length around 22 mm. The gear covered the free water surface and was used with a view to fishing as regularly as possible over the whole surface, the flies riding on the surface and the spoons dipping a short way down into the water.

To obtain information about the abundance of different species in different habitats, the selectivity of the different types of gear will be discussed.

As shown in Chapter 2 fishing with artificial flies is highly selective. Fly fishing connected with the experiment took 162 (81 per cent) trout in July in the years 1959—62 of 201 checked catches with this gear, whether put back or not. In other sport fishing with fly the percentage of trout varied between 71 and 97 in the yearly catches in 1958—65. This fly fishing was centred on habitats where the trout was known to stand, *e.g.*, outside the *Carex* reeds, but otter fishing with flies that swept over the whole clear surface of the lake as regularly as possible also caught a high percentage of trout (Table 2).

The trout dominance was reversed into a char dominance when the fly otter was replaced by an otter with small spoons, working over the same clear surface in the same regular manner (Table 2). Such small spoons were known from general Swedish experience to be effective in catching char.

The catches in suspended gill nets, set in the surface in the middle of the lake, are not influenced by the attractiveness of a lure but by the ability of

Table 3. Total number of trout and char in suspended gill nets set between the surface and about half way down to the bottom in the middle part of the lake. Mesh size (2.50—1.67) is knot-to-knot measure in cm.

	August 1961, nights				October 1961, days & nights			
	2.50	2.14	1.67	% trout	2.50	2.14	1.67	% trout
Trout	6	3	0	12	0	1	0	7
Char	16	27	21	—	0	4	9	—
Efforts	3	3	3	—	2	2	2	—

the fish to avoid the net. The char dominance is here still more pronounced and increases for the more fine-meshed nets (Table 3). The trout-char proportions should not be greatly influenced by the fact that the nets did not fish well on all nights. As the nets extended to a point about half way from the surface to the bottom, it may be suspected that another kind of fish fauna exists deeper in the lake. Echo sounding in the middle of the day in July 1962 did not indicate many fish below the uppermost 2—3 metres — where the sounder does not work — down to the bottom layer, but the bottom layer was not investigated with this gear as "white line" was not used.

Seining was almost exclusively concerned with the surface region. It was performed with a floating seine and started from a point well outside the half-way point between two opposing shores. The seine thus at first advances over the deepest, middle part of the lake and any fish that are sufficiently observant can escape below or outside the seine. In the last stage of a haul the fish has less opportunity to escape, and as the seine was often hauled towards a *Carex* reed region — in order to obtain an easy landing — the gear should catch littoral fish more effectively.

A successful catch of pelagic fish in this lake should depend on the weather and light conditions that regulate all pelagic fishing, controlling the vertical distribution of fish and affecting the inclination of the fish to dive when gear approaches. The seine catch of pelagic fish could thus *a priori* be expected to vary more than the catch of littoral fish. Accepting this postulate the results of the seining, Table 4, are consistent with the results from gill-net

Table 4. Total number of trout and char in seine catches, whether the fish was put back into the lake or not. Most results from sections A, B and G. In all 17 hauls.

	July 1960	July 1961	July 1962	% trout, 1960—62
Trout	75	56	91	47
Char	35	26	187	—
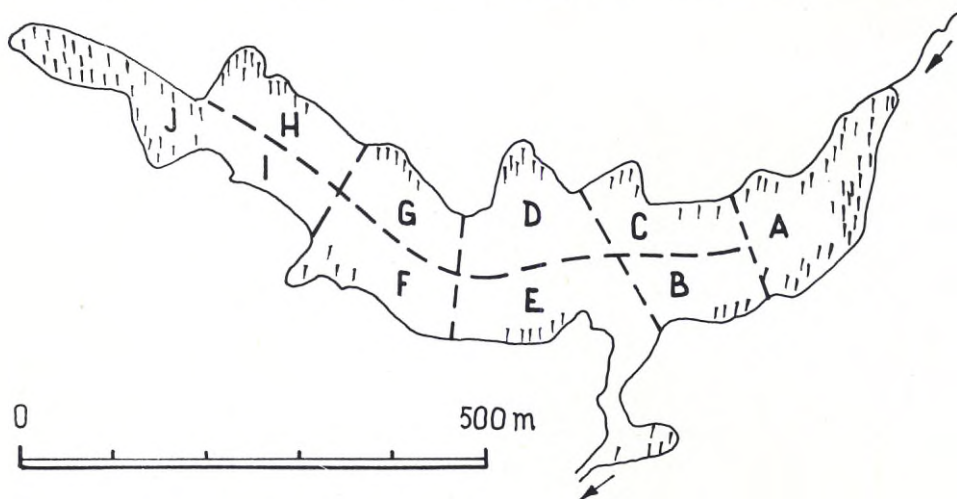
Fig. 2. The different sections in Lake Långbjörsjön. The distribution of *Carex* reeds is also sketchily indicated.

fishing (Table 7 and 3): char inhabits the pelagic region too, while trout appears closer to the shores.

As a preliminary result of a study of the heterogeneity, it was stated in a mimeographed report (LINDSTRÖM 1961) that the proportion of trout was high in the sections A and B between the main affluent and the exit of the lake (map, Fig. 2) and low in the northern arm of the lake (section J), and that the proportion of trout decreased and the proportion of char rose in

Table 5. Number of trout and char in two different parts of the lake, totals for the different kinds of gill nets.

Date	Knot-to-knot measure, cm	Section J		Section A—B	
		Trout	Char	Trout	Char
1961, August 9—11	2.50	2	5	7	5
1965, July 9—11	—	2	4	16	7
1966, July 1—3	—	0	4	14	5
		4	13	37	17
1961, August 9—11	2.14	1	8	21	9
1965, July 9—11	—	3	10	9	10
1966, July 1—3	—	3	13	13	34
		7	31	43	53
1961, August 9—11	1.67	2	13	53	18
1965, July 9—11	—	3	18	6	39
1966, July 1—3	—	7	21	17	31
		12	52	76	88
Grand totals	—	23	96	156	158
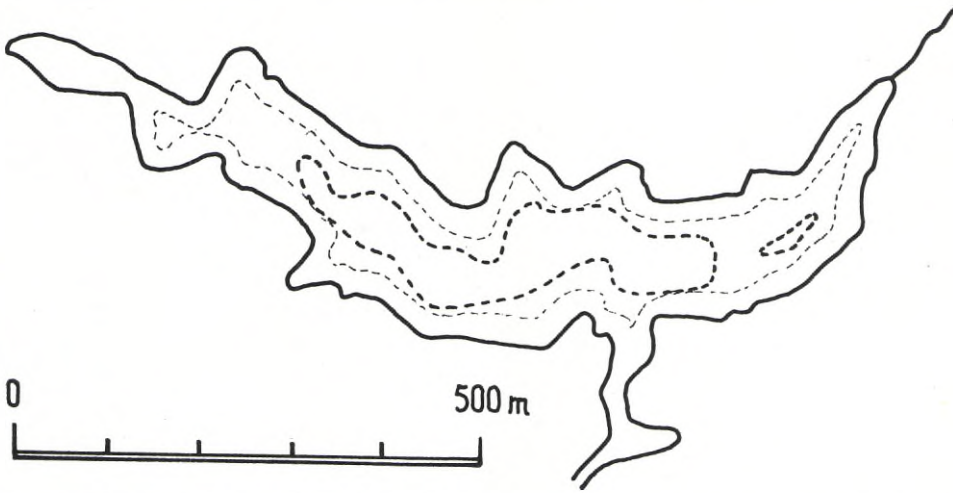
Fig. 3. The first net area is bounded by the shore line and the thin contour of short dashes, 25 metres out in the lake. The 5 metres depth is indicated by two coarser contours of dashes.

the direction from the region close to the shore where the first gill net is placed towards the deeper area further out in the lake where the second gill net in each gang of two nets was placed.

The catch of char and trout in sections J and A—B is reported in Table 5 for the years 1961, 1965 and 1966, when the gill-net fishing was more complete and regularly distributed than in other years. Section J is shallow as a whole, whereas sections A and B contain deeper parts, and only in the latter sections would the habitat difference between the "first net place" and "second net place" in a gang be obvious (cf. Fig. 3). Section J was fished with nine fine-meshed nets, and sections A—B with eighteen fine-meshed nets, totals for all three years.

There is a higher proportion of nets in first place in section J than in sections A—B. This is important for the results in Table 5, as an excess of nets in first place should in other circumstances favour the catching of trout. This is confirmed by the results in the years 1961—66, Table 7, when the

Table 6. Distribution by first and second place in the gang of gill nets, which gave the catches in Table 5.

Knot-to-knot measure, cm	Section J		Sections A—B	
	First place	Second place	First place	Second place
2.50	2	1	2	3
2.14	3	0	4	2
1.67	1	2	3	4

Table 7. Total numbers of trout and char in gill nets, regularly distributed round the lake. Place of net in gang and mesh size is indicated.

	Gill net knot-to-knot measure, cm	Place in gang:	
		first place	second place
1961, Aug. 9—11	2.50		
efforts		5	4
trout		25	18
char		34	38
1965, July 9—11			
efforts		5	4
trout		35	13
char		11	10
1966, July 1—3			
efforts		4	5
trout		30	11
char		7	12
1961, Aug. 9—11	2.14		
efforts		5	4
trout		43	7
char		24	43
1965, July 9—11			
efforts		4	5
trout		24 or 18	12
char		27 or 47	37
1966, July 1—3			
efforts		4	5
trout		16	5
char		45	43
1961, Aug. 9—11	1.76		
efforts		4	5
trout		73	27
char		47	68
1965, July 9—11			
efforts		4	5
trout		24	6 or 12
char		78	59 or 39
1966, July 1—3			
efforts		5	4
trout		45	6
char		86	56
Totals for all the years:	2.50 efforts	14	13
	trout	90	42
	char	52	60
	2.14 efforts	13	14
	trout	80	24
	char	106	123
	1.67 efforts	13	14
	trout	142	42
	char	211	173
Totals for all three net types:	1961 trout	141	52
	char	105	149
	1965 trout	80	34
	char	126	96
	1966 trout	91	22
	char	138	111
Grand totals	efforts	40	41
	trout	312	108
	char	369	356

catches were obtained with 3×3 efforts each year of each net type, distributed as regularly as possible over the ten sections of the lake and as first and second net in each gang of two nets.

The action of the gill nets thus gives a consistent and plausible picture of trout as preferring the shallowest, most littoral border of the lake and proximity to running water, though they also occur further out in the lake, whereas char is about equally abundant in the "first net zone" as in the "second net zone", widely outnumbering the trout in the second zone and in the central pelagic zone. Section J forms an exception to the rule. Owing to the circumstances mentioned at the beginning of this chapter, a final description in actual densities cannot be given, but the smallest fish seems most inclined to keep close to the shore.

IV. The movements of trout and char

Trout and char were tagged and/or fin-cut in the first part of July in the years 1959, 1960 and 1961. In Table 8 the number fin-cut and the number of recaptures during the subsequent netting in July 1959 are reported. During the recapture period the netting was a little more frequent in the northern part of the lake than in the southern part.

The table indicates a certain amount of stationariness. Furthermore, about half the number of fish moving into the opposite part of the lake before recapture were caught near the boundary between the two parts. Tagging in 1960 and 1961 gives a more detailed picture of the movements, as tagging and recaptures have been specified for the ten sections of the lake shown in Fig. 2. Recaptures have been classified in the following categories.

A. Fish recaptured in the section where they were tagged.
B. Fish recaptured in the nearest sections, including those meeting the tagging section corner to corner.
C. Fish recaptured further away from the tagging section.

Table 8. Number fin-cut (pelvic fin or pelvic and adipose fin) and number of recaptures during netting in July 1959, Lake Långbjörsjön.

	Recaptures of:			
	Trout		Char	
	northern part	southern part	northern part	southern part
67 trout and 26 char fin-cut in northern part of lake	7	1	3	0
110 trout and 44 char fin-cut in southern part of lake	9	11	5	5

Table 9. Recapture of tagged trout from Lake Långbjörsjön, grouped according to the text. Tagging in 1960 and 1961, total length of trout 24 cm or less at tagging.

	Recapture category:		
	A	B	C
Recapture within one week	11	3	2
Recapture from one week to one month, after tagging, mean 27 days	11	11	8
Recapture after about three months	2	0	2
Recapture in July, one year after tagging	6	2	3
Recapture later	1	1	0
Total	31	17	15

Table 10. Recaptures of trout from Lake Långbjörsjön, tagged in 1960 and 1961, total length exceeding 24 cm at tagging.

	Recapture category:		
	A	B	C
Recapture within one week	7	6	2
Recapture from one week to one month after tagging, mean 26 days	13	14	1
Recapture after about three months	2	3	1
Recapture in July, one year after tagging	11	6	3
Recapture later	0	2	0
Total	33	31	7

Table 11. Recapture of char from Lake Långbjörsjön, tagged in 1960 and 1961.

	Recapture category:		
	A	B	C
Recapture within one week	3	1	1
Recapture from one week to one month after tagging mean 31 days	6	23	11
Recapture after about three months	2	5	3
Recapture in July, one year after tagging	1	5	4
Total	12	34	19

Trout is also classified according to total length at tagging and the limit is fixed at 24 centimetres in order to obtain enough trout in each group. Many trout in the longer group are mature. One year later the mean lengths in the two groups have of course increased.

Tagging and fishing for recapture were not quite regularly distributed over the ten sections of the lake. The tagging is reported in Table 12 and

Table 12. Distribution of the tagging over the different sections of Lake Långbjörsjön.

	Sections									
	A	B	C	D	E	F	G	H	I	J
Trout, tagged 1960	54	18	8	0	15	6	24	10	15	12
Trout, tagged 1961	55	24	7	3	27	9	11	5	6	6
Char, tagged 1960	28	5	0	1	2	1	5	4	4	5
Char, tagged 1961	32	16	10	6	14	8	11	7	10	16

the line "tagged 1961" also shows the distribution of the catch in which recaptures of fish tagged 1960 were obtained, one year after the tagging.

In 1962, one year after the tagging in 1961, most catches were obtained in sections A, B, E, G and J but all sections were fished to some extent. The distribution of catches and recaptures in July one year after tagging is thus produced by the distribution of fish and of fishing and also by the unequal vulnerability. The netting, on the other hand, was distributed as regularly as possible in the years 1960 and 1961, and gave almost all the recaptures up to about three months after tagging reported in Tables 9—11. During the summers the proportion of the recaptured trout recaptured in the section of tagging is higher than the corresponding proportion of char. (When considering the possible features of the mixing process, it should be noted that category B refers to from two to five tenths of the total area, Fig. 2.) Three months after the tagging (Tables 9—11) the mature char had just spawned and the spawning time for mature trout was approaching.

The fish in these waters do not migrate far away. No fish tagged in Lake Dörstjärn was recaptured in Lake Långbjörsjön or in the Björsjöarna lakes downstream. No fish tagged in Lake Långbjörsjön was recaptured in Lake Dörstjärn and only one char was recaptured in Lilla Björsjön and one trout in Stora Björsjön (map, Fig. 1). The areas of the lakes and the yield during the years of the experiment are shown in Table 13 below and in a table in a following paper (MS).

Table 13. Surface areas and yields from adjacent lakes where the existence of tagged fish could be effectively checked.

Lakes	Area, square kilometres	Species	Catch in numbers:				
			1959	1960	1961	1962	1963
Dörstjärn	0.04	trout	171	124	195	67	272
Stora Björsjön	0.35	trout	333	291	578	462	512
		char	108	132	486	267	1,111

V. Discussion

The habitat segregation within lakes between char and trout is treated by NILSSON (1955) and a general survey of interactive segregation is given by the same author (1967). New material on char and trout segregation in Lake Kultsjön (LINDSTRÖM and NILSSON, 1964) showed that the catch in fine-meshed gill nets increased for char and decreased for trout in the direction from the first net at the shore towards the deeper region, even in this deep lake where the trout population is small compared to the char population. The study concerned only the first and ninth nets in a gang set from the shore with the ninth net 12—17 metres below the surface. The material included catches from June to October, and more exceptions to the stated rule occurred in September and October than in the other three months.

In Lake Långbjörsjön the density is considerably higher and falls by something like three or four catchable fish per ten square metres (in MS), to which must be added fish from two to four age classes of young fish. Young trout seem to spend only a very short period in the brooks, and the possible spawning areas of the brooks are in fact of very limited extent. The mean density thus approaches one fish per square metre in a lake with a maximum depth of 11 metres. The interchange of fish between the different sections of the lake — particularly that of char — and the occurrence of both trout and char in the first as well as in the second net region ensure frequent encounters between trout and char, even though the char distribution pattern must be conceived of as patchy.

The demand for habitat segregation is not so great that this interspacing of char and trout could not be tolerated, although there is a general trend in the trout and char distribution reported in Table 7. Moreover, one particular section forms an exception to the rule and has a very high char proportion in spite of its being very shallow and rich in aquatic vegetation (J). The main contribution from this material is thus the incompleteness in habitat segregation that could be tolerated in a natural lake. From Table 14 it can be concluded that the incompleteness is not the result of a temporary superabundance of some resource, as the catches in Table 14 were taken in the autumn and after the necessary redistribution of the mature specimens during the char spawning and the redistribution of mature trout as a preparation for the trout spawning.

The char accumulation in section J is not observed in this autumn fishing, but otherwise the pattern is the same as in July and August.

MAGNUSSON (1962) stated that aggressive behaviour is a mechanism for reserving a greater portion of a limited food supply and is not the expression of a competition for space *per se*. A study of food abundance would be a logical sequel to the present study but has not yet been undertaken.

Table 14. Distribution between first and second place in gang. Total number of trout and char in gill nets in the autumn of 1961 after the main char spawning. The 2.50 cm knot-to-knot and 2.14 cm knot-to-knot nets were regularly distributed round the lake if the whole period is considered, but the day catch must be included to give this fair distribution; the day results reduce the segregation somewhat. The 1.67 cm knot-to-knot nets were few in number and two of them were set in section J.

	2.50 net		2.14 net		1.67 net	
	first place	second place	first place	second place	first place	second place
Oct. 5, day						
efforts	2	1	1	2	2	1
trout	11	5	2	10	5	1
char	2	0	2	4	4	4
Oct. 6 and 7, nights						
efforts	3	3	3	3	0	1
trout	38	10	29	13	—	22
char	10	11	17	28	—	14
Totals:						
efforts	5	4	4	5	—	—
trout	49	15	31	23	—	—
char	12	11	19	32	—	—

VI. Summary

The distribution of trout and char in a small high mountain lake is studied. The study concerns the action of different types of gear and the movements of the fish. The degree of habitat segregation, or rather the interspacing of char and trout that is tolerated, constitutes the main contribution from this study.

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Reactions of Young Salmonids to Sudden Changes of pH, Carbon-dioxide Tension and Oxygen Content

Continuous-flow and alternating-environment experiments performed with feed waters rich and poor in bicarbonate (Uppsala water and River Dalälven water)

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Contents

Abstract

Starting with aerated Uppsala tap-water (originally hard, bicarbonate-rich, subsoil water; see Tables 1—2 and Fig. 1), environmental fluctuations of pH and pCO_2 were obtained by the addition of hydrochloric acid to this feed water, which flowed continuously through an alternating-water-quality test apparatus (Fig 2). Two or three isolated specimens of fish were observed simultaneously. Decreasing pH, combined with increasing CO_2 tension, provokes changes in locomotor behaviour and ventilation among second-summer parr of the Atlantic salmon (*Salmo salar*). Extremely adverse pH/pCO_2 conditions, in combination with low O_2, also give rise to delayed chromatophore reactions. The locomotor reactions, mainly due to CO_2, are particularly intense on the introduction of a changed water quality into the test apparatus. The intensity of reaction depends on the time of contact, the combination and the quantity of the external factors varied, *i.e.* the degree of deviation from the pre-acclimatization water quality, which is the control condition of the tests. Similar reactions but reversed in time and less intense also occur after a test period in conjunction with and after the return once again to control conditions. Pure oxygen depletion — down to a critical value in the range of 1—3 mg O_2/l — provokes hyperventilation but does not initiate any changes in behaviour, *e.g.* any locomotor activities. Nor does any "initial peak" appear on the ventilation curve (see experiment VII in Fig. 7), which is characteristic of most of the pH/pCO_2 deviation and combination tests performed (Figs. 3—6). Similar experiments, starting with a softer surface water from the River Dalälven (Table 1), confirm that the reactions displayed in Uppsala water are mainly due to CO_2 (cf. Fig. 8).

The parr — which under control conditions remain on the whole immobile in resting positions on the bottom of the test chamber — accordingly display characteristic behavioural reactions of the whole organism, but also reactions on the organo-physiological and cell-physiological levels of integration. The intensities of these reactions may be taken as measures of an exogenously released or *environmental stress* (sensu FRY, 1947; BRETT, 1958; thus disregarding the definition of stress proposed by SELEY, e.g., in 1952). In the present experiments this was mainly a *respiratory* distress. In the cases of pH/pCO_2 and $pH/pCO_2/O_2$ changes towards lowered pH and O_2 content — but to a less extent in the cases of environmental changes of pH or O_2 *per se* (Figs. 7—8) — the respiratory stress is reinforced by a working or *loading stress* (sensu BRETT, 1958), on account of the muscular activity exterted during the *emergency reaction* (HÖGLUND, 1961) which is induced in the fishes' confrontation — without being given any choice — with unpleasant or adverse environmental conditions, as regards the abiotic factors studied (cf. SAUNDERS, 1962).

From an ecological and physiological point of view two main principles are discerned which make it possible for the parr to withstand high external carbon-dioxide tensions, viz. (1) *avoidance reactions* (HÖGLUND, 1961; cf. "orientational homeostasis" (MITTELSTAEDT, 1964) and "comfort for survival" (WOODBURY, 1956)) and (2) the *regulation of a CO_2-induced acidosis* to obtain normal pH in the blood by a presumable new acid-base equilibrium during retained CO_2 supersaturation in the ambient water (HÖGLUND &

BÖRJESON, unpublished manuscript). The latter compensatory mechanism is an example of an "internal regulatory homeostasis" (CANNON, 1932; HALDANE, 1935; PROSSER, 1958; HUGHES, 1964; and others). In the case of environmental O_2 depletion the respiratory stress involves first of all, an *immediate reflex response,* which is easily recognized from the ventilatory rate (HUGHES, 1963, 1964) but in the second place and only at critically low O_2 contents an *emergency behaviour* (LINDROTH, 1947; HÖGLUND, 1961) is also provoked.

It cannot be decided, from the observations made in the present experiments, whether or to what extent the immediate hyperventilation response and the "initial peak" on the ventilatory curve connected with excited locomotor activities due to raised pCO_2 in the respiratory water is attributable to external and/or internal stimulation of CO_2-sensitive receptors. This will be discussed in more detail in a future paper by HÖGLUND and PERSSON. The responses may be the result of direct stimulations of exterior receptors in the gills and/or receptors in the aortic arches, as well as in the central nervous system, after a rapid diffusion of CO_2 into the blood. Anyhow, there is reason to believe that CO_2 deriving from the metabolic processes is more likely, from a teleological point of view, to act upon receptors in contact with the bloodstream than in contact with the respired medium. On the other hand, the avoidance reactions in a spatially CO_2-differentiated water, especially the instantaneous rebound reaction (HÖGLUND, 1961, p. 102), when fish swim towards higher pCO_2, are difficult to explain without hypothesizing the existence of external CO_2-sensitive receptors (see HÖGLUND, 1961, p. 108 ff.). HUGHES (1963, p. 85) remarks that "The responses increasing ventilation in the environments containing high levels of CO_2 but normal levels of O_2 appear inappropriate, since they will not apparently lead to an increased oxygenation of the blood". The explanation of this seeming inappropriateness may be connected with the acid-base regulatory mechanism and the prompt avoidance reaction displayed by fish in environmental pCO_2 gradients.

Finally the consequences of the acidification of fish-producing waters by air and water pollution and the possible additional effects of artificial abiotic factors are emphasized (ODÉN, 1968; EIFAC, 1968). The reader is further referred to the concluding remarks attached to the particular sections in which the results are presented.

I. Introduction

The aim of the present study is to give some physiological background information which may elucidate the biological and ecological consequences of the present acidification by air and water pollution of fish-producing natural waters with different ionic compositions. On that account the reactions and compensatory functions of salmon parr (*Salmo salar*), which are provoked at the confrontation and displayed during the course of contact with temporarily fluctuating but spatially homogeneous environmental conditions of stress, as regards the abiotic factors of basic physiological interest, viz. pH, carbon-dioxide tension, and oxygen content, are described and discussed in certain detail. The conception of *environmental stress* is not used here in the sense of the word *stress,* as suggested by SELEY (e.g., 1952), but rather in accordance with the views presented by FRY (1947) and BRETT (1958). *Environmental stress reactions* provoked by the adverse combination

of these factors in the ambient water are easily demonstrated to exist on three functional integration levels, *viz.* in the form of *chromatophore reactions* at the cellular level (Section VI), in the form of respiratory distress or *hyperventilatory* compensation at the organ-physiological level (Sections V and VII), and in the form of various kinds of behaviour — excitement, locomotor activation and temporary *hyperactivity* (emergency reaction, Höglund 1961), and even exhaustion — at the whole organism (or ethological) level (Sections IV and VII).

This study was also performed in order to follow up some observations presented and suggestions made by Höglund (1961) on the reactions of freshwater fish, among them salmon parr, in stable concentration gradients in a laminar flow. In the latter study the question was raised as to the existence of appropriate adaptability in the preference reactions displayed, as regarded the factors mentioned as well as the artificial ecological factors deriving from human civilization, such as sulphite waste liquor, and to what extent the effects of products discharged by man may affect fish life in receivers with various water qualities. The purpose of this investigation is also topical on account of the present discussion, above all in Sweden, about the acidification of rain-water by sulphurous compounds in waste products deriving from industrial and urbanized areas in western Europe. There is, finally, one more question raised by modern civilization on which light may be shed by the present experiments, *viz.* what effect intermittent discharges in large quantities of acid pollutants into the rivers, the lakes and the sea may have upon the fish fauna in comparison with a more continuous dilution. In this respect it is of great interest to compare the effects of pH upon the reactions of fish kept in and pre-acclimatized to waters with various bicarbonate-buffering capacities and oxygen contents. One of the aims of the present paper is to further study the effects of each of these factors and some combinations of them (cf. Höglund, 1967; EIFAC, 1968).

Tolerance to fluctuating environmental conditions is due to *homeostasis* (Cannon, 1932). The reactions and physiological mechanisms which contribute to "the maintenance and co-ordination of the whole organism" (Haldane, 1935) may, with regard to temporal relations, be grouped in the following way (partly after Hughes, 1964, p. 83):

(1) *Responses,* i.e. immediate reflexes and co-ordinations.
(2) *Regulatory processes,* which after a temporary but well-manifested disorder in the internal fluids, re-adjust the organism almost to the functional state which is normal under optimal environmental conditions. *Acclimation* means a physiological adaptation to one or more definite environmental factors in the course of hours, up to a day and a night; *acclimatization* means functional adaptation to a more complex change in the environment for a longer period.

(3) *Adaptation* is used by HUGHES (1964), as by FRY (1958), to mean "responses at the phylogenetic level which enable a species to exist in a given environmental stress". On the contrary, adaptation is used here in the same sense as acclimation, with regard to the functional adaptation of a specimen for days, weeks, or months.

The present tests are intended to show how the salmon young react to the change from acclimatization conditions, i.e. control conditions in the tests, to new pH/pCO₂ and/or O₂ test conditions which are held constant for a couple of hours and vice versa at the change back again to control conditions. Reactions in single fish are followed during the entire contact with the test solution (5 or 10 hours; see Figs. 3—10). This report is restricted to the descriptions of pH-, CO₂-, and O₂-provoked responses induced in the hard and bicarbonate-rich subsoil water supplied by the Uppsala waterworks (aerated Uppsala tap-water) and in the softer surface water from the Salmon Research Laboratory at Älvkarleby (River Dalälven water). The reactions obtained by fluctuations of these environmental factors in the two kinds of natural waters are accompanied by a comparative analysis. The responses are recorded to sudden changes as well at to changes during the periods of time when these factors are varied from the control conditions described.

The ventilation rate has been continuously registered without regard to amplitude. But it is obvious that the amplitude is more affected by increased hydrogen-ion concentration in Uppsala water than in River Dalälven water. This means that carbon dioxdie is the controlling factor in the amplitude as well as in the frequency responses (cf. also HÖGLUND, 1961, Figs. 31—38). Thus the frequency-rate function of the period of contact with variously arranged, diverse qualities, as regards pH/pCO₂ and/or O₂, is plotted in the form of *ventilation curves* (Figs. 3—8). The moment of altering the supply conditions in order to obtain a new water quality in the test chamber is marked in each case by an arrow and vertical dashes. Periods with the same water quality as in the pre-acclimatization aquaria are called control periods. They are indicated by a C and a sequence number. Periods with different environments are called test periods and are designated by a T and a sequence number. The latter are to be found, with the applicable experimental conditions, written in the diagrams.

II. Materials and methods

A. Test fish and acclimatization

Second-summer parr (*Salmo salar*) were employed in all experiments, with the exception of No. VIII (see Table 3). The fish were supplied by the

salmon-breeding plant in Älvkarleby, where the fry were hatched in April 1965, and later reared in River Dalälven water (Table 1). They were transported to Uppsala on 24 September 1966 and kept in the laboratory for at least a month before being used in the experiments. The same quality of oxygenated Uppsala tap-water was later used in the test chamber under control conditions (see enclosed data in Tables 1—3 and Figs. 3—7 and 9—10). No losses occurred in conjunction with the transport or later. The fish were fed daily, as at Älvkarleby, with EWOS pellets, standard fodder, size 3. They ate well all the time and grew quite noticeably.

B. Water-quality definitions

The tests in the present study were made in well-defined and, in principle, homogeneous media. The feed water to the test chamber was ordinary tap-water supplied to the Institute (experiments I—VIII) and River Dalälven water at the Salmon Research Station at Älvkarleby, about 90 kilometres north of Uppsala (experiment IX). The mains water in Uppsala is subsoil water from the esker running through this area. This water has a fairly constant composition and high ion strength (predominantly Ca^{++} and HCO_3^-) (see HÖGLUND, 1961, Table 3, p. 33, and MARCSTRÖM, 1967, Table 7, p. 457)[1]. It was unchlorinated and odourless and contained no suspensions or *coli-aerogenes* bacteria. The oxygen content originally was less than 1.0 O_2/l. The temperature did not vary too much with the seasons (during the course of the present experiments around 9°C; see Table 1). After aeration the oxygen content was determined by ordinary WINKLER titration (KARLGREN, 1962) and the pH was measured with a BECKMAN pH meter, type 22. Alkalinity determinations were performed according to BERGER (KARLGREN, 1962). The oxygen content was regulated by injecting air through a water-suction tube controlled by a water manometer. The variation in oxygen content at its maximum was 3.2 % in those cases in which the oxygen content was kept around 9.3 mg/l, 6.5 % at about 6 mg/l and 5 % at about 3 mg/l and the pH was not appreciably altered by the injection of air.

Carbon-dioxide over-saturation in the test chamber is conveniantly achieved by mixing hydrochloric acid and water containing bicarbonate in definite proportions. This is done in the inlet tube (see Fig. 2). CO_2 does not evade to any noticeable degree. Thus, knowing the total alkalinity, $C_{tot.\ CO_2}$, of the feedwater and the pH of the incoming water, the pCO_2 can be calculated from the following formula (cf. HÖGLUND, 1961, p. 38):

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[1] According to data from RUTTNER & NÜMANN presented by CZENSNY (1960, p. 167) about 90 % of the electrolytic conductivity in Uppsala tap-water is due to this pair of ions.

Table 1. Chemical characteristics of the two kinds of feed water continuously supplied to the storage aquaria and test apparatus, these being the "acclimatization conditions" (equal to the "control conditions") for the test series (see Table 3).

Original feed water	Date	pH	HCO_3^-, mg/l	pCO_2, mm Hg	O_2, mg/l	Temp., °C	Electrolytic conductivity, 10^6	Total Fe, mg/l
Aerated	28/10/1966	7.40	—	ca. 8	9.2	9.3	550—580 [1]	—
Uppsala	17/11/1966	7.30	323	10.8	9.4	9.2	—	0.1 [1]
tap-water	8/12/1966	7.30	326	9.8	9.3	9.0	—	—
	12/ 1/1967	7.40	324	8.6	9.4	8.3	—	—
River Dalälven water	16/11/1967	6.4— —6.5	8	1—2	10.8— —11.6	8.3— —8.5	32—33 [1]	0.23 [1]

[1] Based on several measurements over a long period.

$$pH = pK_1' + \log \frac{C_{tot.\ CO_2} - c_s \cdot pCO_2}{c_s \cdot pCO_2}$$

where pK_1' (10°C) = 6.47 and c_s (10°C) = 0.054, according to BUCH (1945, 1951). The relationship between pH and pCO_2 thus obtained on the basis of three determinations of the total alkalinity of aerated Uppsala water is graphically shown in Fig. 1.

pK_1' is the negative logarithm of the "apparent" or "incomplete" dissociation constant of carbonic acid. c_s is the carbon-dioxide concentration, expressed in moles, in the liquid phase in equilibrium with the CO_2 tension of 1 atmosphere in the gas space above.

In order to ascertain whether the fishes' sojourn in the chamber affected the quality of the medium, samples were taken before and after the water had passed through the test chamber. With three fishes the tests showed no difference in either oxygen content of pH (Table 2). Attempts to identify ammonia with NESSLER's reagent in the outgoing water yielded a negative result.

C. Test technique

Fig. 2 is a sketch showing the simple continuous-flow and alternating-environment apparatus.

The test chamber, made of acrylic plastic, is divided into three compartments, the outer ones having dimensions of $16 \times 20 \times 10$ cm, and the centre being $8 \times 20 \times 10$ cm. A fish was placed in each compartment to prevent visual stimulation of territory-defending behaviour. The results shown in Figs. 3—10 apply only to fish placed in the outer sections. The size of the
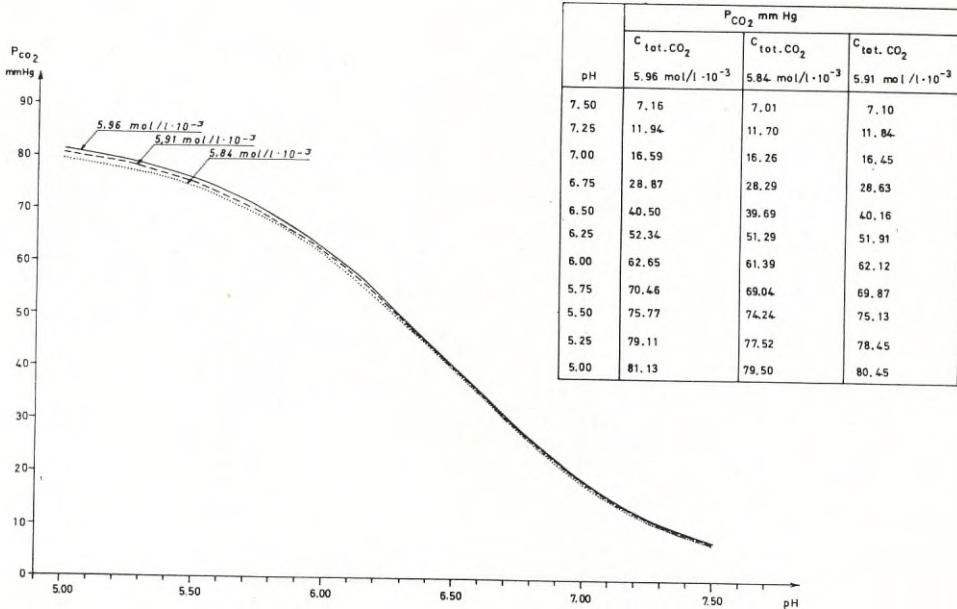
Fig. 1. The relationship between pH and pCO_2, calculated according to the expression on p. 83 for the total carbon-dioxide/bicarbonate/carbonate contents ($C_{tot. CO_2}$), determined on three different occasions in samples of aerated Uppsala tap-water which was used as feed-water in the tests in experiments I—VIII (Figs. 3—7 and Table 3).

compartment may affect behaviour. Thus a too small compartment provokes excitation. In a very narrow chamber the parr show obvious unease, which may be because of tactile sensations and the inability to turn and move freely. GEYER and MANN (1939; according to FRY in BROWN, 1957, p. 30) state that a test chamber for respiration trials with perch should be at least three times as big as the fish. The question of a connection between the size of the test chamber and the behaviour of the salmon parr has not been closely examined but in the compartment size used here second-summer parr of the Atlantic Salmon take resting positions on the bottom and usually ventilate

Table 2. Tests of pH and oxygen content in aerated Uppsala water before and after passing through the fish chamber sketched in Fig. 2.

Date 1966/67	Before test chamber		After test chamber	
	pH	O_2 mg/l	pH	O_2 mg/l
28/11	6.78	9.35	6.78	9.35
13/12	6.06	9.40	6.08	9.35
19/1	6.80	3.00	6.80	3.00
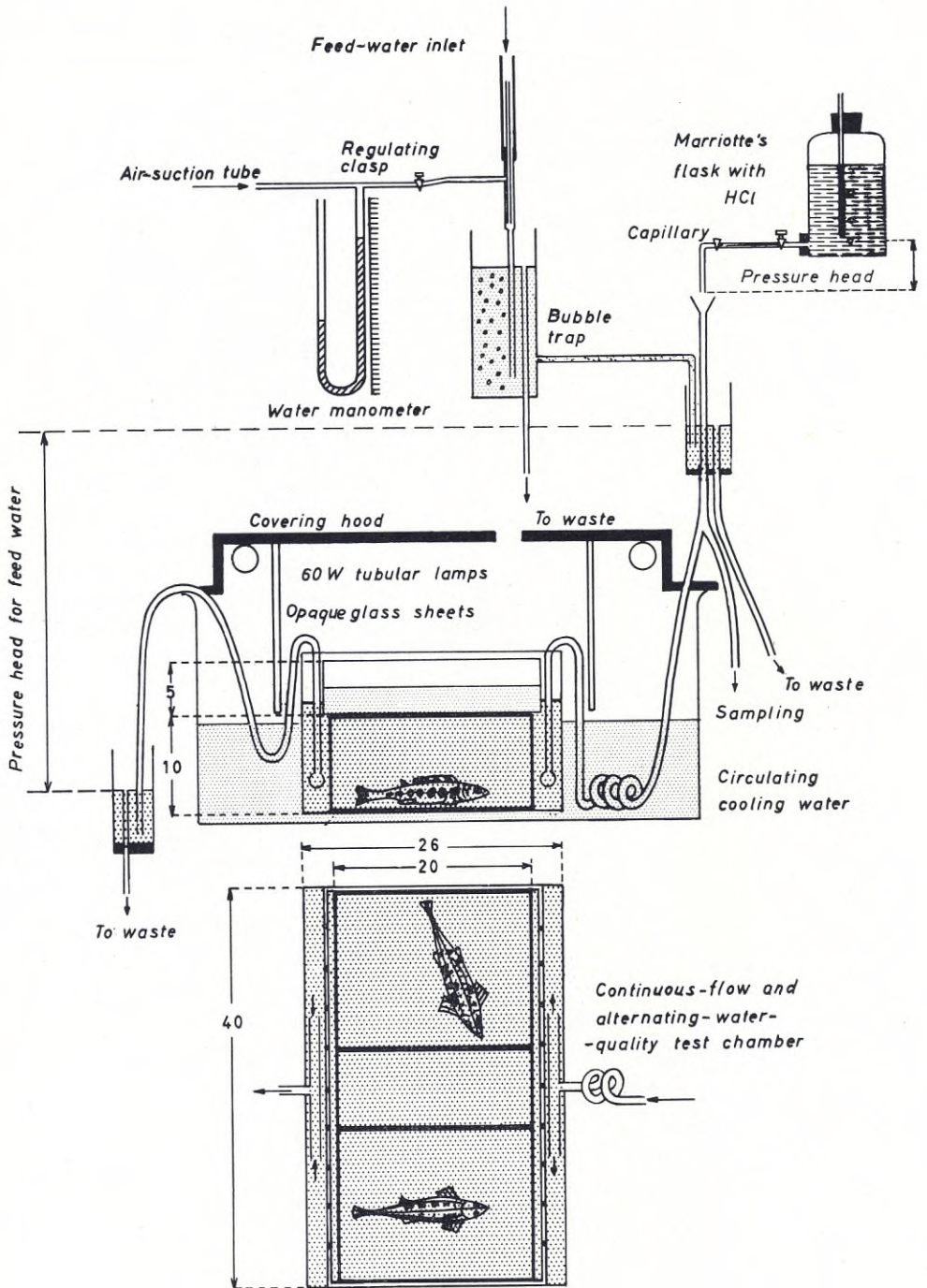
Fig. 2. Sketch of the continuous-flow and alternating-environment test chamber. Some details are not drawn to scale. The measurements are given in centimeters.
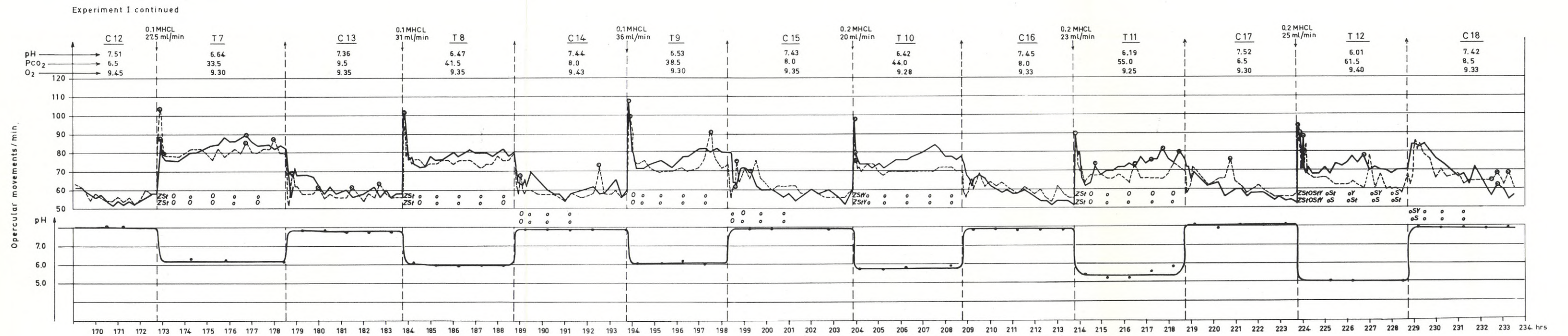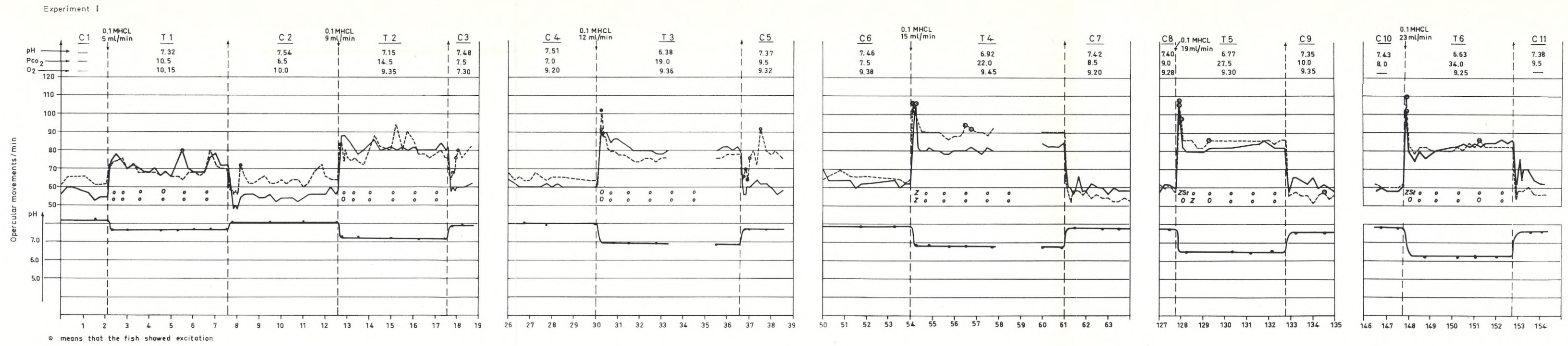
Fig. 3. Experiment I, according to Table 3. The behaviour and ventilation of two second-summer parr (*Salmo salar*) tested in parallel in the course of 10 days for consecutive 5-hour periods with pH and pCO$_2$ deviating more and more from those of the intervening control periods with the pre-acclimatization water quality (pH about 7.4, pCO$_2$ about 8 mm Hg, and O$_2$ about 9.4 mg/l in aerated Uppsala tap-water). The symbols designating the predominant behaviour are explained in Table 4.

with a frequency of around 60 opercular movements per minute. The observations were made through a narrow crack in the lid of a protective cover when the fish were indirectly illuminated by two tubular lamps (each 60 watts).

The chamber was immersed in a tank of circulating water. The temperature variation during any one test period never exceeded 0.1°C. A constant pressure head was maintained. For all tests the drainage was 1.5 l/min, which corresponds to an average rate of flow in all parts of the chamber of about 0.0625 cm/second. The water is led into the fish compartments via a transversely placed outer compartment, separated from the test compartments by a perforated plate (nine rows of seven holes with diameters of 5 mm), and drained via a similarly arranged outer compartment. This gives a satisfactorily even distribution of flow.

The addition of acid from a MARRIOTTE flask via a horizontal capillary was regulated according to POISEUILLE's law. The time taken to attain new, constant, environmental conditions in the test chamber after an alteration of the acid admixture in the inlet was about 20 minutes. Samples of the continuous side-stream (25 ml/min) of watery solution near the inlet of the fish chamber were taken, as a rule, once an hour during the test periods and at longer intervals during the control periods. The environmental data presented in Figs. 3—8 are based on samples taken at this place.

III. Observed reactions and the presentation of experiments

Provided that it encounters the pre-acclimatization water quality, the parr remains quite still in its compartment after being transferred to the test chamber. In a couple of hours it seems to be well acquainted with the new environment and assumes a fixed resting position on the bottom. The ventilation is even at a comparatively low rate. The fins and the entire body are kept almost immobile. After the restlessness of more or less short duration earlier provoked during a test period, it also reverts to this quiescent appearance when confronted anew with the acclimatization conditions during intermediate- and post-control periods.

This behaviour bears a considerable resemblance to that found in nature and in the storing aquaria in the laboratory or in the large troughs at the breeding station. Young salmonids, viz. the trout and the Atlantic salmon, stay at the bottom for long periods orientated against the current. Occasionally they make brief and quick excursions, particularly on being fed (cf. LINDROTH, 1955; KALLEBERG, 1958). This resting on the bottom, in the centre of a "territory" which is defended against intruders, is energy-saving in the constant flow.
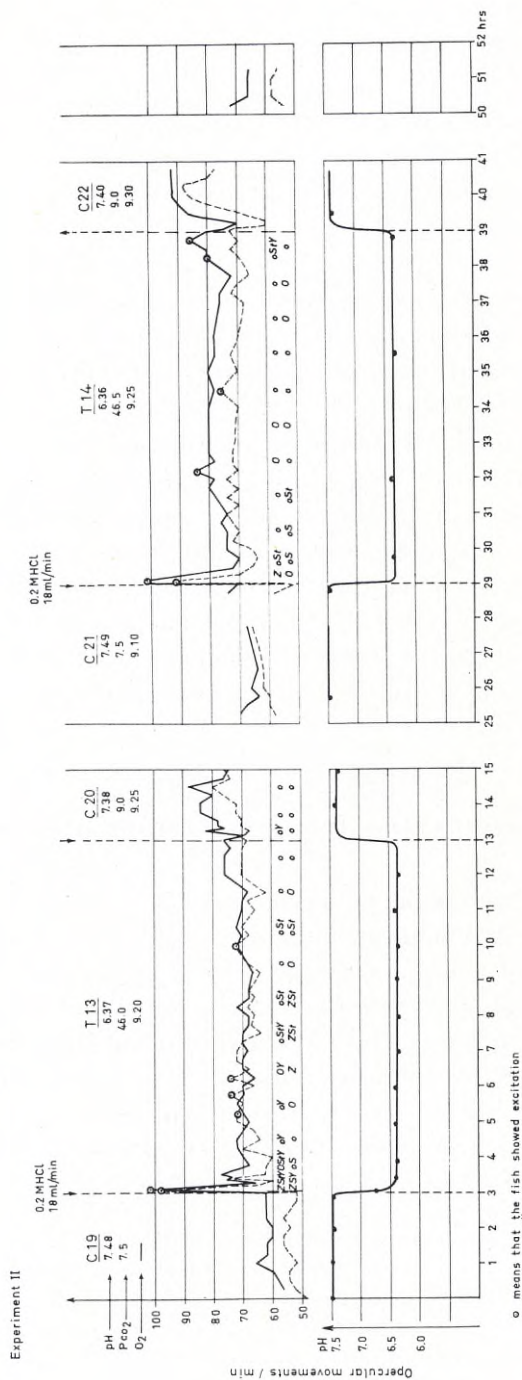
Fig. 4. Experiment II (see Table 3). Behaviour and ventilation of two salmon parr tested in parallel on sudden changes of pH and pCO_2 and in the course of two similarly arranged 10-hour periods with constantly lower pH and higher pCO_2, viz. 6.4 and 46 mm Hg, than during pre- and post-control periods in aerated Uppsala water. The symbols used for the prevalent behaviour are explained in Table 4. The O_2 content was kept constant at about 9.3 mg/l.
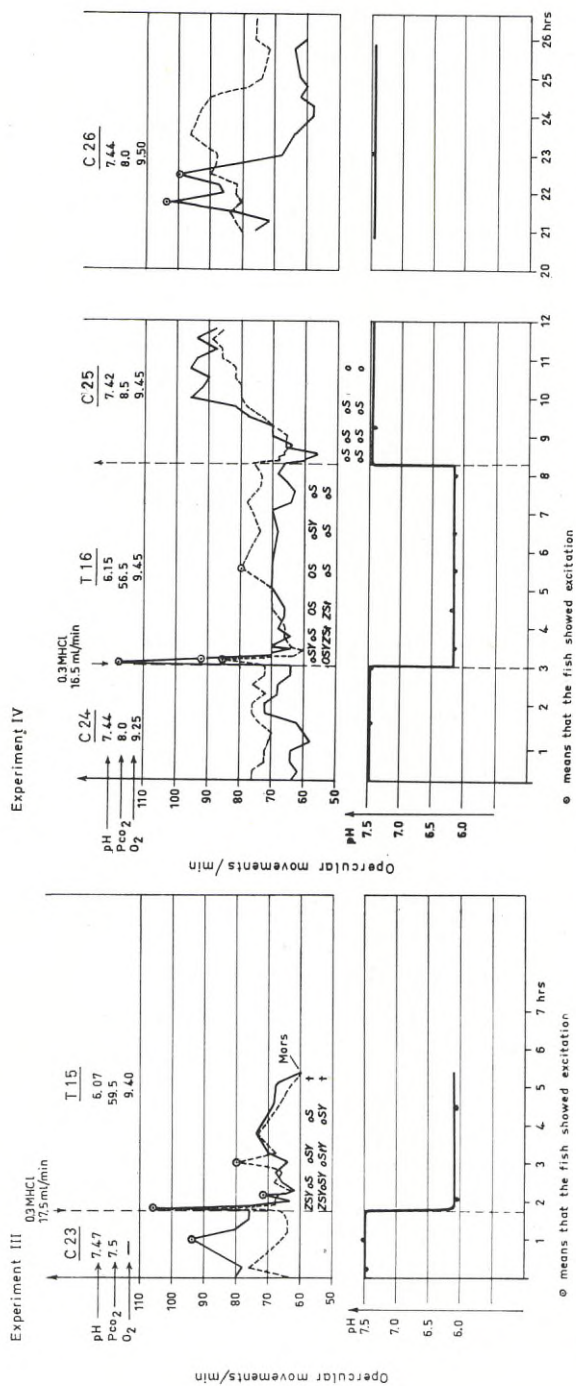
Fig. 5. Experiment III and IV (see Table 3). The behaviour and ventilation of four salmon parr at extremely low pH and high pCO_2 values, viz. about 6.0 and 60 mm Hg respectively in aerated Uppsala water. For the explanation of the symbols of behaviour, see Table 4.

This immobile behaviour under control conditions in the test apparatus is a suitable prerequisite for an ethological and physiological analysis of the performance of the parr upon environmental stimulation provoked by chemical agents, *viz.* the respiratory gases and pH. This basis of reference facilitates quantitative estimates of the intensity factors of locomotor and ventilatory responses as a function of time. The mutual connections of these responses and the environmental changes are studied here. The question of external and/or internal stimulation, released reflexes and other regulatory mechanisms cannot be discussed with any certainty on the basis of the present results. It will be discussed in two future papers.

Some typical and prevalent behaviour patterns in the *emergency reaction* chain provoked in certain experiments have been described in Table 4 (cf. HÖGLUND, 1961, pp. 90 ff. and 102 ff.). Certain symbols have been suggested and have been used in the diagrams in order to visualize the predominant behaviour in the course of the tests, with special regard to muscular activity.

A complete survey of the experiments performed is given in Table 3. The test fish were changed between each experiment (designated I—IX; see Table 3). With the exception of the experiments numbered III and VIII, no lasting effects on the fish were observed after the tests.

In the first series of experiments (I—IV in Table 3) the pH and pCO_2 were varied, while the oxygen content was kept constant at about 9.4 mg/l. This series covers 13 five-hour and 2 ten-hour test periods and one additional test period (T1—T16 in Figs. 3—5). The parr were tested in Uppsala water at consecutively reduced pH and increased pCO_2. Between tests they were kept undisturbed in their compartments within the test chamber for at least 5 hours under control conditions. The detailed planning and the results of the experiments are shown in the diagrams.

A second series was run in Uppsala water (experiments V—VI in Table 3). In this series both pH/pCO_2 and oxygen content were varied simultaneously. The water quality used and the results arrived at in the combination tests are shown in Fig. 6.

In a third series performed in Uppsala water (experiments VII—VIII in Table 3) the oxygen content was varied, while the pH and pCO_2 were kept constant at 7.4—7.5 and 8.0—8.5 mm Hg, respectively. The oxygen content was altered from approx. 9.3 to 6.0, 3.0, or 0.9 mg/l. (Results in Fig. 7.)

Finally, one experiment (IX in Table 3) was performed with two second-summer parr in River Dalälven water with a quite different ionic composition from that of aerated Uppsala water (for the quality, see Table 1). In these tests the pH was varied, while the oxygen content was kept at a constant high level. The results are shown in Fig. 8.
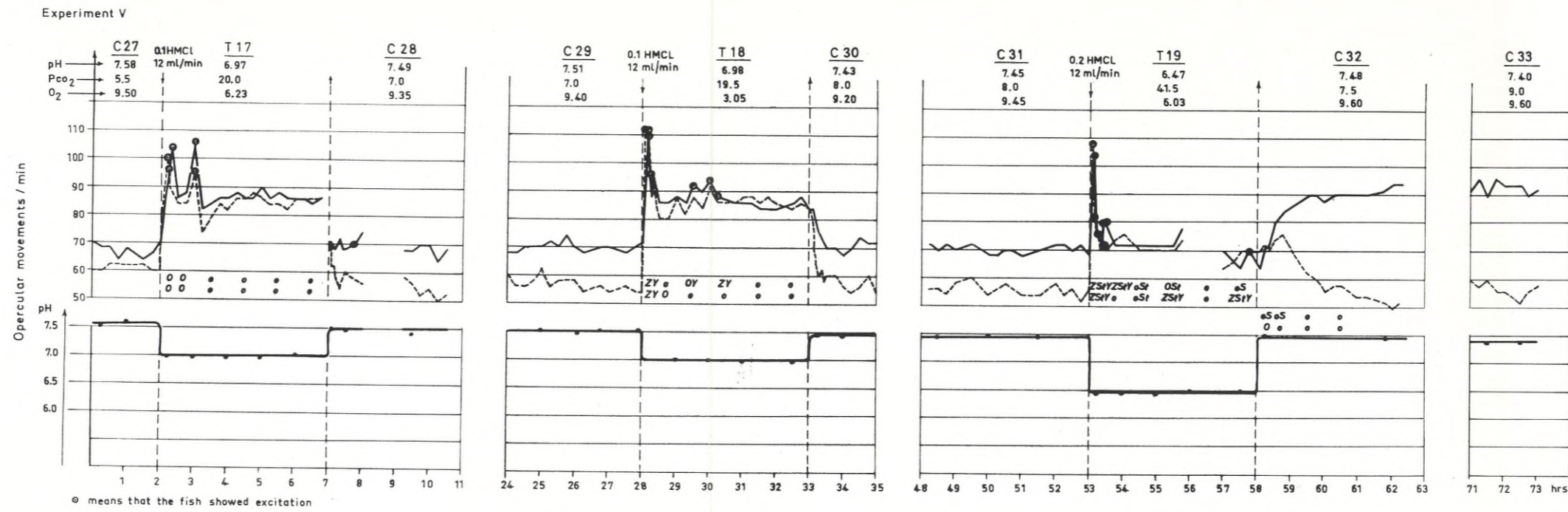
Fig. 6. Experiment V and VI (see Table 3). The behaviour and ventilation of four salmon parr on sudden changes of pH/pCO₂ and O₂ in Uppsala water. The symbols of behaviour are explained in Table 4.
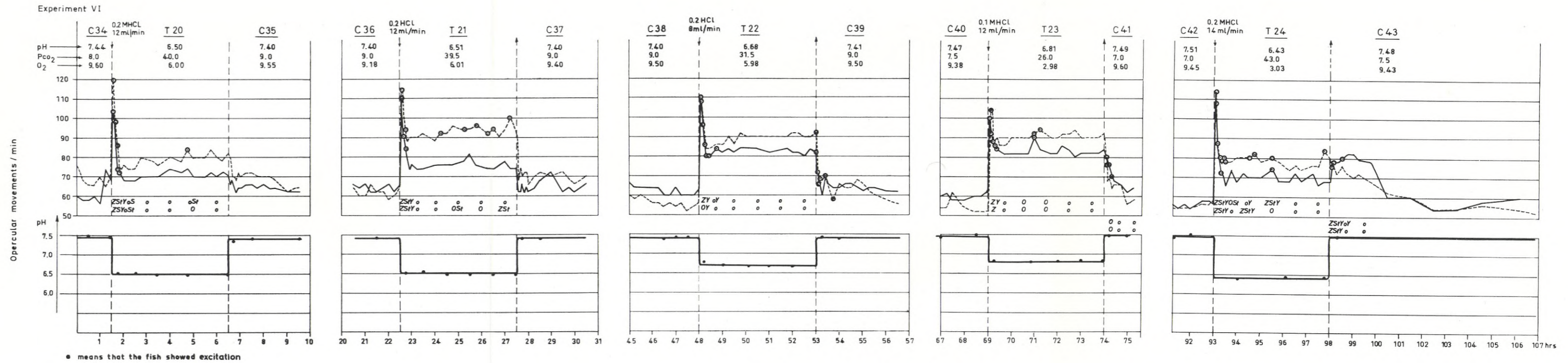
Table 3. Survey of the experiments performed.

Feed water and the actual value of the parameter (s) kept constant	Limits for the variation of parameter (s) changing from one test period to another	Number and duration of experiments in hours	Designation of test and control periods	Date 1966/67 in chronological order	Size of test fish		Results in Fig.
					Weight in g	Length in mm	
Aerated Uppsala water	pH: 7.51—6.08	I 235	T1 —T12 C1 —C18	23/11— —3/12	19 27	124 138	3
O₂=9.4 mg/l	pCO₂: 7.0— —61.5 mm Hg	II 52	T13—T14 C19—C22	8/12— —10/12	39 43	158 162	4
		III 5	T15 C23	13/12	41 31	154 145	5
		IV 28	T16 C24—C26	15/12— —16/12	32 24	140 128	5
Aerated Uppsala water	pH: 7.59—6.43 pCO₂: 5.5— —43.0 mm Hg	V 75	T17—T19 C27—C32	10/1— —13/1	31 42	150 165	6
	O₂: 9.4, 6.0 and 3.0 mg/l	VI 107	T20—T24 C33—C43	16/1— —20/1	33 40	148 156	6
Aerated Uppsala water pH=7.46 pCO₂= =7.5 mm Hg	O₂: 9.5, 6.0, 2.9, 0.9 mg/l	VII 32	T25—T26 C44—C47	30/1— —31/1	— —	—[1] —[1]	7
		VIII 4 ½	T27, C48	9/5	29.4 34.7	155[2] 165[2]	7
River Dalälven water O₂=10.8— —11.6 mg/l	pH: 6.4—3.6 pCO₂: 1.6— —3.3 mm Hg	IX 53 ½	T28—T33 C49—C57	15/11— —17/11	31.0 32.5	160 165	8

[1] Approx. the same size as other fish tested.
[2] Parr of sea trout; in all other experiments parr of the Atlantic salmon were used.

The experimental material described so far is based on observations of 16 second-summer salmon parr and two second-summer sea-trout parr (experiment VIII). Sixteen parr were tested in Uppsala water and two parr in River Dalälven water (experiment IX). This material was supplemented in experiments I—VIII with observations of first-summer salmon parr (eight in all), which were kept in the central compartment of the test chamber. It was further supplemented by observations of a number of perch and roach (small specimens of *Perca fluviatilus* and *Leuciscus rutilus*), which stayed in an identical chamber, connected to and supplied by the fluids from the test chamber proper. All these observations were made in Uppsala water. They correspond in many respects to the results of the main experiments. Perch and, to an even greater degree, roach show more tolerance to extreme pH/pCO₂ and O₂ conditions than do second-summer salmon parr. One-year-old salmon also pass through the behavioural phases noted in Table 4,
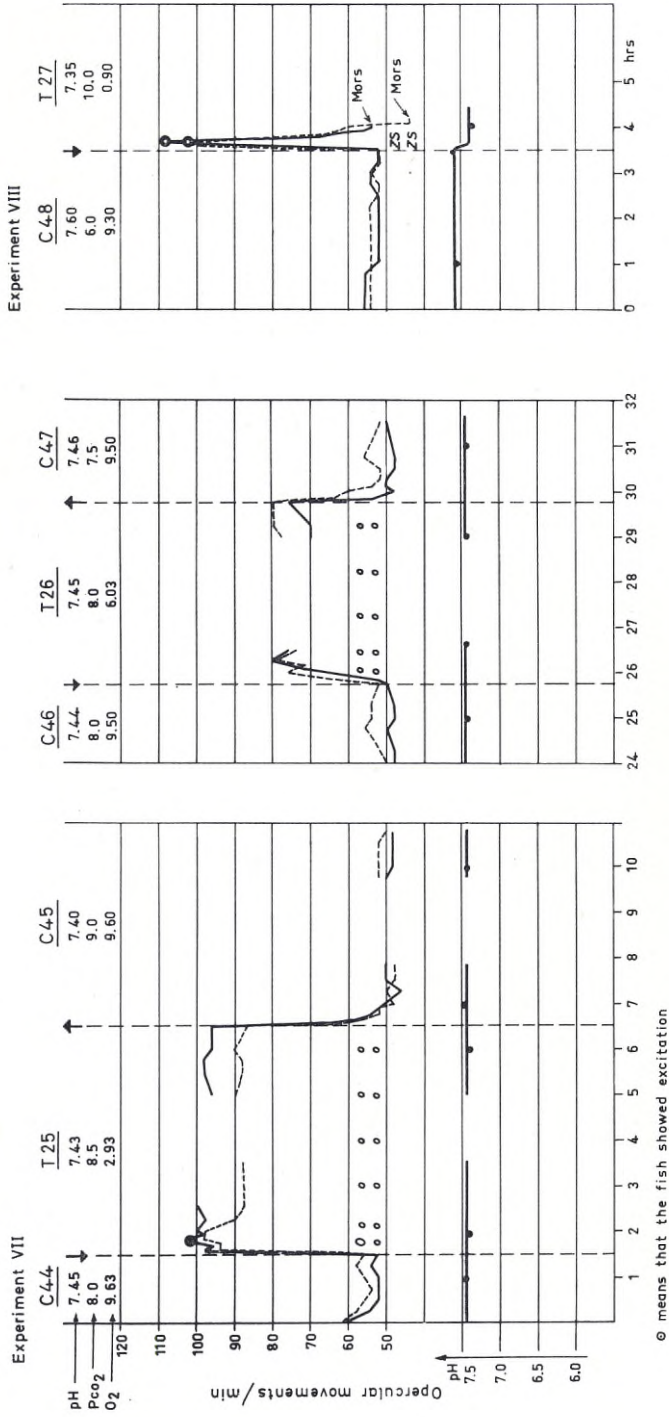
Fig. 7. Experiment VII and VIII (see Table 3). The behaviour and ventilation of two salmon parr (experiment VII) and two sea-trout parr (experiment VIII) on sudden changes in and with various oxygen contents of Uppsala water. As will be seen from the symbols of behaviour (cf. Table 4), the parr did not leave their resting positions in the course of experiment VII.
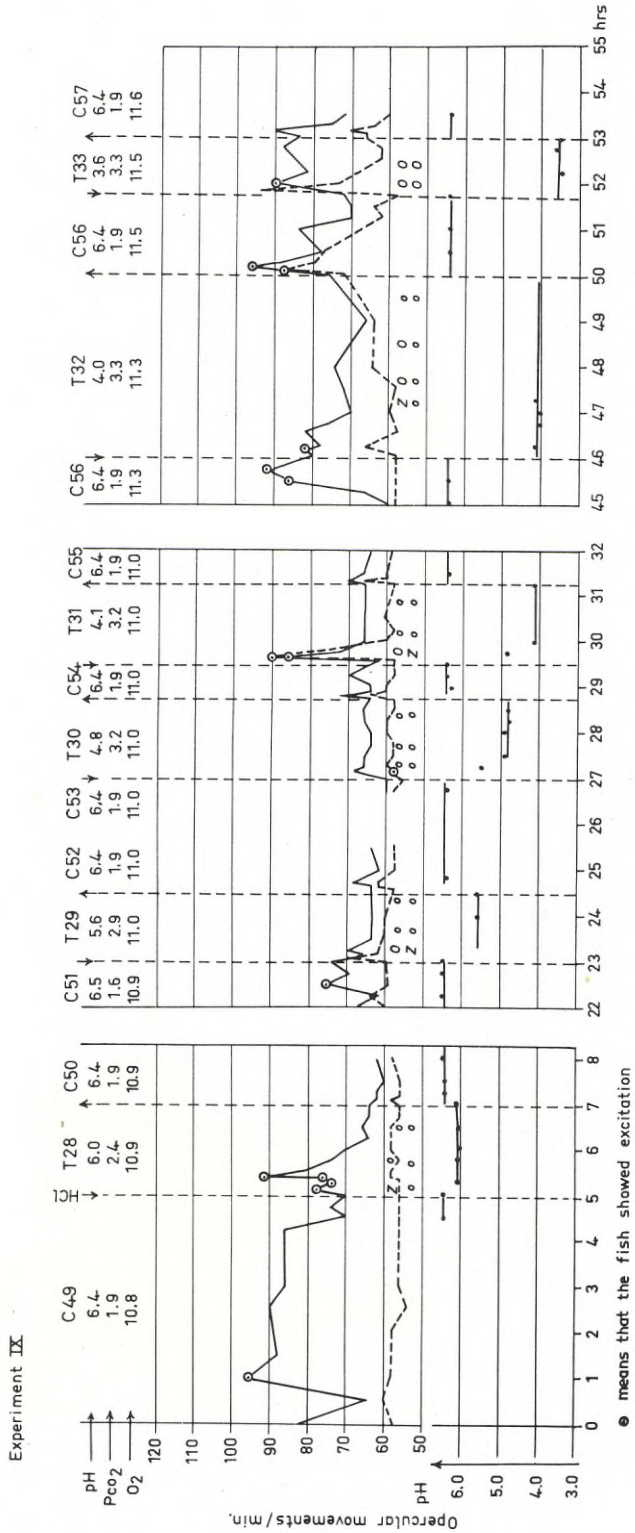
Fig. 8. Experiment IX, according to Table 4. The behaviour and ventilation of two salmon parr on sudden changes of pH in River Dalälven water and during a series of 5-hour periods with constant pH but consecutively increasing deviation from the pre- and post-control conditions. The changes of pH in River Dalälven water are not accompanied with any considerable pCO_2 fluctuations, as in the case of Uppsala water, which is much richer in bicarbonate (Table 1, Figs. 1, 3—7, and 9). One of the parr tested in parallel in this experiment behaved calmly. It was acclimatized to the test chamber for a day and a night, but the other one, wich behaved more inconstantly, was transferred to its compartment just before starting the recordings. The symbols of behaviour are explained in Table 4.

somewhat faster than older parr. They would appear to be rather more sensitive than larger specimens. However, this point calls, for closer investigation.

IV. Results and discussion of behaviour

A. The effects of decreased pH and increased pCO_2 in aerated Uppsala water

On transition to CO_2-tension-raising pH/pCO_2 in the otherwise unchanged respiratory medium of aerated Uppsala water, the parr display the behavioural reactions summarized in Table 4. When a parr in Phase 1 of Table 4 is confronted with increased pCO_2 and lowered pH, the ventilation and the movement of the pectoral fins increase (Phase 2). In a brief transitory phase between Phases 2 and 3 of Table 4 the fish often moves a short distance (a few centimetres) along the bottom. This is achieved by paddling movements of the pectoral fins. It was suggested by Dr. T. G. NORTHCOTE, of British Columbia, (personal communication) that the alternating movements of the pectoral fins may play a rôle in the parr's detecting and swimming away from unfavourable carbon-dioxide tensions, as shown by HÖGLUND (1961) in steep "concentration gradients". The *directive avoidance* response to carbon dioxide demonstrated with the aid of the fluviarium technique may accordingly involve the alternate tasting of water from each side of the head by means of a paired receptor system, located, for example, in the gills. The idea is attractive but needs further investigation. In the present apparatus the parr leaves its bottom position and, when given no choice, swims in a more or less restless fashion around the entire compartment (Phase 3). Phase 3 is characterized by various swimming activities. The intensity of locomotion is not easily described in quantitative terms but is always most pronounced in the initial stage of a test period. Then brief rushes with relatively calm swimming at minute pH/pCO_2 deviations is replaced in more acute pH/pCO_2-stress conditions by highly erratic or even panic behaviour. This becomes in extreme cases so lively that the fish repeatedly collides with the walls and the lid. The smaller the deviations in pH/pCO_2 the fish is exposed to, the sooner it returns to a resting position. The initial erratic stage of behaviour is accompanied with a very striking *initial peak on the ventilatory curve*. This is clearly demonstrated in most test periods by the diagrams in Figs. 3—6. When it has taken up a resting position once more, the parr remains on the whole immobile at the bottom for the rest of a test period. To what extent this is due to possible anaesthesia ("carbon-dioxide depression of the locomotor activities"), regulation of a

presumed acidosis and/or adjustment of external reception mechanisms will be discussed in more detail later on.

An ethological analysis of the behavioural reactions in the trial series, T1—12 in Fig. 3 and T13—16 in Figs. 4—5, gives rise to the following conclusions. A relatively small deviation from the acclimatization conditions provokes most clearly the behavioural phases described in Table 5. With a greater deviation from the acclimatization conditions to pH 6.8 and less ($pCO_2 > 26$ mm Hg), the behavioural changes occur faster. Phase 1 leads more directly over to Phase 4 (surface-breathing attempts) or Phase 5 (difficulties in keeping the body in a normal position). An extreme pH/pCO_2 deviation leads even more directly to overturning tendencies (Phase 6—8 in Table 4), when the fish lies overturned or shows a tendency to overturn. By the end of the test period (see T12, T16, and T19 in Figs. 3—5, the fish has become so deeply anaesthetized by CO_2 that this effect lasts on into the following control period, e.g. C18, C25, and C32 in Figs. 3, 5, and 6. The time required for the fish to re-acquire a normal position is a measure of the pH/pCO_2 effect, viz. the degree of anaesthesia established during and carried over from the previous test period. The transition from overturned to normal position proceeds fairly directly without any transient excitation phase.

B. The effects of oxygen depletion in aerated Uppsala water

In the tests with a moderate oxygen depletion during the test period (down to 3 mg O_2/l in experiment VII in Table 3; T25—26 in Fig. 7) there were *no behavioural changes at all* of the kind demonstrated in the pH/pCO_2 deviation tests. So long as the oxygen content exceeds a minimum of 3 mg/l, the fish assume a bottom position during the entire test period (at least 5 hours). They are immobile, as under the pre-control conditions. The behaviour does not change at all, either on transition from control to test period or vice versa. The ventilation frequency — without any initial peak on the ventilation curve — increases directly with a lower oxygen content in the water (cf. Fig. 7 and LINDROTH, 1949). An extreme oxygen reduction (to less than 1 mg/l in T27) provokes in the fish an immediate, transient and extreme restlessness, combined with maximum ventilation. They turn over and soon die on account of lack of oxygen.

C. Combined effects of oxygen depletion and changed pH/pCO_2 in aerated Uppsala water

In the combination tests in Fig. 6 a fairly large individual variation has been noted. The reactions and behavioural changes predominate that occur in the pH/pCO_2 tests performed at the unvaried oxygen content of about 9.3 mg/l. At the beginning of T24 the fish show a tendency to overturn but

Table 4. Behavioural reactions induced in second-summer parr in the pH/pCO$_2$ tests. The phase numbers show the prevailing order of the behavioural changes after pH reduction and pCO$_2$ rise in the aerated Uppsala tap-water flowing through the test chamber. Combinations of the behavioural categories noted below also occur.

Phase	Symbol	Prevalent behaviour with special regard to muscular exertion
1.	o	The salmon fry remains immobile in a resting position on the bottom of the test chamber. It stands supported by the pectoral and pelvic fins.
2.	0	The fish is still resting on the bottom but exhibits distinct body movements and, above all, undulating pectoral fin movements.
3.	Z	The fish swims about in the test compartment.
4.	Y	The fish remains relatively constantly with its front tip near to or on the under side of the glass lid (cf. the surface-breathing reflex). It then assumes a more or less oblique angle to the horizontal.
5.	St	The fish is unable to keep its body in the normal position and shows a tendency to overturn.
6.	S	The fish displays opercular movements and lies still on its side on the bottom of the tank.
7.	Sy	The fish displays opercular movements and floats, belly up, on the under side of the glass lid.
8.	† or *mors*	The fish shows no signs of life.

later on they stay still at the bottom in a normal position. This behaviour is probably to a large extent the effect of "carbon-dioxide depression". Initially during the following C43 an abnormally high muscular activity is displayed for about 15 minutes. The parr swim about, make surface-breathing attempts and also show rolling. These reactions are possibly due to the fish giving off CO$_2$ more easily on return to the control conditions. The fish pass through an obvious excitation phase, later superseded by a calm behaviour that is normal under control conditions. Similar behavioural changes also appear in conjunction with the transition from test to control conditions in C14—15, C20, and C41. These are, however, not so marked as during the beginning of C43.

The main conclusion of the combination experiment is that a reduction of the oxygen content along with lowered pH and raised carbon-dioxide tension in Uppsala water accentuates the behavioural reactions provoked by pH/pCO$_2$ when the O$_2$ is kept constant at about 9.3 mg/l. As will be shown in experiment IX in Fig. 8, — this is essentially due to increased pCO$_2$ and not to decreased pH (see the next subsection).

D. The effects of lowered pH in River Dalälven water

One of the two parr observed in River Dalälven water was accustomed to the test chamber for about 24 hours. The other one, which showed a higher ventilation rate and behaved less calmly, was placed in its compartment a

short while before the start of the recording. As regarded the first-mentioned parr, it seemed to be, on the whole, unaffected by the lowering of the pH until a certain limit of about pH=4.0. Below that value it gave rathers light responses on the introduction of acid water into the test chamber (see Fig. 8).

This supports the conclusion by HöGLUND (1961) that fish respond to environmental CO_2 separately from the accompanying pH. Hydrogen ions do not provoke behavioural reactions when the environmental pH in River Dalälven water is decreased from about 6.5 to 4.0. Accordingly, acidity and carbon dioxide are either perceived by different receptor systems or by the same receptors at essentially different thresholds. These conclusions are also true as regards ventilatory responses. This means that CO_2 rather than pH may exert an "immediate environmental stress" or working stress on account of the increased ventilation provided by more water passing over the gill membranes (HUGHES, 1961, 1963).

V. Results and discussion, mainly regarding ventilation

A. The effects of pH/pCO₂ changes in aerated Uppsala water

1. *Comments on experiment I in Fig. 3*

(a) *Initially CO_2-provoked excitation and the initial ventilation peak.* In experiment I in Fig. 3 two salmon parr were observed for nearly 10 days and nights. It is clear from the figure that the reduction of pH and the accompanying increase of CO_2 tension in aerated Uppsala water immediately gave rise to a powerful hyperventilation response. It was temporary, however, and shows up on the ventilation graph as an initial peak of more than 100 opercular movements a minute. It is then followed in most cases (T2—12) by a continued hyperventilation at a fairly constant level around 80, as compared with the normal value for the intervening control periods of around 60. This *initial ventilatory peak* is found for all test periods, with the exception of T1 and possibly T2. Apart from the initial peak, the course of the ventilation curve shows better agreement with the pH/pCO₂ conditions than with the fishes' behaviour. Despite the otherwise immobile behaviour the fish continue to hyperventilate. This is accordingly a respiratory stress which is probably mainly due to the carbon dioxide tension in the water.

(b) *CO_2-provoked depression of the ventilatory rate.* In T6—11 the ventilation rate decreases rapidly after the initial peak and reaches values under 80, after which it slowly increases once more. The dip in the curve that appears in this way is probably due to CO_2 anaesthesia, localized to the

central nervous system. This interpretation is supported by the fact that the "dip" is deepened when the pH reduction or CO_2-tension rise is increased in T6, T7, T8, etc., up to and including T11 in Fig. 3. On return to control conditions in C2—9 after T2—5, the course of the curve suggests an "undershoot reaction". This transient reduction in ventilation rate resembles an apnoea state, which may be due to previous over-ventilation. It further suggests a feed-back mechanism, maintaining *internal homeostasis*. Finally the ventilation frequency generally reaches a stable level near 60. This restoration of the pre-acclimatization normal ventilation level is steadily more delayed during C11—17. This may also be due to various degress of CO_2 anaesthesia, established during previous test periods, while the delay is probably due to the degree of pH/pCO_2 deviation in the medium.

In T12 and the subsequent C18, the ventilation curve follows a course which differs greatly from the picture hitherto described. The frequency in T12 never reaches 80 after the peak. Nor is there in C18 the otherwise normal reduction in the ventilation rate to about 60. The ventilation rate increases instead at the beginning. It also shows other irregularities.

The anomalies imply more deep-seated disturbances in the central nervous system than in those cases in which the pH/pCO_2 deviations were smaller. The high CO_2 tension during T12 (about 60 mm/Hg) is nearly fatal. This agrees very well with the CO_2 tensions given as critical for *Homo Sapiens* (see, *e.g.,* GUYTON, 1956, Fig. 333, on p. 474) and with reports as early as REUSS (1909, 1910; HÖGLUND, 1961, Table 14, on p. 107) regarding *Salmo iridaeus* and *Salmo trutta*. The results of T15 in Fig. 5 also show that the boundary between reversible anaesthesia and fatal CO_2 tensions for second-summer *Salmo salar* under the experimental conditions used here lies around 60 mm Hg at pH 6.0—6.1. This presumable anaesthetic effect of a high external pCO_2 upon the locomotor and ventilatory action of the fish will be called *CO_2-provoked depression*.

2. *Comments on experiment II in Fig. 4*

(a) *Reactions on repeated pH/pCO_2 stimulation.* T13 and T14 in experiment II in Fig. 4 are two ten-hour periods with pHs of about 6.35 and PCO_2s of about 46 mm Hg, differing identically from the intervening control conditions in C20 and C21. The water qualities during these test periods resembled those in T10 in Fig. 3 (pH=6.4 and pCO_2=44 mm Hg). The experiment was intended to illustrate (1) the importance of a longer contact period than 5 hours, and (2) the effects of a pH/pCO_2 stress repeated under the same conditions as demonstrated by the ventilatory and locomotor responses provoked.

During the first 8 hours of T13, the parr displays some difficulties in

keeping its normal position. This is interpreted as being mainly the effect of CO_2 anaesthesia or CO_2 depression. The behaviour becomes more normal for the concluding 2 hours. In T14 the tendency to overturn is less frequent and occurs only during the first 3 hours.

(b) *Possible regulation and adaptation.* The results imply that the environmental conditions affect the young fish less at the end of the two trial periods. They are apparently also less affected during the latter period. This implies some kind of adjustment, *viz.* regulation or adaptation. Toward the end of T13 the frequency level rises slightly and is higer throughout in T14 than in T13. In T14 it reaches its highest level at the middle of the period, sinks thereafter and rises again for one of the two parr tested simultaneously, as appears at the end of the period, due to swimming activity. Whether a presumable compensatory ability of the parr to adjust themselves to the actual environment is sufficient to enable them to survive similarly critical conditions for any length of time is still open to question. Perhaps the physiological compensatory capacity is limited with regard to time. It may be so strenuous that the fish can tolerate adverse water quality of the present kind only for a transitional period. It would appear, anyhow, that the strain is less if the fish are introduced gradually to increasingly different pH/pCO_2 conditions, as compared with the pre-acclimatization, as happened in the long series of tests performed with the same fish in experiment I. A long-term experiment was therefore preliminarily made with three second-summer salmon parr. It was run in aerated Uppsala water from 2 February to 26 March, 1968, and the water quality was kept constant within the following ranges in accordance with daily measurements, viz. pH 6.6—6.8 (corresponding to a pCO_2 of around 30 mm Hg), O_2 10.0—10.4 mg/l, and temperature 8—9°C.

The reactions displayed during this considerably long period of contact with essentially less stressful conditions, as regards pH/pCO_2 than in experiment II do not, however, give a more definite answer to the question of long-term adaptation, because the three speciments tested showed quite diverse reactions. One fish died after 12 days. Before that this parr displayed a very pronounced hyperventilation. Especially during the last 4 or 5 days, the rate was nearly 100 opercular movements a minute. Both the other parr survived the whole test, which was interrupted after 32 days. As compared with its reactions under control conditions, one of these displayed a significant hyperventilation, fluctuating during the course of the testing period between 80 and 95. Judging from the gradually decreasing ventilation rates, the third fish seemed to acclimatize quite well to the high carbon-dioxide tension. After 15—20 days the frequency levelled off at around 60. This

indicates the existence of a regulatory and/or adaptational mechanism.[1] It has since been strongly supported by a study made by HÖGLUND and BÖRJE-SON (unpublished MS.). In their study the occurrence of temporary extreme acidosis is manifested. An extreme decrease of pH in the blood is indicated in young salmon after confrontation with a water similar in quality to that used in the present long-term experiment. It is not accompanied with any very significant lactate concentration. Notwithstanding the unchangeable external CO_2 supersaturation in the respiratory fluid, the normal blood pH is then restored withtin a couple of hours. Furthermore, preliminary measurements also made on salmon parr with the aid of the *Astrup technique* (Radiometer Micro Electrode Unit, Copenhagen) even indicate a possible rise in blood pH, compared with normal values after the parr has been subjected to pH/pCO$_2$ stress over a period of more than a month in aerated Uppsala water at a pH of about 6.6—6.9.

(*c*) *Recovery from a provoked CO_2 depression.* The recovery from the presumable carbon-dioxide anaesthesia can be studied by following up ventilation and behaviour after the return to control conditions. During the initial phase of both C20 and C22 in Fig. 4 an increased ventilation rate appears. This is also seen in the curve from C18 in Fig. 3. Such a ventilation increase is typical after a fairly strong pH/pCO$_2$ depresison in aerated Upp-sala water, when the parr are confronted anew with the acclimatization water conditions. The revival comes about visibly via an excitation phase, which then in turn passes over into a stage with normal ventilation. After test periods with relatively small deviations, however, the fish show no changes in behaviour in conjunction with the return to the original control conditions in the apparatus. After greater deviations the behavioural changes at the beginning of the following control conditions resemble the phases of Table 4, although they appear in reverse order.

3. *Comments on experiments III and IV in Fig. 5*

(*a*) *The effects of a presumable adaptation on the reactions to critical CO_2 tensions.* The pH/pCO$_2$ conditions of T15 in experiment III in Fig. 5, *viz.* pH=6.0 and pCO$_2$ around 60 mm Hg, were chosen to correspond closely with those of T12 in experiment I in Fig. 3. As the conditions in T12 turned

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[1] This concept seems to be in good agreement with some observations made by SAUNDERS (1962). According to this author (p. 817), "The effects of moderate increases in ambient carbon dioxide on non-swimming fish may be temporary only. If the rise in the pCO$_2$ is slight to moderate, the percentage utilizations of oxygen at given respiratory volumes are at first depressed but may return, after 3 to 5 hours, to the levels they held before the pCO$_2$ was raised."

out to be almost lethal, and as the period comes when the fish has been repeatedly exposed to pH/pCO$_2$ stresses and if the tolerance to high hydrogen-ion concentration in combination with high carbon-dioxide tension is raised after a gradual adaptation, as suggested above, the salmon taken directly from the storage tank to the test in experiment III would probably not survive, because they would be confronted more abruptly with the same pH/pCO$_2$ environment as was found to be critical in T12.

In T15 the fish died after about 3 hours, with wide-open operculars. This is in contrast to the results of experiment I, in which both fishes survived T12, in spite of having gone without food for about 9 days in the test chamber, and notwithstanding the fact that the environmental conditions in T12 had differed somewhat more from those in T15. This supports the hypothesis of adaptation advanced here.

In order to further verify this, T16 in experiment IV (Fig. 5) was made. In its details it corresponded closely to T11 in experiment I, but the values differed somewhat less than in T15 in experiment III, viz. the pH was about 6.2 and the pCO$_2$ around 55 mm Hg.

The typical initial peak on the ventilation curve is found in all cases, *i.e.* in T11, T12, T15, and T16. The following parts of the curves show large and irregular fluctuations. The ventilation curve for T16 in Fig. 5 resembles most that for T12 in Fig. 3, but under the post-control conditions, C25 in Fig. 5 and C18 in Fig. 3, the cources of the curves, particularly at the beginning of the periods, are more at variance with one another. The curve in C25 climbs more slowly, and reaches higher values than in C18. This may be due to the hang-over of varying degrees of anaesthesia which was established during the preceding test periods. The fish lie on their sides for the whole of both T12 and T16. This condition lasts in both cases for a time in the corresponding post-control periods, but despite the fact the conditions in T16 are less extreme than in T12, the fish return to a more normal position earlier in C18 than in C25. In both cases the ventilation increases parallel to the "return to consciousness". The respiratory control is evidently more disturbed during T16 than during either T12 or T13. The course of the ventilation curve during C26 in Fig. 5 even implies more lasting disturbances. This is manifested primarily by a powerful hyperventilation, which is evident for long periods. The intermittent over-ventilation continues in this case up to about 12 hours after the return of pre-acclimatization conditions in the chamber. These results, too, support the theory that the fish possess a certain capacity for adjustment to low pH values in combination with high carbon-dioxide pressure. In experiment I the physiological adjustments obviously have time to develop through the gradual de-escalator arrangement of the pH conditions during the consecutive test periods which precede T12.

(b) *Lethal limits.* Judging by experiments I, III, and IV, the lethal limit for second-summer parr under present conditions lies somewhere between pH 6.20 and 6.15, corresponding to a carbon-dioxide tension of 55—60 mm Hg. This applies if, without previous conditioning, the fish are confronted with and kept for a 5-hour period under the stated pH/pCO_2 conditions in well-oxygenated Uppsala water. Gradual conditioning (as in experiment I) moves — under otherwise identical conditions and disregarding individual variations in tolerance — this limit to pH around 6.0, which in Uppsala water, with 325 mg HCO_3^-/l, corresponds to a carbon-dioxide pressure of just over 60 mm Hg.

4. *Concluding remarks*

What has emerged from experiments I—IV can be summarized as follows:

(1) Parr of the Atlantic salmon display immediately typical changes in behaviour and ventilation frequency, when exposed to distinct variations of pH and carbon-dioxide pressure in aerated Uppsala water (Table 1).

(2) The second-summer parr tested reacted characteristically also in the course of a 5—10-hour test period, when the pH/pCO_2 conditions were kept constantly different from their pre-acclimatization conditions, as regarded the water quality.

(3) In the environmental fluctuations, as regards pH and pCO_2, described here, the parr tested in parallel react mostly simultaneously and in the main display reactions according to a rather set pattern, as shown in Table 4.

(4) The intensity of reaction depends on the degree of pH/pCO_2 deviation.

(5) The environment-provoked reactions are dependent on the time factor.

(6) The parr show an increased tolerance to high carbon-dioxide pressure in combination with low pH values, after gradual adjustment and after a certain period of contact. Points (5) and (6) imply the existence of a regulatory capacity and/or adaptational mechanisms, but the change in the tolerance or incipient lethal limit seems to be relatively insignificant.

(7) The adaption (cf. p. 98) cannot yet be quantitatively expressed. In particular, the importance of the time factor, i.e. the effect of a longer period of adjustment on the degree of response, and the incidence of individual variations ought to be more closely investigated.

(8) The capacity for adaptation of second-summer Atlantic salmon parr is not sufficient for survival during a couple of hours' contact with extreme pH/pCO_2 conditions in aerated Uppsala water, i.e. beyond approximately 6.0 pH units and 60 mm Hg.

(9) The immediate response provoked by an external change of pH/pCO_2 to unnaturally high CO_2 tensions can be regarded as measure of the *exogenous stress* caused by the abiotic environment. This is most pronounced

at the confrontation with the new water quality and shortly afterwards. It implies a *respiratory distress* caused by CO_2 supersaturation in the respiratory medium, either directly by the stimulation of exterior receptors and/or by the action of CO_2 within the animal after a rapid diffusion into the blood-stream and before a new acid-base equilibrium is obtained to re-establish the normal blood pH. This may occur by the retention of blood bases and/or some other *compensatory mechanisms, e.g.* the transformation of CO_2 to bicarbonate.

(10) The early part of the contact period is characterized by an *initial peak* on the ventilation curve. This is partly due to excited swimming activity or restlessness (emergency behaviour) which constitutes a super-imposed *working or loading stress* (BRETT, 1958). *Internal homeostasis* is then very probably obtained. As these reactions and compensatory mechanisms all require energy, the physiological state may be regarded as an *internal regulatory homeostasis,* on account of the environmental conditions of stress (*sensu* FRY, 1947 and BRETT, 1958).

B. The effects of O_2 depletion

1. *Comments on experiments VII and VIII in Fig. 7*

(*a*) *Observations in experiment VII.* Changes in the oxygen content produce no behavioural changes in experiment VII. Both the salmon parr tested take up their typical resting positions and remain quiescent also during the transitions between periods C44—T25—C45 and C46—T26—C47. A reduction of the oxygen content from 9.3 mg/l in C44 to 3.0 mg/l in T25 produces an immediate increase in ventilation rate from below 60 to between 90 and 100 opercular movements a minute. A reduction from 9.3 mg O_2/l in C46 to 6.0 mg/l in T26 raises the ventilation rate from about 50 to between 70 and 80. So long as the oxygen content is unchanged during a period, the frequency remains largely constant. The ventilation rate reflects the oxygen depletion in the medium (see also Fig. 1 in LINDROTH, 1949, p. 50). As soon as water containing more than 9 mg O_2/l is introduced once more into the test apparatur, the ventilation goes back again to about 50. The reason for this decrease of the basic ventilation level in the post-control conditions has not yet been made clear. Hyperventilation during the tests, changes in the blood's capacity to take up oxygen, the discharge of erythrocytes from the spleen, etc., may be parts of the phenomenon.

(*b*) *The absence of "initial excitation and an initial peak on the ventilation curve".* A striking feature of the ventilation curve of experiment VII is the absence of "an initial peak" on the transition from high to low oxygen con-

tent, i.e. as long as the depletion is not critical enough to provoke an "emergency reaction" (cf. LINDROTH, 1947; HÖGLUND, 1961). This is most interesting in comparison with the experiment with raised pCO_2 (Figs. 3—6). This disparity may be attributed to the different behaviour following the changes of the two kinds of environmental parameters, but it may also a least partly be due to the different ability to perceive the ambient CO_2 and O_2 (HÖGLUND, 1961, pp. 135 ff.). This is not the right place to discuss in more detail the controversial and somewhat confusing views of the effects of increased CO_2 and decreased O_2 in the ambient water upon the respiratory functions of fish. The reader is referred to the discussions and references given by SAUNDERS (1962) and HUGHES (1963, 1964).

(c) *Experiment VIII*. In experiment VIII second-summer sea trout were employed, but experiments on salmon parr give the same results. An abrupt reduction from 9.3 mg O_2/l to less than 1 mg/l, combined with a pH reduction from 7.60 tod 7.35, very soon produced a powerful hyperventilation (maximum? cf. SAUNDERS, 1962) and the fish die shortly after, either with open or closed mouths.

2. *Concluding remarks*

The results arrived at in the oxygen-depletion tests performed in Uppsala water lead to the following conclusions.

(1) Under the present circumstances the ventilation frequency varies in parallel with the oxygen content in the medium. The greater the oxygen depletion, the greater the increase in the ventilation frequency. This is at least valid within the range 3.0—9.3 mg O_2/l.

(2) No apparent counterpart to the initial peak on the ventilation curve, a common occurrence in the pH/pCO_2 experiments, was found in the O_2-depletion tests.

(3) A reduction of the oxygen content to a manifestly critical level (under 1 mg O_2/l) provokes restless swimming behaviour of short duration, called an *emergency reaction* ("the restlessness on account of hard respiratory distress", according to LINDROTH, 1947). It contributes to brief and very powerful ventilation before the fish succumb.

C. The effects of simultaneous changes of pH/pCO₂ and O₂

1. *Conditions and the grouping of tests*

The combined effects of decreased pH, raised carbon-dioxide tension and lowered oxygen content on the ventilation of salmon parr kept in Uppsala water are demonstrated in Fig. 6. The basis for comparison for the following

discussion is given in Table 5. Within each group numbered 1—3 in this table the pH/pCO_2 conditions are identical, but the oxygen content is varied (9.3, 6.0, and 3.0 mg O_2/l respectively). The pH/pCO_2 values for the test periods in group 1 are, approx. 7.0/19, in group 2 6.75/28, and in group 3 6.45/42. In the additional "repeat-test" group (group 4), in which the oxygen content is kept at 6.0 mg/l during both T20 and T21, the pH/pCO_2 is 6.15/40.

2. Comments on experiment groups 1—3 in Table 6

In group 1 in Table 5 the ventilation curves are similar. The response pattern provoked in the pH/pCO_2-deviation experiments (see above) predominates. This also applies to the behavioural reactions. The oxygen content has thus less effect on the fishes' reactions than the pH/CO_2 in the ambient water. This supports the assumption that the response is not merely due to the lowered oxygenation of the blood presumably caused by the BOHR and ROOT effects after the diffusion of CO_2 over the gill lamella into the bloodstream. On the contrary, it seems more probable that CO_2 also has an action upon some unknown receptor system, which in turn influences the behaviour. To what extent the CO_2-provoked behaviour alone gives rise to the "initial peak" on the ventilation curve or to what extent this is due to external and/or internal stimulation more directly by the ambient CO_2 or by changes in the pH, CO_2 and/or O_2 in the blood is still an open question (cf., e.g., the discussion in HEYMANS and NEIL, 1958, pp. 188 ff., and FRY, 1957).

The hyperventilation and increased locomotor activity, which from the present experiments seems to be primarily induced by the supersaturation with CO_2 of the originally bicarbonate-rich Uppsala water, is accentuated by a simultaneous oxygen reduction. This applies to a greater extent if the oxygen content is reduced to 3.0 mg/l than when it is left at 6.0 mg O_2/l (T17 and T18 in Fig. 6 should be compared with T3 in Fig. 3).

To this can be added certain remarks from a comparison of the results of T18 in experiment V in Fig. 6 with those of T25 in experiment VII in Fig. 7. The oxygen content in both test periods is kept at about 3 mg/l, but the pH in T18 is 7.0, while in T25 the oxygen depletion is not combined with any deviation from the control conditions, as regards pH/pCO_2. The ventilation curves are clearly different in two ways. The "initial peak" of the ventilation curve in T18 is missing in T25. Further, in the later part of T18, the ventilation (after the "initial peak") lies at a lower level, i.e. around 85, than in T25, where it is nearly 100. This slowing-down effect, which is clearly demonstrated by a comparison of the ventilation curve in T18 and T25 must be attributed to the difference in the pH/pCO_2 conditions and may

Table 5. Survey of tests in studying the combined effects of environmental changes in pH, pCO_2, and O_2 content in Uppsala water upon the reactions of salmon parr.

Test-group no.	Test period in experiment no.	Oxygen content in mg/l	pH	pCO_2 in mm Hg	Results in Fig.
1.	T3 I	9.3	6.98	19.0	3
	T17 V	6.0	6.97	20.0	6
	T18 V	3.0	6.98	19.0	6
2.	T5 I	9.3	6.77	27.5	3
	T22 VI	6.0	6.68	31.5	6
	T23 VI	3.0	6.81	26.0	6
3.	T8 I	9.3	6.47	41.5	3
	T19 V	6.0	6.47	41.5	6
	T24 VI	3.0	6.43	43.0	6
4. "Repeat test"	T20 VI	6.0	6.50	40.0	6
	T21 VI	6.0	6.51	39.5	6

therefore be called a CO_2-*provoked depression*. However, as was pointed out above, it is impossible to decide whether and to what extent this is due to CO_2 anaesthesia, as the difference may also be due to an antagonistic stimulation by O_2 and pH/pCO_2 of receptors connected with blood vessels and/or the gills and as the the oxygen load of the blood is very probably affected (cf. BLACK, according to FRY, 1957, p. 18, Fig. 7).

In experiment group 2 (periods T5 in Fig. 3, T22 and T23 in Fig. 6) the courses of the ventilation curves are similar, but the behaviour and activity are reinforced and prolonged somewhat more pronouncedly after each oxygen reduction, as compared with the experiments in group 1. From tests T8 in Fig. 3 and T19 and T24 in Fig. 6, finally, it appears that the effect of oxygen reduction on the fishes' ventilation and behavioural response is still greater if the pH/pCO_2 deviation from the control conditions is increased to a pCO_2 of approx. 42 mm Hg. At this CO_2 tension, according to data from E. C. BLACK presented by FRY (1957, Fig. 7, on p. 18), the percentage of HbO_2 in the blood of *Salvelinus fontinalis* equilibrated at 15°C with air (pO_2 about 160 mm Hg) never exceeds 50 % (as the result of the ROOT effect). Judging from these data from the speckled char, the percentage of HbO_2 at a pCO_2 of 40 mm Hg and pO_2s corresponding to the external O_2 contents used in the present experiments, *viz.* about 6 and 3 mg O_2/l, would be about 45 % and 35 % respectively. The dissociation curves at pCO_2s of about 20 and 30 mm Hg — which are actual values in the present experiments — would probably not differ too much from the dissociation curve at a pCO_2 of 40 mm Hg. Probably none of these curves would reach above the level of about 75 % HbO_2 saturation, even with high O_2 contents in the

ambient water. This may explain, at least partly, the responses to lowered oxygen values observed in the present three groups of tests (see Table 5). The reactions are not more strikingly intensified because the response pattern characteristic of the environmental CO_2-stress conditions are predominant. The possible adaptation during the course of each experiment is then neglected.

3. Comments on the "repeat test" (T20 and T21) of experiment VI in Fig. 6

At the beginning of both T20 and T21 — representing two 5-hour periods with idenically different pH/pCO_2/O_2 conditions, separated by a 16-hour-long control period — the fish show a tendency to overturn. This state (cf. T9 and T10 in Fig. 3) lasts for about 1 hour in T20, but for about 30 minutes in T21. The ventilation rate is higher during T21 than during T20. This means that the CO_2 depression (p. 96) is less pronounced in the latter period. This result is in line with what has emerged in the pH/pCO_2-deviation experiments, viz. that the tolerance is somewhat greater on renewed contact with the pH/pCO_2 (or in this case pH/pCO_2/O_2) deviation in question. A moderate physiological adaptation is developed, presumably on account of the regulation of a new acid-base-equilibrium after the transformation of CO_2 to bicarbonate, which is then retained in a certain degree (HÖGLUND and BÖRJESSON, unpublished work).

4. Concluding remarks

(1) A reduction of the oxygen content in Uppsala water from 9.3 to 6.0 mg/l and from 9.3 to 3.0 mg/l lowers the motivation threshold for the provocation of the typical reaction pattern, which is also displayed by salmonid parr after a reduction of pH and an accordingly raised CO_2 supersaturation in Uppsala water if the oxygen content is kept constant throughout at 9.3 mg/l (section V, A). In the series of pH/pCO_2 deviation from the control conditions of 7.0/20, 6.8/27, and 6.45/42 (expressed in pH units and mm Hg) the lowering of the threshold caused by oxygen depletion becomes more obvious when the pH reduction is greater. This can hardly be explained by the lowered oxygen-holding capacity of the blood on account of the increased CO_2 tensions only.

(2) During a test period of 5 hours, with a constant lowered pH of 7.0 in Uppsala water, the threshold shift caused by the oxygen depletions produced is best seen in the "initial peak" on the ventilation curve (test group 1 in Table 5).

(3) If the pH in Uppsala water is kept constant at about 6.8 for a 5-hour test, the threshold shift does not show up so clearly in the shape of the ventilation curve. However, the shift appears more evidently as an increase of the fishes' behavioural activity during the initial stage of the test period, corresponding to the "initial peak" on the ventilation curve. This means that the initial peak — so to say — "covers" various degrees of locomotor and other behavioural activities (Table 4). See test group 2 in Table 5.

(4) With the pH kept constant in Uppsala water at about 6.45 for a 5-hour period, the threshold shift due to simultaneous oxygen reduction appears once more as variations in the shape of the ventilation curves (test group 3 in Table 5).

(5) Judging from the degree of CO_2 depression (cf. p. 97), the parr show a certain capacity for adaptation to unnaturally adverse $pH/pCO_2/O_2$ conditions in the ambient water (test group 4 in Table 5).

D. The effects of lowered pH in River Dalälven water

1. *Conditions*

It is a well-known fact that living cells and organisms can withstand large variations in the pH of the external medium. This is also true of fish (for the literature, see DOUDOROFF and KATZ, 1950; BISHAI, 1962; ERICHSEN JONES, 1962, p. 258; MARCHETTI, 1962; McKEE and WOLF, 1963; WOOD, 1968, p. 110). "Cell membranes are very impermeable to hydrogen ions because the positive charge on their external surface repels the positively charged ions. But the chief cause of the maintenance of body fluids in the region of neutrality, and of the ability of cells and organisms to tolerate changes in the external pH, is the possession by them of chemical *buffer* system . . ." (quoted from WOOD, 1968, p. 111). And according to ERICHSEN JONES (1962, p. 272): ". . . there is a fair amount of evidence suggesting that unionized substances enter the bodies of fish most readily. Freshwater fish normally swallow little or no water but have a dilute and copious urine. The elimination of much water is necessary, because the osmotic pressure of their body fluids is much higher than that of the surrounding medium; this results in considerable quantities of water entering the body, mainly through the gills . . ."

Accepting the ideas quoted above experiment IX was carried out. This was performed in River Dalälven water, with the aim of studying the reactions of salmon parr to sudden changes of pH in a feed-water with a considerably lower bicarbonate content than Uppsala tap-water. The results are shown in Fig. 8.

2. *Comments on experiment IX in Fig. 8*

As the changes of pH in River Dalälven water (cf. Table 1) are not accompanied by any considerable pCO_2 fluctuations, as compared with the tests carried out in Uppsala water, the following discussion refers mainly to the effects of pH *per se*. The fish were pre-acclimatized to River Dalälven water of the same quality as that used in the controls (C49—C57 in Fig. 8), i.e. pH about 6.4, pCO_2 less than 2 mm Hg, and O_2 about 11 mg/l. The addition of HCl produces a rapid decrease in pH but only a slight increase of pCO_2 in this unbuffered water (see T28—T33 in Fig. 8). With regard to the parr, which was pretty well pre-acclimatized to the test chamber (the curve of short dashes), a more pronounced "initial peak" on the ventilation curve does not appear until the pH is lowered to a level well below 5.0. This seems to be in agreement with the statement by DUODOROFF and KATZ (1950), that 5.0 is approximately the critical pH value for most species of fresh-water fish. According to ERICHSEN JONES (1962, p. 258): "Higher hydrogen-ion concentration apparently produces a coagulation of gill secretions and asphyxia, or may exert an astringent or corrosive effect upon the gill tissues with similar results." The rather inconstant reactions displayed in experiment IX in C56, T32, C56, T33, and C57 can probably be explained along these lines as the result of persisting damage to the gill lamella. It is interesting in this connection to note that Atlantic salmon parr show avoidance reactions in a differentiated space (concentration gradient) to pHs lower than about 5.3 (HÖGLUND, 1961, Fig. 37, on p. 105) and are able to detect and avoid CO_2 separately from the accompanying pH (ibid., p. 135).

3. *Concluding remarks*

(1) The statement by HÖGLUND (1961) that salmon parr are able to detect and avoid CO_2 separately from the accompanying pH is supported by the absence of an initial peak on the ventilation curve and excitation at the confrontation with lowered pH, at least to the value of 5, in a soft water, in which the pCO_2 is increased at the same time by less than 2 mm Hg from about 1—2 mm Hg.

(2) A decrease in environmental pH not accompanied with an increased pCO_2 seems to have no influence *per se* on the ventilatory rate. At a pH lowered below 4.8 an initial peak on the ventilatory-rate curve may occur (T31 in Fig. 8) but this is probably due to an emergency reaction, owing to suffocation on account of damage to the gills caused by the low pH.

(3) The results arrived at in Section V suggest that CO_2 but not H_3O^+ easily penetrates the gill membranes (in both directions) when pH is changed suddenly in soft river Dalälven water as well as in well bicarbonate-buffered Uppsala tap water.

VI. Chromatophore reactions

A. Results

In experiment VI (Fig. 6), towards the end of T24, when the pH was kept at 6.4, the carbon-dioxide pressure at about 45 mm Hg, and the oxygen content at around 3.0 mg/l, the parr changed colour and this colour effect was intensified during the following control period (C43). These reactions were localized to the chromatophores, primarily the erythrophores, containing red pigments, such as carotenoids, including astaxantines, and the melanophores, with black melanine pigment (FOX, 1957; YOSHIMURA, 1964; DILL, ADOLPH, and WILBER, 1964, p. 112).

The phenomenon is striking, above all, on the pectoral fins. It appears on the fin bases and expands slowly outward along the fin rays, mainly as an increasing red flush. It culminates during C43 and peters out again in the course of half an hour. The darkening of the back and sides of the animals increases and decreases in a similar way. The melanophore reaction lasts longer than the erythrophore reaction. A temporarily increasing melanisation has also been observed in the outer edges of the fins.

These colour reactions ran parallel in both the parr tested in the experiment. They appeared at the same time in six other fish, which were tested alongside them in another chamber fed by the outflow water from the test chamber proper. Colour reactions of the kind described here do not appear under similar pH/pCO_2 conditions in Uppsala water when the oxygen content is kept constantly high at 9.3 mg/l (cf. T8 and T10 in experiment I). Nor did any such reactions appear during the still more extreme pH/pCO_2 conditions in T15 of experiment III or in T16 and T25 of experiment IV. Nor did the colour reactions appear before the fish died in experiment VIII, after being reached by Uppsala water with a very low oxygen content (about 0.9 mg/l) and pH 7.4.

B. Discussion

Regardless of whether the chromatophore reactions have a hormonal and/ or nervous background, they most certainly give an interesting indication of what, in the present study, we have called environmental stress. Similar reactions occur in various stages of poisoning, for instance, after the rotenone treatment of fish (Dr. K.-E. ÖBERG, personal communication). The extent to which a quantitative connection exists between such reactions and various environmental conditions producing stress, is, to judge from a study of the literature, still largely unexplored (ABOLINS, 1925; BURGERS, 1956; and Prof. L. ABOLINS, personal communication). The physiological interpretation is, however, complicated (cf. e.g., Chapter 15 in FLOREY, 1967).

C. Concluding remarks

(1) In tests for 5 hours in Uppsala water with reduced pH, increased pCO_2 and low O_2 content, Atlantic salmon parr manifest one or more of the following reactions, depending on the combination of parameters mentioned and the degree of deviation from the pre-acclimatization water quality.

(*a*) A lowering of the motivation threshold for the provocation of certain typical behavioural reactions;

(*b*) A change in the appearance of the ventilation curve; and in extreme cases

(*c*) Chromotophore reactions.

(2) A pH reduction (and the accompanying pCO_2 increase) from approx. 7.5 to approx. 6.4, and a simultaneous reduction of the oxygen content in Uppsala water from 9.3 to 3.0 mg/l causes typical colour changes. Such chromatophore reactions have in this study been observed in first- and second-summer Atlantic salmon parr (*Salmo salar*) and also in perch (*Perca fluviatilus*) and roach (*Leuciscus rutilus*). The reactions are intensified for a time after the test conditions are re-adjusted to the control conditions chosen for the experiment and equivalent to the fishes' pre-acclimatization environment.

(3) The chromatophore reactions are not forthcoming in parr of *Salmo salar* which have been kept for 5 hours at a correspondingly low and even lower pH level, when this has not been combined with oxygen depletion. Nor have colour changes of this kind been observed before the parr die after an extreme reduction of the oxygen content.

(4) The chromatophore reactions are probably, at least partly, a result of an intensified carbon-dioxide depression and are partly due to a low oxygen content in the blood. The normal innervation of the chromatophores may have been disturbed by CO_2 anaesthesia and/or by changed pH in the blood. But other explanations are also possible.

(5) The combination of pH reduction, pCO_2 increase, and oxygen reduction in the ambient medium aggravates the stresses that deviations from the normal conditions of these parameters each exert separately on second-summer parr.

VII. Summary and final discussion

A. Type division of ventilation curves

A summary of the results obtained in the pH/pCO_2- and O_2-deviation experiments (Figs. 3—7) is presented in Figs. 9 and 10. Somewhat idealized, the two series of ventilation curves (1—4) may be typical of various degrees of environmental stress provoked by increased CO_2 tension (in Fig. 9) and lowered oxygen content (in Fig. 10).
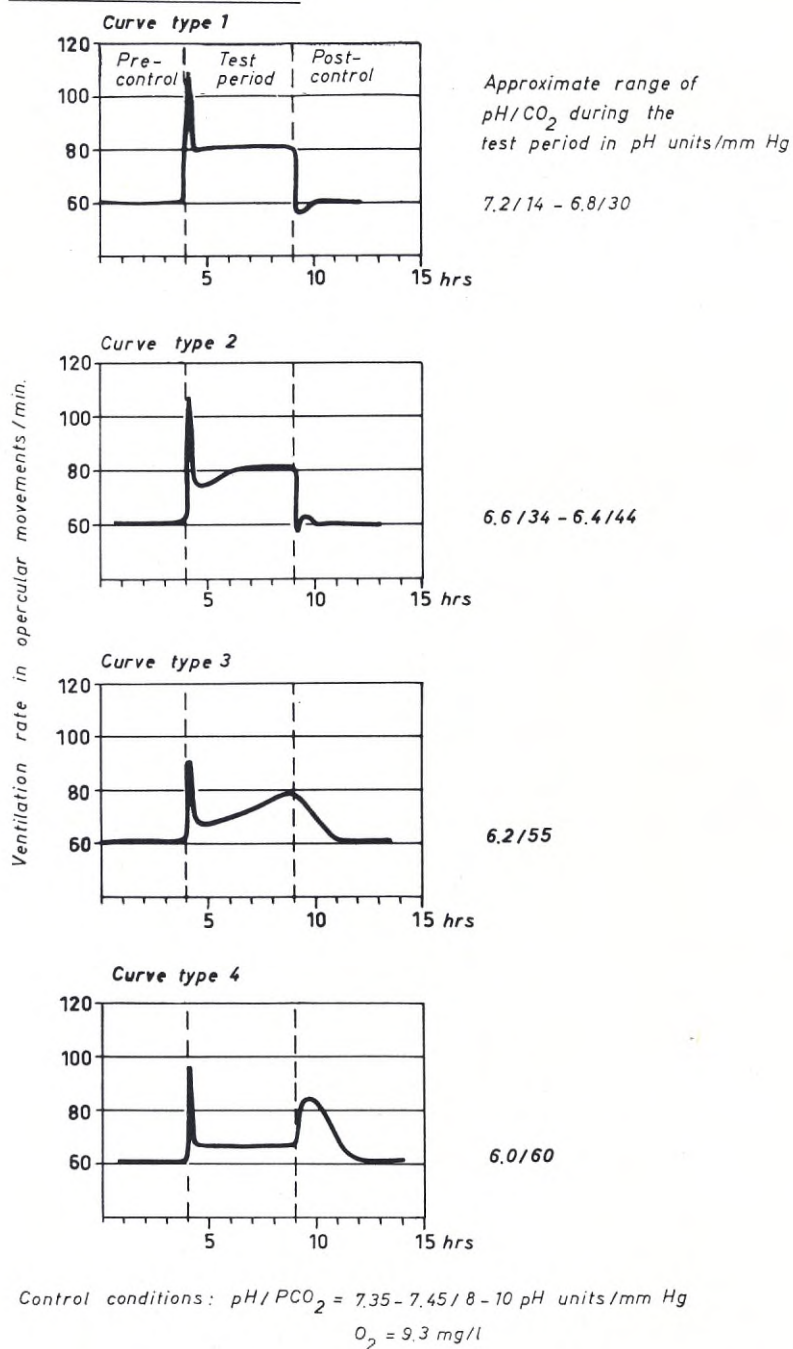
Fig. 9. Generalized curves of the ventilatory responses to sudden changes in aerated Uppsala water (Fig. 1 and Table 1) of pH and pCO₂ (cf. Figs. 3—5) and the ventilation during a 5-hour period with a constantly lowered pH and raised pCO₂. These parameters deviated by various amounts from the pre- and postcontrol conditions in the sequence of curves from 1 to 4. The oxygen content was kept constant at 9.3—9.4 mg/l and the pH and pCO₂ in the control conditions were kept at 7.4 and 8 mm Hg respectively.
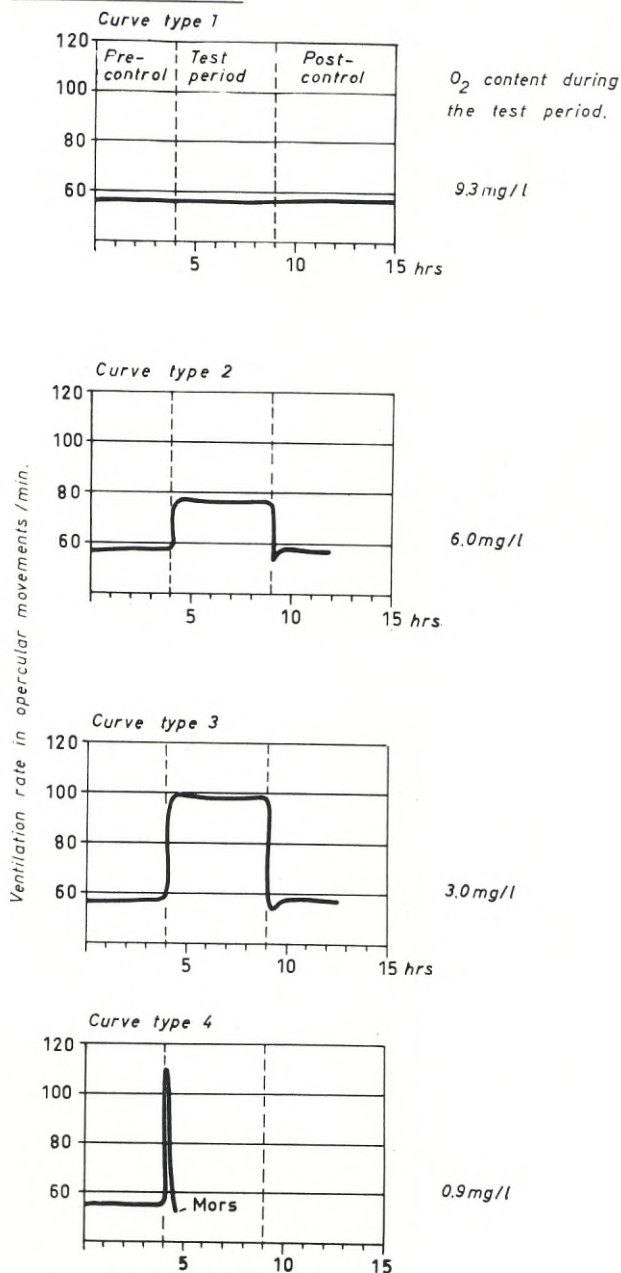
Fig. 10. Idealized ventilation curves in response to environmental oxygen content in Upp-sala water (cf. Fig. 7). The absence of an »initial peak» is striking in comparison with the ventilatory reactions recorded in the pH/pCO₂-deviation experiments visualized in Fig. 9. The oxygen content during the period of pre-acclimatization, as well as under the pre- and post-control conditions, was about 9.3 mg/l, while the pH and pCO₂ were kept constant at values at about 7.4 and 8—10 mm Hg respectively.

All the fish are characterized during the pre- and post-control periods by a basic respiration at about 60 opercular movements per minute. This corresponds to the resting metabolism, when the parr under the control conditions are immobile on the bottom of the test chamber. The control periods embrace a 5-hour test period, which in the ordinal series of curve types, designated 1—4 in Figs. 9 and 10, contain more and more extreme environmental conditions, which are kept constant during the test period.

The initial ventilation peak of curve type 1 in Fig. 9 is followed by a fundamentally constant hyperventilation at the rate of about 80. This is probably largely due to central-nervous-system regulation modified by reflexes caused by carbon-dioxide stimulation. The physiological interpretation is, however, much more complicated, *inter alia,* because the carbon-dioxide content in the blood to a great extent affects its capacity to take up oxygen (the BOHR and ROOT effects). Thus the effects of oxygen depletion in the blood cannot be disregarded as a respiratory stimulus. The direct (external) and indirect (after diffusion into the blood) effects of the carbon-dioxide supersaturation in the water cannot be discerned, nor the effects of metabolic CO_2 or other metabolites, on account of the provoked swimming activity. This question will be further discussed in two future papers, one by HÖGLUND and PERSSON and the other by HÖGLUND and BÖRJESON.

In curve type 2 a hollow dip appears after the initial peak. This effect — here called a carbon-dioxide depression in the ventilatory rate and in the behaviour — is gradually increased with increasing differences in the pH/pCO_2 conditions. The type of curve is, on the whole, valid within the pH/pCO_2 ranges stated in connection with each curve (1—4 in Fig. 9). In curve type 4 this depression becomes through-shaped, squeezed in between the initial peak and the new hyperventilation "hump" that appears at the beginning of the following post-control period. This may be connected with a retarded acid-base regulation, as observed by HÖGLUND and BÖRJESON (unpublished work). It may also be interpreted as the consequence of a presumable CO_2 depression (and/or acidosis effect), probably affecting the respiratory centre. Yet, with deep CO_2 depression caused by the external pH/pCO_2, which is nearly fatal after only a brief contact, there still appears, strangely enough, a faint hyperventilation (about 65/min.). When the fish have again been reached by water with the pH which normally occurs during the control periods (about 7.5), they revive from the presumed CO_2 depression. They then display unease reactions resembling those that appear at the beginning of a test period; the ventilation increases, passes a maximum and sinks again to normal rates.

These results may also be regarded in the light of the studies made by SAUNDERS (1962). According to this author, a moderate rise in the ambient level of CO_2 frequently gives rise to an increased oxygen consumption, presumably owing to the increased energy devoted to ventilation resulting

from high respiratory volumes (*op. cit.* p. 857). SAUNDERS continues: "After a period of 3 to 5 hours, with ambient carbon dioxide at a fixed level, the respiratory volume and percentage utilization of oxygen often, but not invariably, return to the levels they displayed before the level of carbon dioxide was raised." These observations seem to be in accordance with the observations made in the present study.

The courses of the curves presented in Fig. 10 (curve types 2 and 3) show that the ventilation in the oxygen tests runs more parallel with the oxygen content in the surrounding Uppsala water. The fish are passive all the time. The absence of an initial peak in the ventilation curves of Fig. 10 is in striking contrast to the results attained in the pH/pCO_2-deviation experiments. As the abrupt reaction in the latter case, judging from experiment IX (Fig. 8), is mainly due to CO_2, it must accordingly be concluded that environmental CO_2 changes are perceived independently of simultaneous changes in pH and oxygen content in the ambient water. This conclusion is also supported by the results of the combination experiments V and VI in Fig. 6.

As the initial peak in the ventilation curve is connected with more or less violent locomotion, there is reason for saying that this phase of behaviour is equal to the first phases described by HÖGLUND (1961, Fig. 38, p. 108). This experiment was made in order to elucidate the physiological and ethological background to the reactions displayed by fish, among them young salmonids, in a water spatially differentiated, as regarded pH/pCO_2 in such a way that high carbon-dioxide pressures were avoided.[1] The spatial differentiation in the continuous-flow chamber used here is, however, of short duration. The parr's opportunities of avoiding the more pH/CO_2-contaminated parts during the time required for water-changing are therefore limited to a few minutes. After a while they resume their bottom position as before the introduction of new water into the chamber. They remain thereafter mainly passive, as under undisturbed control conditions. Accordingly,

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[1] Appropriate preference reactions in fish have been demonstrated in gradient trials (or rather ten concentration steps), which were carried out with a laminar flow in an aquarium by what is known as "fluviarium technique" (HÖGLUND, 1961). With reference to pH/pCO_2 gradients, obtained by proportioned additions of hydrochloric acid to Uppsala water, a number of fresh-water fish showed pronounced (directed) reactions. They avoid those parts of the gradient which show critical conditions. Even lower carbon-dioxide pressure is promptly avoided. With a spatial differentiation of the oxygen content, on the other hand, in the range of 0.9—9 mg/l in flowing Uppsala water, the reaction among roach (*Leuciscus rutilus*) is less pronounced and "undirected". The fish are observed more often in the parts of the chamber which show higher oxygen content. The more often they encounter low, critical oxygen values in some part of the chamber, they behave livelier and accordingly they are not observed as frequently in these parts of the test space. Thus the preference is a "pseudo-attraction" due to orthokinesis (FRAENKEL and GUNN, 1940). Parr stages of *Salmo salar* avoid low oxygen concentrations more promptly (HÖGLUND and LINDQUIST, unpublished results; *cf.* also WHITMORE *et al.*, 1960 and BISHAI, 1962 a, b).

the ventilation does not exactly follow the behavioral changes. The hyper-ventilation continues, for instance, in many cases for a long time after the fish has settled down quite passively on the bottom.

Curve types 1 and 2 in Fig. 9 show an "undershoot" reaction on return to normal conditions after a test period. After this the intensity of respiration becomes normal within an hour. Already in curve type 2 there appears a clear tendency to hyperventilation during the corresponding period of time; this occurs after a very brief period. Hyperventilation at the beginning of a post-control period becomes more striking in those cases in which the pCO_2 has been kept at higher values during the test period. In curve types 3 and 4 this effect lasts for up to 3 hours.

B. The biological meaning of the reactions displayed

Compensatory reactions released by environmental abiotic stress conditions occur on different integration levels (PROSSER, 1958). The principle of "comfort for survival" (WOODBURY, 1956) occurs on the whole organism level in spatially pH/pCO_2 differentiations established in Uppsala water. This is valid for the salmon parr as well as for other fish, as regards un-favourably high CO_2 pressures in the ambient water, which — if possible — are distinctly avoided (HÖGLUND, 1961). This *avoidance homeostasis*[1] (cf. MITTELSTAEDT, 1964) also prevails in pH gradients, starting from a maximum value of about 5.5, which were established in a less buffered water (HÖGLUND, 1961). In both cases the avoidance is prompt and seemingly directed and protects the fish from getting into the parts of the differentiated space which contain adverse pCO_2 as well as adverse pH conditions. Low oxygen below a critical value releases an *emergency reaction*. But the latter is much more vague and the fish is evidently not aware of the direction of spatially differentiated oxygen content (HÖGLUND, 1961). The reactions obtained in changing environments, as regards pH, CO_2, and O_2, produced as far as possible without any spatial differentiation in the present study, support the statements by HÖGLUND (1961). When the fish are given no choice or allowed no preference, increased pCO_2 provokes an initial excite-ment, shown by increased locomotor activity. This is connected with a marked initial peak in the ventilation curve, which reaches its maximum in the course of about a quarter of an hour, even though the environmental pCO_2 is kept constant at a raised level, compared with the pre-acclimatization water quality of aerated Uppsala water. This may be interpreted as *emer-gency behaviour,* which occurs during a phase after the introduction of the new environment which corresponds to the parts of the gradient experiments in which the parr make their choice. In the present experiment it is also

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[1] This protective mechanism on the ethological-physiological level is called *avoidance homeostasis* in contrast to the concept of *orientational homeostasis* used by MITTELSTAEDT (1964).

worth noting that salmon parr can tolerate a high pCO_2, which can hardly occur in natural surface water, when compelled to stay in it. This seems to be connected with the capacity of the acid-base regulation to prevent a more delayed acidosis after the diffusion of CO_2 from the respiratory water to the blood (HÖGLUND and BÖRJESON, unpublished report). This *internal regulatory homeostasis* is a second principle which protects fish against adverse environmental pCO_2 tensions. It is conceivable that the considerable amount of carbonic anhydrase which is reported to occur among fresh-water fish may play a part in this regulation. Great activity of this enzyme has been reported from the erythrocytes, gills, pseudobranchs, swim bladder, retina, choroid layer and choroidal gland of the eye, and pyloric caeca, and smaller amounts in the kidney of teleosts (MAREN, 1967, p. 667).

Both these types of compensatory reactions to high environmental pCO_2 are favourable to the parr. But it is less probable that a persistent ventilation increase on response to raised external pCO_2 has any biological significance. In a medium with a higher pCO_2 than the blood, a hyperventilation response instead must be a disadvantage, as it exerts a *loading stress* on account of the increased muscular activity of the ventilatory apparatus. This is probably compensated for by acid-base regulation in the blood, which, it is suggested, may occur by means of bicarbonate retention or, less probably, by the active excretion of carbon dioxide, *e.g.*, via the swim bladder.

An adversely low pH certainly leads to suffocation by the destruction of the gas transporting function over the gill lamella. For this reason the *avoidance homeostasis* seems to be the only way to survive such conditions.

On the other hand, the only appropriate response to a lowered oxygen content, from a biological point of view, is increased ventilation and a reduction of the metabolic need for oxygen by inactivation or a decrease in the locomotor activities. This is also demonstrated in the present experiments, until a flagrant oxygen deficit is reached in the tissues on account of the lack of a supply of oxygen from the environmental water.

C. Concluding remarks from the ecological and fishery biology points of view

Impaired water quality has certainly in the long run — directly or indirectly — effects upon the internal functions of fish which retroact on their biological activities, such as spawning, egg and larval development, feeding, and growth (cf. GERKING, 1967). Limitations in reproduction or distribution are usually not discovered suddenly (HARDEN JONES, 1968). The populations may rather fade away indiscriminately (cf. BRETT, 1958). One has therefore to be careful in adopting water-quality criteria based upon survival tests only. Most important are the combined effects of various environmental factors, especially such factors as give rise to hyperventilation. Excessive ventilation is a loading stress *per se* (FRY, 1947; BRETT, 1958; HUGHES, 1963,

p. 5). The contact between the respired water containing pollutants and the blood probably increases, even though there is no simple relationship between respired water volume and oxygen uptake (SAUNDERS, 1962; HUGHES, 1964). But, on account of the counter-flow principle, the contact and interchange between the exterior and the interior of fish are very intimate. The loading stress due to increased ventilation thus very probably also implies an indiscriminate chemical or poisonous stress, which further restricts the scope for normal activities.

Judging from the present results, the acidification of fish-producing or fish-containing waters by air or water pollutants will not probably have a primarily restrictive effect on the distribution of fish owing to lowered pH only. In experimental conditions — as in the present study — the Atlantic salmon parr tolerate each on its own account, a moderate decrease in pH to about 4.5—5.0, a rise in pCO_2 up to 50—60 mm Hg, and a oxygen depletion at least down to 3 mg O_2/l in the environmental water. The tolerance to a high pCO_2 does not decrease disastrously in combination with moderate oxygen depletion. These statements are valid at least for contact periods up to five hours.

Two protective mechanisms exist in response to high CO_2 tension, viz. (1) *avoidance homeostasis,* when the salmon parr are given a chance of selecting the most favourable environment, and (2) *internal regulatory homeostasis,* when they are compelled to stay in water with a high pCO_2. Besides, raised carbon-dioxide pressure in the ambient water always provokes (3) *initial stress,* which at the first confrontation is characterized by excited locomotion and respiratory distress. Hyperventilation in relation to high external pCO_2 is a disadvantage (p. 78). Oxygen depletion *down to a critical value* [1] seems not provoke anything like (1), (2), and (3), but increased muscular work is recognized, as regards the hyperventilation, which is a mirror image of the deviations from the pre-acclimatization conditions with high oxygen content. It forms a contrast also with the pCO_2-provoked hyperventilation in the absence of any initial peak as well as in the absence of erractic movements. The hyperventilation response to lowered oxygen content is advantageous, coupled with the fact that the parr remain quiet as long as the oxygen supply is sufficiently high for their metabolic needs at rest. An extreme oxygen depletion, however, provokes erratic swimming behaviour or an *emergency reaction,* which soon leads to collapse, if a space with a sufficiently high oxygen content is not encountered seemingly *by chance.*

Among conceivable ecological-physiological reasons for the exclusion of more sensitive fish on account of lowered pH in natural waters with low bicarbonate-buffering capacity (*cf.* DAHL, 1926; DANNEVIG, 1966, 1968), the following suggestions seem to be of noticeable interest but need further investigation.

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[1] cf. foot-note on p. 113.

(1) Increased hydrogen concentration leads to a shift in the equilibrium of proteolytic systems, such as the bicarbonate and the bisulphite systems. The latter is of current interest when sulphite waste liquor is discharged into unbuffered river water. Free carbon-dioxide and sulphur dioxide will then appear molecularly dissolved in the receiver. They may act upon living organisms, as uncharged gas molecules penetrate the potential barriers of biological membranes much more easily than ionic compounds. Sulphur dioxide, for example, may inhibit the activities of enzymes because it is readily oxidized.

(2) The chance of "microchemical contaminants" (WARNER, 1964), i.e. pesticides and other poisons occurring in low concentrations, to penetrate and accumulate in the tissues increases, owing to hyperventilation.

(3) The protective functions on response to substances interfering on each physiological level are energy-expending and restrict the scope for normal activities in biological, ethological, and functional respects.

(4) Organisms such as arthropods and molluscs having carbonate shells and serving as fish food may be more sensitive to environmental pH and pCO_2 alterations than fish.

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Occurrence of Triaenophorus spp. in Lake Mälaren fishes

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I. Introduction

Members of the genus *Triaenophorus* are widely distributed throughout the northern temperate regions of the world and usually occur wherever the northern pike *Esox lucius* is found. The life cycle of these cestodes involves three hosts; the first larval stage is found in copepods; the second intermediate host is one of several species of fishes which may eat an infected copepod; the final or definitive host, in which the tapeworm reaches sexual maturity, is the pike which becomes infected by eating the parasitized second intermediate host.

Three species of the genus *Triaenophorus* have been described from several species of fish: *Triaenophorus crassus* Forel, *T. nodulosus* (Pallas) and *T. stizostedionis* Miller. *T. crassus,* is of economic importance as the encysted parasite in the flesh of coregonine fishes affects the marketability of these fish because of the objectionable appearance of these cysts. The second intermediate stage of *T. nodulosus* is found in the liver and viscera of many of the same or closely related species of fish in North America, Europe and Asia including; burbot *Lota lota,* perch, *Perca fluviatilis,* ruffe *Acerina cernua,* grayling *Thymallus thymallus,* smelt *Osmerus eperlanus* and several other species. According to Bauer (1959) *T. nodulosus* can seriously affect the health and marketability of valuable fish. He cites records of mass deaths of perch and smelt which were attributed to the degeneration of liver tissue caused by the presence of *T. nodulosus.* Burbot livers in one region were so heavily infected with *T. nodulosus* that they were rejected for canning purposes. In trout rearing ponds *T. nodulosus* can cause the death of young fish (Bauer ibid). No evidence of the harmful effect of *T. nodulosus* has been noted in Canadian fishes.

Triaenophorus stizostedionis Miller which has not been reported from European fishes has a slightly different life history in that the final host is the pike-perch *Stizostedion vitreum* and the second intermediate host is the troutperch *Percopsis omiscomaycus.*

Very little data have been published on the occurrence of *Triaenophorus* in Sweden since Bergman (1923) noted the presence of *T. robustus* Olsson= *T. crassus* in American brook trout, rainbow trout and salmon in the Kälarne

fish culture station and NYBELIN (1924) reported the presence of *T. tricuspi-datus*=*T. nodulosus* in burbot and other freshwater fishes other than roach-like fish. NYBELIN (ibid) also remarked on Alm's investigation of Hjälmaren in 1914 in which he found that the whitefish were heavily infected with *T. robustus* and pointed out that the low catches in the following year were due to the infection with this parasite.

This paper describes the results of the examination of several species of Lake Mälaren fish for *Triaenophorus*.

II. Materials and methods

The fish that were examined for *Triaenophorus* infection were captured in the Lambarfjärden region of Lake Mälaren and in the vicinity of the Freshwater Laboratory between December 1968 and May 1969. The fish were taken in nylon gill nets set on the bottom where the depth ranged from 12 to 50 m. The mesh size of the nets measured knot to knot ranged from 25 to 50 mm. with the exception of the nets (10 mm. across) used to catch smelt. Fyke nets were used to catch fish in the shallow water near the Fresh-water Laboratory.

All fish were examined immediately after they were removed from the water. Length and sex was recorded for each fish. The number of each species and the parts of the body examined are shown in table 1.

III. Results

Perca fluviatilis

T. nodulosus was found in 69 % of the 111 perch examined. Most of the cysts were located in the liver but occasionally cysts were found attached to the peritoneum. Usually only 1 or 2 cysts were found in one liver but in two heavily infected livers 12 cysts were noted. Some individual cysts were very large and seemed to have replaced almost all the liver tissue.

The incidence of infection in perch of different sizes is shown in Table 2. The length range of female perch was 14—27 cm ($\bar{x}$=18.48 cm) and for the male perch 14—22 cm ($\bar{x}$=16.37 cm). The incidence of infection is usually higher in the very young fish which are feeding almost exclusively on zoo-plankton and thus are more likely to acquire more parasites than the older perch, which have somewhat more omnivorous feeding habits. Unfortunately no small perch (< 12 cm) were captured in the nets, however, the smaller perch (14—17 cm) in the sample from Lake Mälaren were more heavily infected than the perch > 17 cm in length. There was an apparent decrease in the incidence of infection with increasing size of the perch.

Table 1. Number of each species of Lake Mälaren fish examined for *Triaeno-phorus*.

Species	Number examined	Flesh	Body part examined		
			Intestine	Liver	Body cavity
Perca fluviatilis Perch	113	×	—	×	×
Osmerus eperlanus Smelt	169	×	—	×	×
Lota lota Burbot	39	—	—	×	×
Abramis brama Bream	6	×	—	×	×
„ *blicca* White or Silver Bream	15	×	—	×	×
Acerina cernua Ruffe	49	×	—	×	×
Leuciscus rutilus Roach	54	×	—	×	×
Esox lucius Pike	155	×	×	×	×
Coregonus albula Cisco	44	×	—	—	—
„ *lavaretus* White fish	1	×	—	—	—
Lucioperca lucioperca Pike-perch	24	—	×	×	×

A higher percentage of infection was found in male (77 %) than female fish (67 %) but the difference was not statistically significant.

Particular attention should be given to the high number of cysts per infected fish in the 14 and 15 cm size groups. As the unusually high infection is confined to these size groups it is quite possible that a strong year-class of *Triaenophorus* had occurred during some stage of their development, and this is reflected in the considerably higher proportion of cysts/infected fish. Age analysis of a small sample of perch scales indicated that fish in the 15 cm. group were in their fourth year of life and those 16 cm long were 4+ years of age. It is quite conseivable that these perch received a large infection of *T. nodulosus* in 1965, a year in which pike were fairly abundant and the water level was high by comparison with years of really low water level.

Osmerus eperlanus

The smelt does not serve as a second intermediate host of *Triaenophorus nodulosus* in North America but *T. nodulosus* has frequently been found in smelt from European waters.

In a sample of 169 smelt from Lake Mälaren, 58 per cent were infected with *T. nodulosus*. The musculature was carefully checked but no *T. crassus* was found.

The majority (68 %) of the infected smelt contained only 1 cyst, however, in one fish 9 cysts were observed. Most of the cysts were located in the liver but occasionally cysts were found attached to the peritoneum and one was found fixed to the stomach wall.

The incidence of infection is shown in Table 3. The smelt ranged in size from 8 to 19 cm and the females were slightly larger ($\bar{x}=12.5$ cm) than the

Table 2. *Triaenophorus nodulosus* in Lake Mälaren perch.

Length of fish (cm)	Number examined	Infection %	Total number of cysts/fish	Number of cysts/ infected fish
14	7	71	1.1	1.6
15	22	90	2.6	2.9
16	14	93	2.7	2.9
17	7	100	1.3	1.3
18	11	73	0.8	1.1
19	19	53	0.5	1.0
20	15	60	0.6	1.0
21	6	66	0.6	1.0
22	2	0	—	—
23	1	100	1.0	1.0
24	2	0	—	—
25	2	50	0.5	1.0
26	2	0	—	—
27	1	0	—	—

males ($\bar{x}=11.7$ cm). No statistically significant difference was found in incidence of infection between the sexes. There did not appear to be any major differences in the incidence of infection between the various sizes, in fact, the incidence of infection appeared nearly uniform. With the exception of the 9 and 13 cm size groups of smelt the number of cysts per fish was less than one.

Coregonus albula

The cisco and closely related forms of this species are the major second intermediate hosts of *T. crassus* both in North America and Europe. For this reason attempts were made to obtain a sample of ciscoes from Lake Mälaren to determine the incidence of infection from a population of relatively deep dwelling fish.

Table 3. *Triaenophorus nodulosus* in Lake Mälaren smelt.

Length of fish (cm)	Number examined	Incidence of infection (%)	Number of cysts/fish	Number of cysts/ infected fish
8	3	33	0.3	1.0
9	2	50	2.5	5.0
10	5	40	0.20	1.0
11	16	56	0.90	1.5
12	49	53	0.70	1.4
13	32	65	1.1	1.7
14	5	60	0.6	1.5
15	5	60	0.8	1.3
16	—	—	—	—
17	—	—	—	—
18	1	0	—	—
19	1	0	—	—

Table 4. *Triaenophorus crassus* in Lake Mälaren ciscoes.

Length of fish (cm)	Number examined	Infection %	Number of cysts/each size					Number of cysts/infected fish
			0	1	2	3	4	
21	3	66	1	2	—	—	—	1.0
22	12	33	8	3	—	1	—	1.5
23	11	54	5	5	—	—	1	1.5
24	13	31	9	3	—	1	—	1.5
25	4	50	2	1	1	—	—	1.5
26	1	100	—	—	1	—	—	2.0
	44	43	25	14	2	2	1	1.5

The ciscoes were obtained after spawning and there was a preponderance of males in the samples. This should not affect the infection statistics, however, as it has not previously been shown that there is differential infection.

In a sample of 44 fish 43 % contained cysts of *T. crassus*. The majority (74 %) of the infected fish contained only 1 cyst, however, one fish had 4 cysts (Table 4). The incidence of infection does not differ greatly from that observed in many Canadian lakes.

Esox lucius

Both *T. crassus* and *T. nodulosus* were found in the intestines of L. Mälaren pike. The larval form of *T. nodulosus* was not found in the liver of pike, a site of encystment recorded by other investigators. In a sample of 51 pike from the deeper Lambarfjärden region 23 % of the fish had *T. crassus* and 88 % contained *T. nodulosus*. Pike from the shallower region near the Freshwater laboratory at Drottningholm had a slightly lower incidence of infection with *T. crassus* (18 %) but the incidence of *T. nodulosus* (83 %) was almost as heavy as in the Lambarfjärden pike. In the combined sample of 155 pike, 18 % of the fish contained *T. crassus*, 85 % contained *T. nodulosus* and 15 % had no *Triaenophorus* parasites.

The interrelations between *T. crassus* and *T. nodulosus* when they simultaneously inhabit the same pike intestine is of interest as some investigators believe that strong invasion of *T. crassus* is only possible in the absence of *T. nodulosus,* whereas others hold the opposite point of view. Joint occurrences of *T. crassus* and *T. nodulosus* were noted in 19 % of the L. Mälaren pike intestines. In only 2 intestines of the 25 in which joint occurrences were observed, did the number of *T. crassus* exceed *T. nodulosus*. In the Lambarfjärden pike the ratio of *T. crassus* to *T. nodulosus* was 1 : 5 and from the shallow water region the ratio was 1 : 10. Some actual numbers from pike with both *T. crassus* and *T. nodulosus* follow: 9 : 59, 4 : 8, 1 : 3, 1 : 18, 4 : 12, 1 : 2, 1 : 17 etc.

Table 5. Incidence of *Triaenophorus* spp in Lake Mälaren pike.

Size range of pike (cm)	Lambarfjärden region			Laboratory region		
	Number examined	Incidence of infection (%)		Number examined	Incidence of infection (%)	
		T. crassus	T. nodulosus		T. crassus	T. nodulosus
30	6	0	83	12	0	50
40	31	22	87	38	10	92
50	8	37	100	37	19	89
60	5	20	80	10	10	75
70	1	100	100	4	33	67
80	—	—	—	2	50	50
90	—	—	—	1	100	100

The maximum number of *T. crassus* found in one pike intestine was 9 in both the Lambarfjärden and shallow water regions. In the Lambarfjärden region there did appear to be a slightly higher average number of *T. crassus* per infected pike (3.0) than in the shallow water region (2.0). The maximum number of *T. nodulosus* observed in one pike intestine was 61 and the average per infected pike intestine was 16.

The relationship between incidence of infection with *Triaenophorus* and size of pike is shown in Table 5. It should be noted that although *T. crassus* was not found in pike under 40 cm. in length, *T. nodulosus* was often found in the smaller pike. There was a trend to higher infection of *T. crassus* with increasing size of the pike.

Other species of fish

Several other species of fish (Table I) caught in the gill nets were carefully examined but no *Triaenophorus* parasites were found.

IV. Discussion

Eleven species of Lake Mälaren fish were examined for *Triaenophorus* and four species were found to act as hosts for this cestode. Only the pike was found to serve as the adult host for both *T. crassus* and *T. nodulosus*. The relative abundance of *T. crassus* and *T. nodulosus* in Lake Mälaren pike was determined by recording the actual number of each of these species present in infected fish. *T. nodulosus* was more abundant than *T. crassus* in pike from both deep and shallow regions of the lake. The pike has more chances of aqquiring *T. nodulosus* as two species of fish (perch and smelt) act as second intermediate hosts of *T. nodulosus* and are likely food for pike whereas *T. crassus* was found only in the cisco. Whitefish are not very

abundant in L. Mälaren and thus even if they were infected they could hardly be considered important second intermediate hosts. *T. nodulosus* was not found in the burbot examined although it has frequently been described as an important intermediate host throughout its range including neighboring Norway (VIK 1959). Personal communication with long time fisherman indicated that cysts in the livers of Lake Mälaren burbot had been prevalent many years ago and although they were unable to recognize these cysts as *Triaenophorus* the fact that *T. nodulosus* has been observed in burbot livers from other parts of Sweden (author's identification) affords support for their observations. The high incidence of infection in perch (69 %) and smelt (58 %) ensures that the life cycle of *T. nodulosus* will be maintained as long as there are pike in Lake Mälaren. Unfortunately there have been no studies made on the food of pike of Lake Mälaren and a full understanding of the interrelationships of pike and its second intermediate hosts in the life cycle of *T. nodulosus* depends on a knowledge of the food and ecology of the final host. It is known that the eggs of the parasite are released from the intenstine of the pike at the same time that the pike is spawning. In order for the life cycle of the parasite to continue it is absolutely necessary that the first intermediate stage, found in a copepod, be consumed by a perch or smelt within a month of the time of pike spawning. This means that these species of fish must be in shallow water where the pike spawn, during the late spring. As the perch and the smelt both move onshore to spawn, the association in this respect is understandable. Annual differences may exist in the incidence of infection in the second intermediate hosts. In 1965 the water level was high, assuring more favorable suitable spawning habitats for pike; pike production was high and there was evidence that the incidence of *T. nodulosus* in perch that year was also high. Ecological conditions then were generally favorable for the early stages of the life cycle of the parasite. As the perch in Lake Mälaren is more littoral than smelt it would be expected that perch would be more heavily infected than smelt because they are in closer contact with infected zooplankton for a longer period in the spring. The pike could conceivably consume perch throughout the year as both species occupy the same or similar habitats, however, the smelt is essentially a fish of deeper pelagic waters, which is not the normal habitat of pike. As large pike are known to move into deeper waters in Lake Mälaren it is possible that the smelt could be consumed even in the deeper waters.

Although the number of cysts of *T. crassus* per infected cisco is not unusually high the overall incidence of infection is high and this enhances the chance of pike becoming infected. The continuation of the life cycle is dependent on the association of the pike and cisco during some period of the year. The cisco must acquire *T. crassus* by eating infected copepods in inshore waters during late spring and early summer and are probably inshore at this time. The larger pike which move to the deeper waters could feed on

the cisco during the summer and autumn but the smaller pike would have to feed on cisco when they are inshore in autumn at spawning time or when they move inshore after the ice leaves the lake. In recent years the demand for Lake Mälaren pike has declined and there has been a concomitant increase in the number of larger pike in the lake which are known to move into deeper water. This could have a serious effect on the incidence of *T. crassus* in ciscoes as it would mean that larger numbers of pike would be coming into contact with ciscoes in the pelagic waters thus creating greater opportunities for transmitting the parasite.

The absence of *T. nodulosus* in ruffe probably results from habitat differences of the pike und ruffe. The ruffe is usually found on or in the bottom ooze of the lake and although it is a common food of the pikeperch in Lake Mälaren, it is not known to be a preferred food of pike. It must be assumed that the pike and ruffe are ecologically isolated in Lake Mälaren and thus are not capable of continuing the life cycle of *T. nodulosus*.

V. Summary

1. 669 specimens of 11 species of Lake Mälaren fish were examined for parasites of the genus *Triaenophorus*.
2. Larval stages of *T. nodulosus* were found in 69 % of the perch and 58 % of the smelt examined but were not found in burbot or ruffe, which are known European hosts of the parasite, or in any other of the species. A possible strong year class of *T. nodulosus* in perch was noted.
3. The larval stage of *T. crassus* was found in 43 % of the ciscoes examined. Only 1 whitefish was examined and *T. crassus* was not found.
4. The adult stage of both *T. crassus* and *T. nodulosus* was found only in the pike. *T. nodulosus* was more abundant than *T. crassus*.
5. Ecological relationships of the pike and the several intermediate fish hosts of *T. nodulosus* and *T. crassus* in Lake Mälaren are discussed.

VI. Acknowledgements

VII. References

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Addendum

Since this paper was written three new species of *Triaenophorus* have been described. KUPERMAN (1968) has produced evidence, based on differences in morphology, host species and biology, that *T. nodulosus* and *T. crassus* from the Anur Basin and *T. crassus* from the southern reservoirs of the U.S.S.R. should be considered as independent species and named them *T. amurensis* sp. n.; *T. orientalis* sp. n.; *T. meridionalis* sp. n. (KUPERMAN, B. I. 1968. New species of the Genus Triaenophorus Rud (Cestoda pseudophyllidea). Parasitologia (Acad. Sci. U.S.S.R.) 2 (6): 495—501.)

Microgradients at the Mud-Water Interface

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I. Introduction

In a previous paper (OIKOS 19,1.) I described a sampling apparatus designed for investigations of the microstratification below and above the mud surface.

The sampler is intended to serve as an alternative to the JENKIN sampler and as a complement to the EKMAN dredge. In principle, it is a long hollow tube of plexiglass, the cross-section of which is almost square. Alongside the tube is a "slide box" containing thin, horizontal metal slides which can be released to cut into the tube and divide it into separate sections. The sampler is submerged to the desired depth, pushed into the mud, then immediately released as the slides enter rapidly into the tube sealing off the sections. The high speed of the closure makes it difficult for even very agile animals to move vertically to any appreciable extent.

As has been proved by aquarium experiments, the stratification of the sediments is kept almost intact. Investigations in situ have been successfully performed throughout more than a year. The sampler can be used for limnological as well as marine investigations of soft bottom sediments and of the water strata close to the bottom.

Each mud and water core with its subdivisions yields samples for chemical and zoological analyses.

The contents of each section pass through a tube into a very dense nylon fabric (60 μ), where the animals and coarse particles are retained. The water passing through the fabric is collected in a polyethene bottle, for chemical analyses. The animals and mud from each section are then put into larger containers. In the laboratory the live specimens are sorted and classified as soon as possible into orders, families etc. Afterwards each classification unit is weighed to obtain the fresh weight. (*Copepoda, Calanoida,* and some *Chironomidae* are not classified alive).

The nylon fabric with the animals collected is placed in a container with water in order to keep the animals alive. Subsequent treatment is the same as for the mud sections.

Portions of the mud from the successive sections are kept in small testtubes which are then placed in a cold box for further analyses. Animals

within the mud may also be sorted by using specially treated equipment in order to avoid contamination. They are placed in a cold box (—20°C), family by family (for instance *Chironomidae* and *Tubificidae* in water-tight containers for future chemical analyses.

Since the animals are sorted alive, it is possible to record every animal within the core retained by the fabrics, even zooplankton. Very often some *Plathelmintes,* and some *Oligochaeta* — especially certain *Naididae* — disintegrate totally if preserved in alcohol or formalin.

To illustrate what information may be obtained from microstratification cores I have chosen the results from three investigations from September 1967. One of them — from Norra Björkfjärden on September 9 (Tables I a and I b) was chosen because preliminary results have previously been published (MILBRINK, 1968). The vertical stratification of the fauna is interesting and worth examining. Unfortunately on that occasion the samples were not tested chemically, but on September 27 in Norra Björkfjärden and on September 28 in Lilla Ullevifjärden both chemical and physical data were obtained.

Most animal species have their maxima of occurrence in the mud-water interface or very close to it. This is a quite well-known phenomenon and a consequence of the "edge-effect". The combined nutrition, oxygen, and temperature conditions are generally most favourable in that region.

The vertical distribution of animals in the mud was previously investigated by, among others, LENZ (1931) and BERG (1938). DELLA CROCE (1955) and PODDUBNAJA (1962) have also studied it, but with rather inconclusive results. Recently KJÄLLMAN & GRIMÅS (1967) have also made investigations in this field.

With the new type of sampler (MILBRINK, 1968) fairly accurate studies appear possible.

The sampler was originally constructed in an attempt to obtain information on the vertical distribution of *Oligochaeta.* The most interesting results, however, would seem to have been obtained on some *Cyclops-* and *Calanoida-* species.

Some animals, for instance *Pisidium sp., Tardigrada,* and certain *Oligochaeta* are known to be quite stationary in the mud or on the mud surface. Others, however, such as many *Crustacea,* some *Chironomidae* larvae (and other *Diptera* larvae like *Chaoborus*), and some *Oligochaeta* (immature *Tubificidae* and certain *Naididae*) are extremely migratory. These circumstances should be well-known to any initiated bottom faunist and are well worth a closer investigation.

Table I a and Table I b (not analyzed in detail) illustrate the results from duplicate samples and Table II the results from one sample out of two. Unfortunately, Table III and Table IV from Lilla Ullevifjärden do not

illustrate duplicate samples. Samples were taken about 100 metres apart, but at the same depth. The chemical data (Figs. 2, 4, and 6) should be compared with the zoological data of Table III, since both instances refer to the same sample.

In the Tables the depth limits for each section are indicated in order to show the position of the mud-water interface. The vertical line between sections Nos. 7 and 8 indicates that from No. 8 onward the sections are four times as voluminous (1.6 litres) as the preceding ones. Consequently for comparison the results have to be divided by four.

As far as possible each animal group is classified into genera and species. Some groups, however, including *Ostracoda, Nematoda,* and *Tardigrada* are not classified into genera.

The sampling apparatus is designed to give an integrated picture of the vertical distribution of animals and chemical variables *at the very moment and at the very spot of sampling.* Effects of horizontal and temporal variation, especially diel migration and heterogeneity may be reflected in the zoological figures. (Isolated samples are naturally not quite representative of an investigated body of water.)

From a statistical point of view it is desirable to take several samples at approximately the same time to get reliable values. Results from duplicate samples, however, generally correspond rather satisfactorily to each other (cf. Tables I a and I b). Even isolated samples may show tendencies in the animal distribution. There always seem to be some pervading characteristics in the pattern.

II. Presentation of material and discussion

Physical factors and chemical concentrations in the water

Norra Björkfjärden and Lilla Ullevifjärden are two separate basins of the central, least polluted part of Lake Mälaren. Norra Björkfjärden is considered to be mesotrophic, with no shortage of oxygen. Conditions prevailing at Lilla Ullevifjärden are more complex. This is a narrow, fairly deep basin with a maximum depth of about 50 metres enclosed by moraine ridges from which it receives its subterranean water supply. There is no visible tributary, but springs are believed to emerge from the steep bottom sides of the basin. The outlet is very narrow. A locally fairly high level of primary production of the epilimnion and an obvious shortage of oxygen in the lower hypolimnion during stratification periods make it tempting to classify Lilla Ullevifjärden as mesotrophic-eutrophic. Other factors, however, indicate a tendency towards oligotrophy.

Owing to rough winds in the last week of September the thermocline in Norra Björkfjärden, which has an exposed position, had been pressed downwards (Fig. 1) and had partly disintegrated. The circulation period was obviously at its start.

Because of its protected position, Lilla Ullevifjärden was still densely stratified (Fig. 2), the thermocline being at a depth of twelve metres.

The collected water was tested to obtain the concentrations of eleven different dissolved substances, five of which are illustrated in the diagrams.

The upper parts of the diagrams (Figs. 1—6) present simplified illustrations of the conditions of the entire water column. The lower parts of the diagrams illustrate the results from each section of the core above the mud surface. The distance between each point of measure is 10 centimetres, or, in the lower parts, only 2.5 centimetres.

Dr. T. AHL "the Lake Mälaren Research" who carried out the analyses, provided the details which follow.

The chemical data — if representative — obtained from the vicinity of the mud on September 27 and 28 are, on the whole, confusing. The concentrations of several cations decrease very strikingly close to the bottom. This might be explained as an absorbtion phenomenon, but some anions decrease as well, which may not be so easily explained as an effect of absorbtion. Another explanation, implying spring water flowing in laminae close to the mud surface, was rejected by Dr. AHL. The water has been tested among other things for potassium and sodium.

This decreasing tendency was not observed in the winter of 1967—1968 and needs further examination before any definite conclusion can be drawn from it.

Near the bottom there generally exists an undulating detritus or silt layer containing organic complexes which may serve as ion-exchangers (cf. MORTIMER 1941/42). This absorbing capacity should be kept in mind when looking at Fig. 5 and Fig. 6, where the total-phosphorus concentrations are plotted in respect to height above the mud surface. Consequently the extremely high concentrations in the interface layer seem less confusing.

At the end of the summer stratification period the oxygen situation in the lower hypolimnion of Norra Björkfjärden is, as was mentioned earlier, better than in Lilla Ullevifjärden. In the deepest parts of the latter basin the oxygen concentrations often reach values critical for animal life. Even the upper sediment layers smell of H_2S, indicating reduced conditions in the mud close to the surface.

The difference is well illustrated in Fig. 1 and Fig. 2. The partial demolition of the thermocline in Norra Björkfjärden may already have caused an increase of the oxygen supply near the bottom.

In Lilla Ullevifjärden the pH surface value of 8.9 was very high, probably owing to a locally high rate of primary production (Fig. 2).
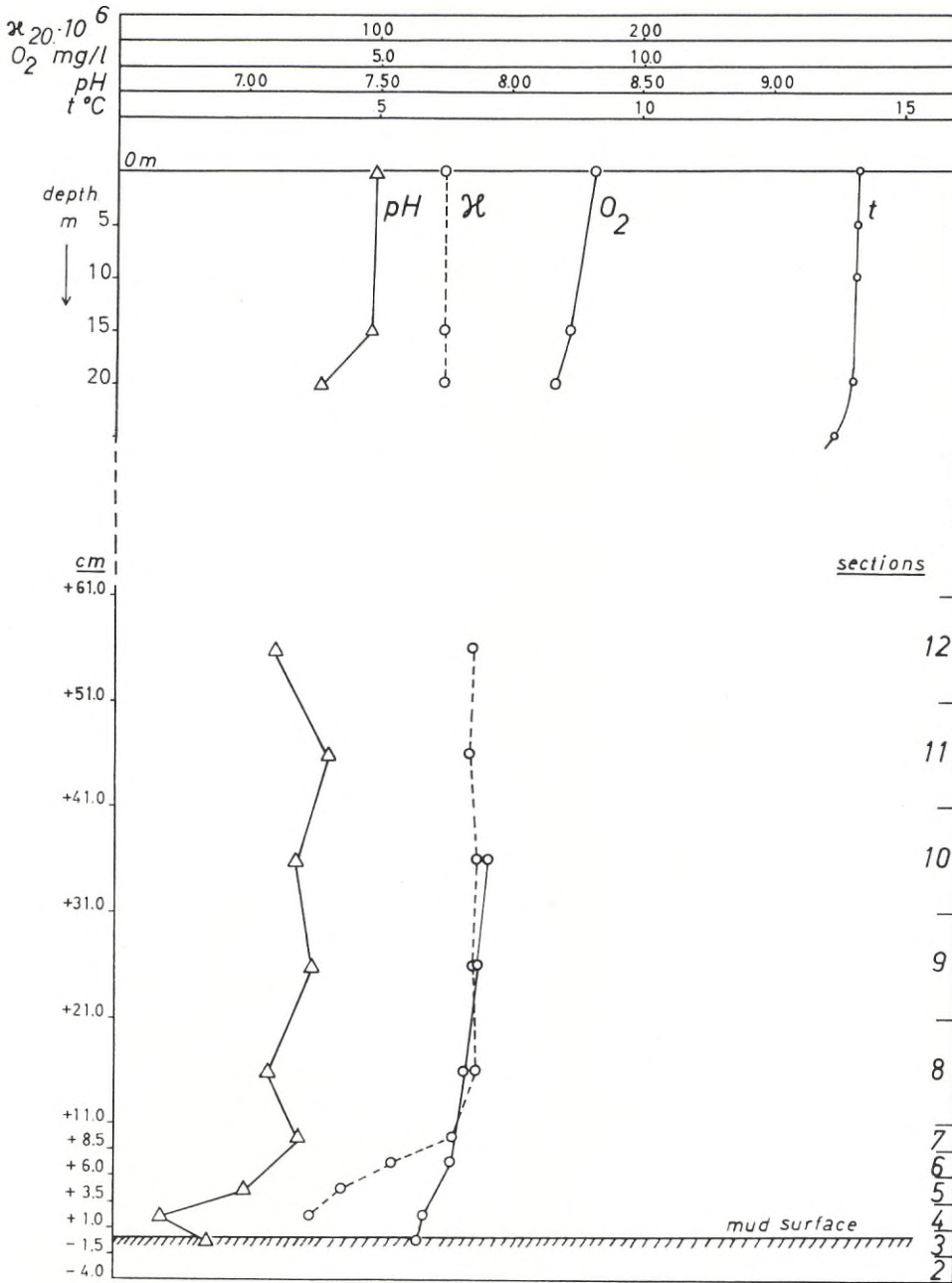
Fig. 1. Some physical factors and chemical variables in Norra Björkfjärden, Lake Mälaren. September 27, 1967. Depth, 26 m.
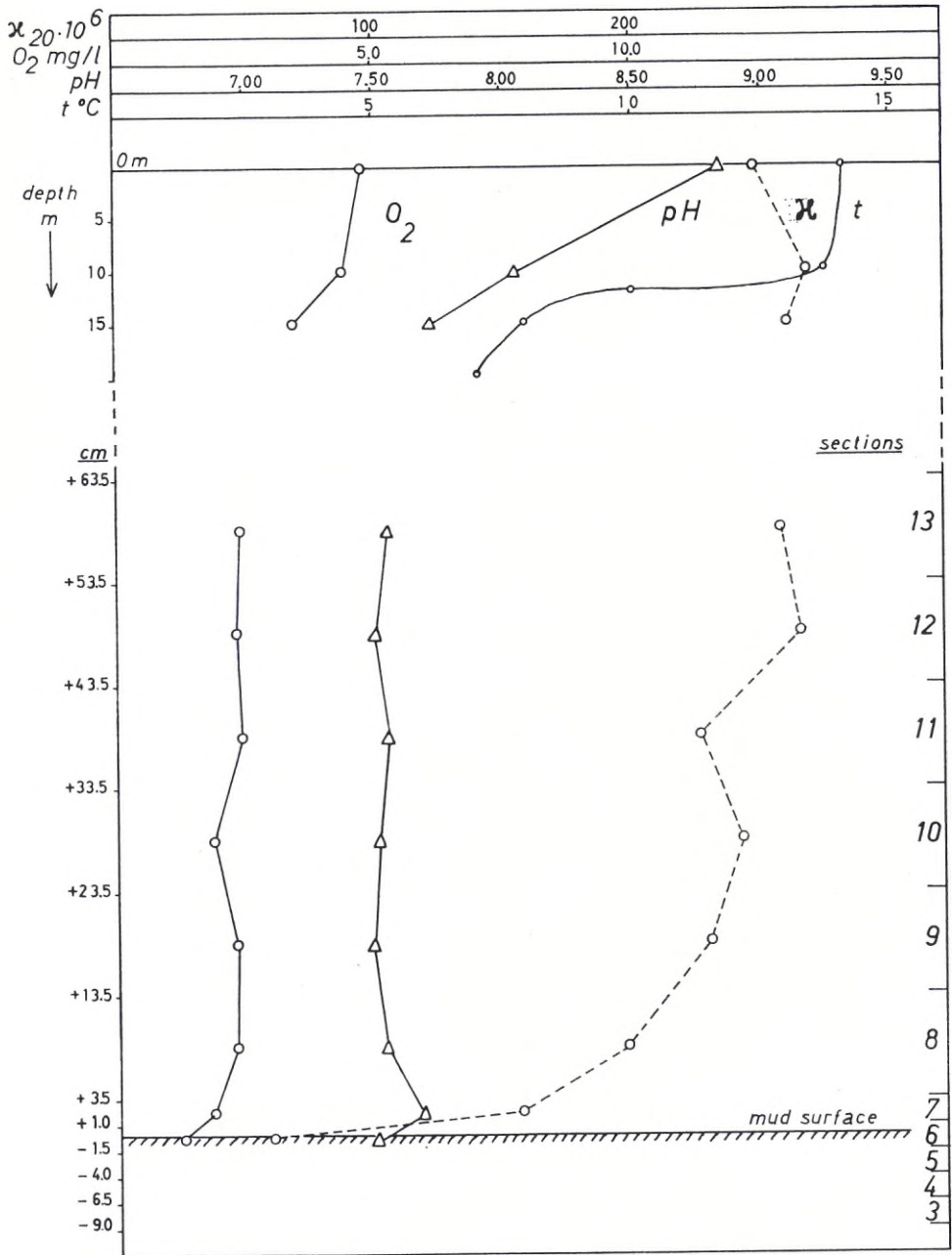
Fig. 2. Some physical factors and chemical variables in Lilla Ullevifjärden, Lake Mälaren. September 28, 1967. Depth, 21 m.
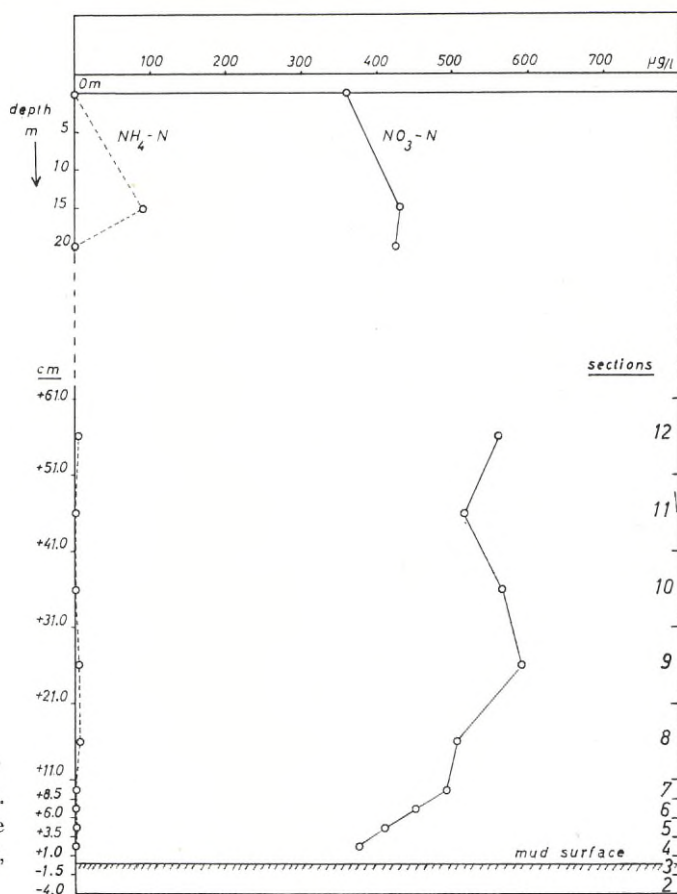
Fig. 3. NO₃-N and NH₄-N. Norra Björkfjärden, Lake Mälaren. September 27, 1967. Depth, 26 m.

Conductivity in the hypolimnion was found to be about twice as high in Lilla Ullevifjärden as in Norra Björkfjärden. Calcium makes up the great bulk of conductivity and consequently, as briefly discussed above, exhibits the same falling tendency towards the bottom.

The concentrations of hypolimnic nitrate-nitrogen in the samples from Lilla Ullevifjärden are just half those of Norra Björkfjärden (almost 500 μg/l) at this time of the year (Fig. 3 and Fig. 4). The hypolimnic supply of oxygen in the latter place is also high. The components probably follow each other.

Probably as an effect of primary production, the concentrations of nitrate-nitrogen in the samples from the epilimnion of Lilla Ullevifjärden were almost zero. On the other hand the supply of ammonia-nitrogen was fairly ample in the epilimnion.

Bottom waters comparatively rich in phosphate-phosphorus characterize Lilla Ullevifjärden as here at heights of about 20 to 30 centimetres above the
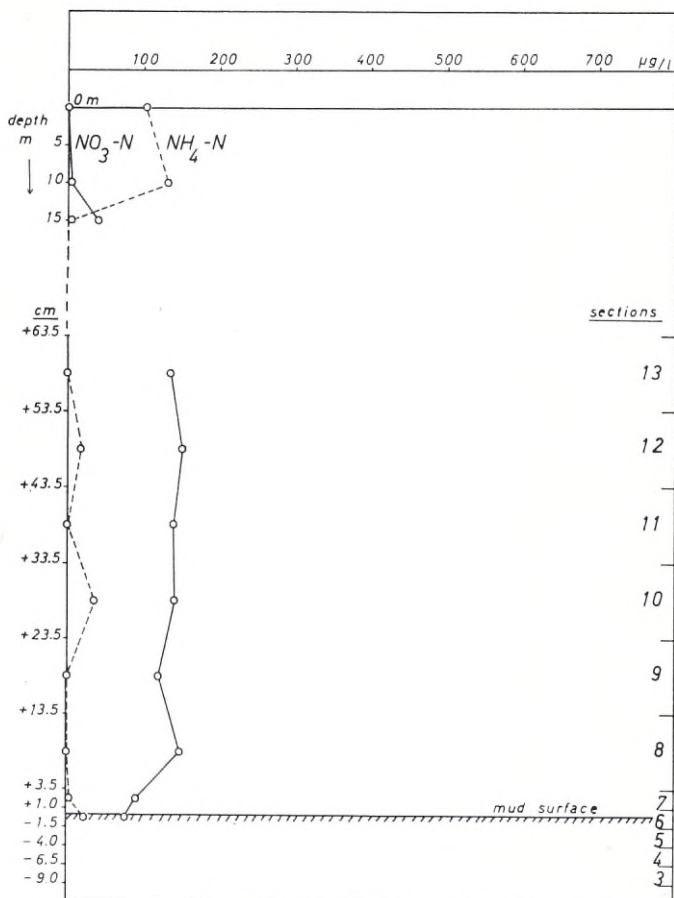
Fig. 4. NO₃-N and NH₄-N. Lilla Ullevifjärden, Lake Mälaren. September 28, 1967. Depth, 21 m.

mud surface. At the time of sampling the concentrations were about five times as high there as in Norra Björkfjärden. The general trend in both localities for phosphate-phosphorus is an increase towards the bottom.

The supply of total-phosphorus just above the interface was extremely high in both basins, especially in Norra Björkfjärden. As mentioned before, the reason is probably the presence of organic compounds at short distances above the mud.

Dr. AHL will continue this discussion in a paper in preparation.

III. Zoological data

Pontoporeia affinis if not found swimming in the water, is repeatedly taken a few centimetres below the mud surface in my cores (cf. Tables I a and I b).
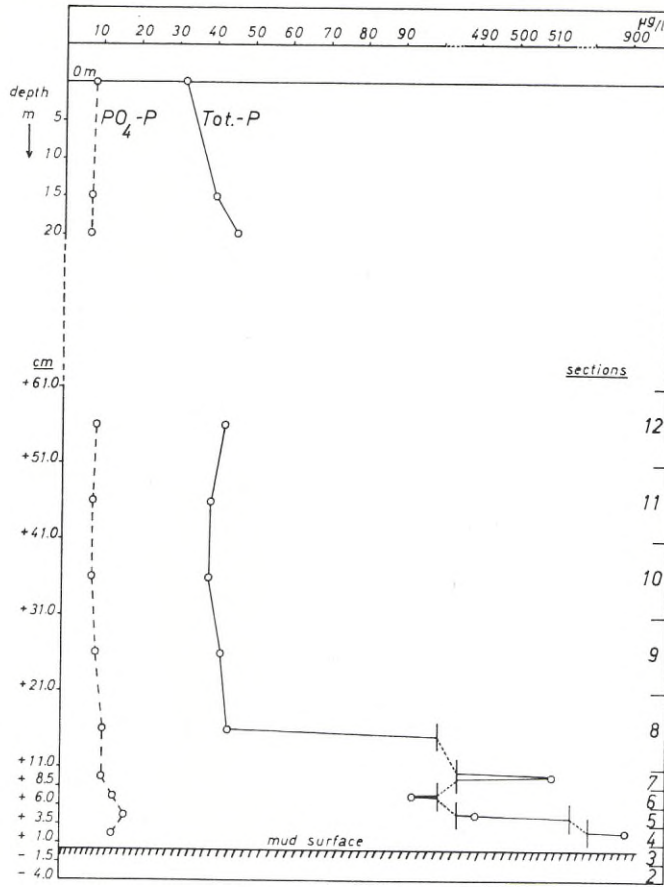
Fig. 5. PO$_4$-P and Tot.-P.
Norra Björkfjärden, Lake
Mälaren. September 27,
1967. Depth, 26 m.

Some *Calanoida* exhibit a very interesting distribution pattern in the cores illustrated in the Tables I a and I b. In view of the fragility of their bodies the mud penetrating capacity of *Eurytemora lacustris* to depths even exceeding 11 centimetres is quite puzzling. A similar tendency for *Eurytemora* has not been observed in other cores. On September 27 (Tabell II) the species was found not in the sediments, but rather close to the mud surface. Effects of diel migration and other temporal variations may explain the different distribution patterns.

The *Copepoda* probably have depth preferences characteristic for each species, varying with external factors such as temperature, light and chemical conditions. The Tables illustrate *examples* of vertical distribution of different *Copepoda*. The most interesting thing is that the species analyzed everytime seem well separated vertically.

Unfortunately fairly little is known about the vertical preferences of different *Crustacea* in the mud.
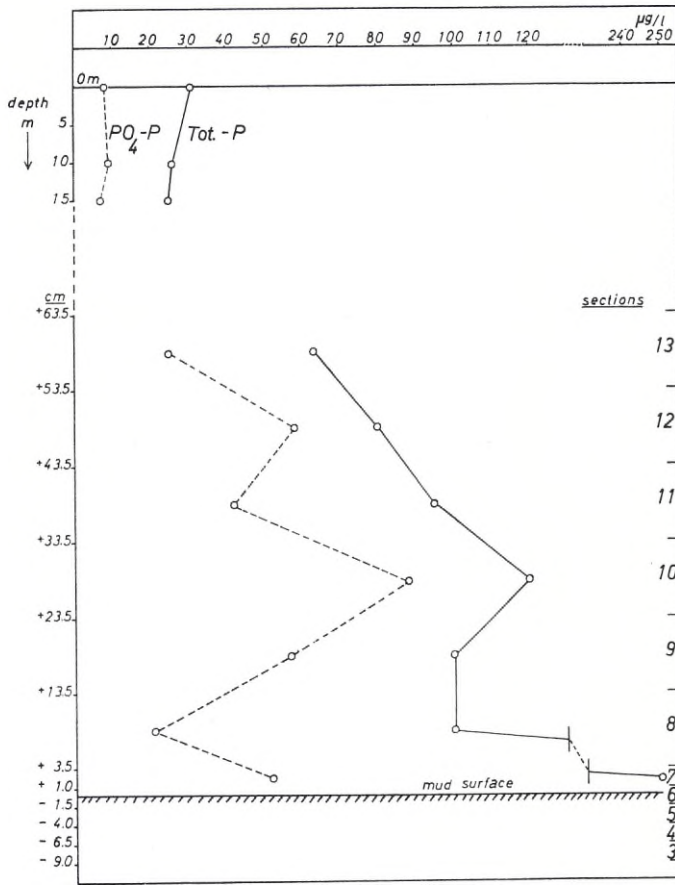
Fig. 6. PO4-P and Tot.-P. Lilla Ullevifjärden, Lake Mälaren. September 28, 1967. Depth, 21 m.

In one of the cores from Lilla Ullevifjärden (Table III) section No. 5 failed. The contents of that section were not properly sieved and could not be analyzed. Most specimens were expected to be found there. For this reason Table IV depicts a complete mud profile, illustrating the distribution pattern. (As mentioned before the spots of sampling were about 100 metres apart.)

As an example of *Cyclops* specimens dwelling in the mud, figures from Norra Björkfjärden as of February 13, 1968, are presented. Most specimens are *Cyclops sp. (strenuus)*. Unfortunately section No. 3 failed because of sieving difficulties.

Depth in cm	−21.5	−19.0	−16.5	−14.0	−11.5	−9.0	−6.5	−4.0	+6.0
Section No.	1	2	3	4	5	6	7	8	
Cyclops specimens	18	19	failed	66	129	127	91	243/4	

The specimens examined seemed very agile. They were not in "deep" diapause, anyhow. Obviously most specimens were concentrated at a mud depth of about 10 centimetres. But there were individuals living more than 20 centimetres deep in the relatively tough mud.

I have found the great bulk of the *Harpacticidae* species *Attheyella crassa* to be bound to the mud surface in these waters. In Norra Björkfjärden on September 9 the position of the *Harpacticidae* (Tables I a and I b) indicates an almost intact mud-water core.

In Table II the specimens were more widely spread, possibly indicating slight turbulence within the core. The specimens examined were extremely tiny. The distribution of *Tardigrada* at the same time may also confirm this (see text below).

Bosmina specimens often show a maximum in the contact layer of the cores (cf. Tables). Unfortunately, since the genus *Bosmina* has not been classified into species, no information can be obtained on the depth preferences of the different species.

Iliocryptus acutifrons is a common species in samples from central Lake Mälaren, usually bound to the mud surface (cf. Tables I a and I b), but sometimes occurring considerably above the interface.

In my cores the *Ostracoda* are usually dispersed through the entire mud- and water column with a distinct maximum close to the mud (see Tables). Since there are generally several *Ostracoda* species in the cores, obviously with different vertical preferences, it would have been interesting to classify the animals into genera or species.

The great bulk of the *Chironomidae* larvae in the cores from Norra Björk-fjärden consists of *Microspectra sp.* and *Sergentia sp.* concentrated in the interface layer (see Tables I a and II). Also *Tanypodinae* specimens are very common.

The presence of a *Cladotanytarsus sp.* in Table I a, in section No. 1, about 10 centimetres deep in the mud, is, however, conspicuous and possibly an error.

Similarly in Table I b is an *Orthocladinae* larva at a mud depth of about 10 centimetres.

In waters where *Chironomus plumosus* larvae are common, however, like in the profundal of Lake Erken or in several basins of Lake Mälaren I have repeatedly found these larvae dwelling 8—20 centimetres below the interface (unpublished material). For comparison see BERG, 1938. Since the sediments of the lakes investigated by BERG are much more soft and less mineralized, the animals recorded by him generally penetrate deeper into the mud.

In Table II one *Tanypodinae* larva and one *Orthocladinae* larva are recorded free-swimming.

Free-swimming *Chironomidae* larvae are a common phenomenon, but seldom described in literature except for *Tanypodinae* larvae, which are known to be very mobile.

But also *Chironominae* larvae, often in young stages, have frequently been found swimming in central Lake Mälaren, as observed in the course of

Table I a. Norra Björkfjärden, Lake Mälaren, Sep. 9, 1967. Depth, 32 m. Individuals per 400 ml. Living material examined.

Depth in cm / Section No.	Mud −11.5 / 1	Mud −9.0 / 2	Mud −6.5 / 3	Mud −4.0 / 4	Interface −1.5 / 5	Interface +1.0 / 6	Interface +3.5 / 7	Interface +6.0 / 8	Interface +16 / 9	Interface +26 / 10	Water +36 / 11	Water +46 / 12	Water +56 / 13	Water +66
Pontoporeia affinis (LINDSTR.)	—	—	—	1	1	—	—	—	—	—	—	—	—	—
Pallasea quadrispinosa (SARS)	—	2	—	—	1	—	—	—	—	—	—	—	—	—
Eurytemora lacustris (POPPE) ad.	20	—	1	3	1	—	—	—	—	—	—	—	—	—
Eudiaptomus graciloides (LILLEBORG) cop. IV	—	—	—	—	—	1	—	—	—	—	—	—	—	—
Limnocalanus grimaldii (DE GUERNE) ad.	—	—	—	—	—	—	—	—	—	—	—	—	2/4	—
Macrocyclops albidus (JURINE) ad.	—	—	—	—	—	1	—	—	—	—	—	—	—	—
Cyclops sp. (strenuus)	20	124	67	51	8	—	—	3/4	2/4	—	—	—	—	—
Megacyclops gigas (CLAUS) ad.	—	—	—	—	2	—	—	2/4	—	—	—	—	—	—
cop. V	—	—	1	6	32	9	1	—	—	—	—	—	—	—
cop. IV	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Mesocyclops leuckarti (JURINE) ad.	—	3	—	2	—	—	—	—	—	—	—	—	—	—
cop. V	—	6	—	—	—	—	—	—	—	2/4	—	1/4	—	—
cop. V	—	—	—	—	—	—	—	—	—	—	—	—	1/4	—
Thermocyclops sp.	—	—	—	—	3	—	—	—	—	2/4	—	—	—	—
Atheyella crassa (SARS)	—	—	—	—	9	2	1	—	—	—	—	—	—	—
Daphnia sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bosmina sp.	—	—	—	—	27	1	—	1/4	—	—	1/4	1/4	—	—
Alona affinis (LEYDIG)	1	—	—	—	29	4	3	2/4	—	—	—	—	—	—
Iliocryptus acutifrons (SARS)	—	—	—	—	18	2	—	—	—	—	—	—	—	—
Drepanothrix dentata (EUREN)	—	—	—	—	—	—	1	—	—	—	—	—	—	—

Depth in cm Section No.	Mud			Interface							Water		
	1	2	3	4	5	6	7	8	9	10	11	12	13
Ostracoda			6	19	187	22	7	10/4	4/4	1/4			
Limnochironomus sp.					1								
Sergentia sp.				2	5								
Stictochironomus histriogroup				1									
Tanytarsini pupae (in general)					2								
Microspectra sp.					11	1							
Cladotanytarsus sp.	1												
Tanypodinae (Procladius sp.)				4	2								
Hydracarina					1								
Euilyodrilus hammoniensis (MICHAELSEN)		2	5	33	29								
Limnodrilus hoffmeisteri (CLAPARÈDE)		2	3	5	2								
Chaetogaster cristallinus (VEJDOVSKY)					2	2							
— Chains					3								
Speciaria josinae (VEJDOVSKY)					1								
Uncinais uncinata (ØRSTED)					1								
Slavina appendiculata (D'ÜDEKEM)						1							
Vejdovskyella comata (VEJDOVSKY)					2	2							
Vejdovskyella intermedia (BRETSCHER)				4	45		2						
Arcteonais lomondi (MARTIN)				1									
Nematoda			2	32	37	1	1	1/4					
Pisidium sp.					2	1							
Hydra sp.					4								
Tardigrada					18								

Table I b. Norra Björkfjärden, Lake Mälaren, Sep. 9, 1967. Depth, 32 m. Individuals per 400 ml. Alcohol-preserved material examined (not analysed in detail).

Depth in cm Section No.	—11.5 1	—9.0 2	—6.5 3	—4.0 4	—1.5 5	+1.0 6	+3.5 7	+6.0 8	+16.0 9	+26.0 10	+36.0
Pontoporeia affinis (LINDSTR.)	—	—	9	—	—	—	—	—	—	—	
Harpacticidae	—	—	—	—	×	—	—	—	—	—	
Cyclops sp.	331	170	51	44	54	10	2	4/4	1/4	—	
Calanoida	—	—	1[1]	5[1]	—	1	—	5/4[2]	5/4[2]	—	
Daphnia sp.	—	—	—	—	—	3	1	1/4	2/4	—	
Bosmina sp. and *Alona affinis* (LEYDIG) ..	—	—	—	1	19	4	1	3/4	—	—	
Iliocryptus acutifrons (SARS)	—	—	—	1	10	1	—	2/4	—	—	
Ostracoda	2	7	5	61	114	8	6	4/4	—	—	
Chironomidae, larvae	1[3]	—	4	12	12	1[4]	1[4]	1/4[4]	—	—	
Oligochaeta	1	6	9	20	26	—	—	3/4[5]	—	—	
Nematoda	2	2	41	57	2	—	—	1/4	—	—	
Plathelmintes	—	—	—	—	1	—	—	—	—	—	
Pisidium sp.	—	1	—	2	2	—	—	—	—	—	
Hydra sp.	—	—	—	—	1	—	—	—	—	—	
Tardigrada	—	—	—	—	×	—	—	—	—	—	

× Very small specimens difficult to discern owing to alcohol-preservation.

[1] *Eurytemora lacustris* (POPPE)
[2] *Limnocalanus grimaldii* (DE GUERNE)
[3] *Orthocladinae larva*
[4] *Tanypodinae larvae*
[5] 2 *Chaetogaster cristallinus* (VEJDOVSKY) and 1 *Vejdovskyella intermedia* (BRETSCHER)

funnel trap investigations in the summer of 1967 (unpublished material). J. H. MUNDIE, for instance (1965) has reported similar results.

In a sample from Görväln (a basin of Lake Mälaren) on June 3, 1967, a young *Microspectra sp.* larva was found in section No. 7, 10 centimetres above and in section No. 12, 60 centimetres above the mud surface, an *Orthocladinae* was collected. Section No. 3, in the interface layer, contained two *Microspectra sp.* and two *Tanypodinae* larvae (unpublished material).

As mentioned before, of particular interest to the author, is the vertical distribution of *Oligochaeta*. Representatives of the families *Tubificidae, Naididae,* and *Lumbriculidae* are fairly well-known from these basins.

Tubificidae as a group are supposed to be mud-preferring organisms, often penetrating into deep mud strata. This is not quite true, since some *Tubificidae,* for instance *Peloscolex ferox,* demand much oxygen (BRINKHURST, 1964 b), which consequently attracts them to the mud surface. As a rule, slender, immature *Tubificidae* are able to swim for short distances and may sometimes be found in the upper sections of my cores.

The behaviour of the *Naididae,* however, is little known. C. SPERBER (1948) mentions only, whether the species are recorded as swimming or non-swim-

Table II. Norra Björkfjärden, Lake Mälaren, Sep. 27, 1967. Depth, 26 m. Individuals per 400 ml. Living material examined.

Depth in cm		Interface						Water					
		−4.0	−1.5	+1.0	+3.5	+6.0	+8.5	+11.0	+21	+31	+41	+51	+61
Section No.		2	3	4	5	6	7	8	9	10	11	12	13
Eurytemora lacustris (POPPE)	ad.	—	1	1	1	3	2	11/4	13/4	20/4	15/4	—	—
	cop. IV	—	—	—	—	—	—	—	—	—	—	1/4	—
Eudiaptomus graciloides (LILLJEBORG)	cop. I	—	—	—	—	—	—	1/4	1/4	—	—	—	—
	cop. IV	—	—	—	—	—	—	1/4	1/4	—	—	4/4	1/4
	cop. II	—	—	—	—	—	—	—	—	—	—	3/4	1/4
	cop. V	—	—	—	1	1	1	—	—	—	—	—	—
Macrocyclops albidus (JURINE)		—	—	—	—	—	—	—	—	—	—	—	—
Cyclops sp. (strenuus)	cop. V	10	32+	20+	1	1	1	6/4	—	—	8/4	—	4/4
Thermocyclops sp.		—	—	—	—	1	—	—	—	—	3/4	—	1/4
Attheyella crassa (SARS)		—	1	+	4+	10	—	—	—	—	—	—	—
Bosmina sp.		—	1	1	1	—	—	—	—	—	—	1/4	—
Alona affinis (LEYDIG)		—	—	—	—	—	—	—	1/4	—	—	—	—
Iliocryptus acutifrons (SARS)		—	—	—	—	—	—	1/4	—	—	—	—	—
Ostracoda		16	40	1	1	—	—	—	—	—	—	—	—
Chironomini (unidentified)		—	1	—	—	—	—	—	—	—	—	—	—
Kribioxenus Brayi (GOETGH.)		—	1	—	—	—	—	—	—	—	—	—	—
Tanytarsus s. str.		—	2	—	—	—	—	—	—	—	—	—	—
Microspectra sp.		4	16	1	—	—	—	—	—	—	—	—	—
Cladotanytarsus sp.		3	3	1	1	1	—	—	—	—	—	—	—
Tanypodinae (*Procladius sp.*)		—	—	—	—	1	—	—	—	—	—	—	—
Orthocladiinae		—	—	—	1	—	—	—	—	—	—	—	—
Hydracarina		—	1	1	—	—	—	—	—	—	—	—	—
Peloscolex ferox (EISEN)		—	1	1	—	—	—	—	—	—	—	—	—
Tubifex tubifex (MÜLLER)		—	—	1	—	—	1	—	—	—	—	—	—
Unidentified Tubificidae		—	1	—	—	—	—	—	—	—	—	—	—
Chaetogaster cristallinus (VEJDOVSKY)		1	4	—	—	—	1	—	—	—	—	—	—
Chaetogaster setosus (SVETLOV)		—	—	—	—	—	—	—	—	—	—	—	—
Slavina appendiculata (D'UDEKEM)		—	2	—	1	1	—	—	—	—	—	—	—
Vejdovskyella comata (VEJDOVSKY)		—	2	2	—	—	—	—	—	—	—	—	—
Vejdovskyella intermedia (BRETSCHER)		—	—	—	—	—	—	—	—	—	—	—	—
Unidentified Naididae		—	2	2	—	—	—	—	—	—	—	—	—
Stylodrilus heringianus (CLAPARÈDE)		1	—	—	—	—	—	—	—	—	—	—	—
Nematoda		13	14	1	1	—	—	—	—	—	—	—	—
Pisidium sp.		—	1	—	—	—	—	—	—	—	—	—	—
Plathelmintes		1	5	—	—	2	—	—	—	—	—	—	—
Tardigrada		—	—	++	+	3	—	—	—	—	—	—	—

++ Plus a great number of very small animals, difficult to count.
+ Plus a few very small animals, difficult to count.

Table III. Lilla Ullevifjärden, Lake Mälaren, Sep. 28, 1967. Depth, 21 m. Individuals per 400 ml. Living material examined.

Taxon / Stage	Mud				Interface					Water			
Depth in cm	−11.5	−9.0	−6.5	−4.0	−1.5	+1.0	+3.5	+6.0	+8.5	+18.5	+28.5	+38.5	+48.5
Section No.	1	2	3	4	5	6	7	8	9	10	11	12	
Eudiaptomus graciloides (LILLJEBORG) ad.	—	—	—	—	—	—	—	1/4	—	—	—	—	2/4
Cyclops sp. (strenuus) ad.	2	7	3	—	9+	5+	12	—	—	3/4	—	—	—
Megacyclops gigas (CLAUS) ad.	—	—	—	—	—	1	—	—	—	—	—	—	—
Diacyclops bicuspidatus (CLAUS) ad.	—	—	—	—	—	1	—	—	—	—	—	—	—
cop. IV	—	—	—	—	1	—	—	—	1/4	—	—	—	—
Mesocyclops leuckarti (JURINE) ad.	—	—	—	—	3	—	—	—	—	—	—	—	—
cop. V	—	1	—	—	8	—	—	—	—	—	—	—	—
Thermocyclops sp. ad.	—	—	—	—	—	4	7	—	—	—	—	—	—
cop. IV	—	—	—	—	—	1	—	27+/4	5/4	1/4	2/4	—	2/4
Daphnia sp.	—	—	—	—	—	—	—	—	—	1/4	—	1/4	—
Bosmina sp.	—	—	—	—	—	1/4	—	—	—	—	2/4	2/4	—
Ostracoda	—	—	—	—	1	1	—	—	—	—	1/4	—	—
Plecoptera	—	—	—	—	—	—	—	—	—	—	—	1/4	—
Tubifex tubifex (MÜLLER)	—	—	—	—	1	—	—	—	—	—	—	—	—
Eutilyodrilus hammoniensis (MICHAELSEN)	2	2	—	—	2	2	—	—	—	—	—	—	—
Nematoda	48	—	—	—	++	+	—	—	—	—	—	—	—

++ Plus a great number of very small animals, difficult to count.
+ Plus a few very small animals, difficult to count.

Table IV. Lilla Ullevifjärden, Lake Mälaren, Sep. 28, 1967. Depth, 21 m. Individuals per 400 ml. Living material examined.

		Mud		Interface Water	
Depth in cm		—6.0	—3.5	—1.0 +1.5	+4.0
Section No.		1	2	3	4
Eudiaptomus graciloides (LILLJEBORG)		—	1	—	—
Cyclops sp. (*strenuus*)		15	326+	190	31+
Megacyclops gigas (CLAUS)	cop. IV	—	—	3	—
Diacyclops bicuspidatus (CLAUS)	ad.	—	—	—	1
	cop. IV	—	—	2	16
Bosmina sp.		—	9	2	—
Ostracoda		—	23	7	1
Euilyodrilus hammoniensis (MICHAELSEN)		9	35	7	2
Tubifex tubifex (MÜLLER)		2	—	—	—
Unidentified *Tubificidae*		—	2	—	1
Nematoda		—	1	+	—
Plathelmintes		—	1	1	—

+ Plus a few very small animals, difficult to count.

ming. The majority are recorded as non-swimming. While many *Naididae* species are certainly not free-swimming, nevertheless several are able to swim a few centimetres or more up into the water column.

The samples from September 9 originate in a slightly enriched sound of Norra Björkfjärden, which is reflected in the composition of *Oligochaeta*, while the samples from September 27 originate in the central *viz.* cleanest part of the basin.

According to Table I a, the *Tubificidae, viz. Euilyodrilus hammoniensis* and *Limnodrilus hoffmeisteri,* are obviously spread from a depth in the mud of about 8 centimetres to the interface, with a maximum a few centimetres below it.

The species mentioned above, esp. *L. hoffmeisteri,* are known to be very tolerant to low concentrations of oxygen and may be dominant under severe conditions. On the other hand they occur frequently even in unpolluted waters, but are then seldom in dominance (BRINKHURST, 1964 b). So is also the case with *E. hammoniensis,* which is very common in all investigated basins of Lake Mälaren.

Peloscolex ferox is a common *Tubificidae* in clean or mildly polluted waters (BRINKHURST, 1966) and is characteristic for Norra Björkfjärden as a whole (cf. Table II). *Tubifex tubifex* occurs frequently in clean waters but can also tolerate very high degrees of pollution (BRINKHURST, 1966). It is not common in this basin.

In view of the severe conditions in the lower profundal of Lilla Ullevifjärden it is no wonder that *E. hammoniensis* at this depth dominates the *Oligochaeta* fauna. *T. tubifex* is tolerant enough to withstand this milieu (Tables III and IV) and will replace the former species at still greater depths.

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Keeping in mind the great mud penetrating capacity of esp. the tolerant species *E. hammoniensis* and *L. hoffmeisteri* I find it remarkable that *Tubificidae* are seldom the deepest burrowing animals in the cores.

Seven different *Naididae* were found in the cores of September 9, but none of them at remarkable distances above the interface.

Vejdovskyella intermedia is generally the dominating *Naididae* on soft bottom sediments in Lake Mälaren. It is very common with a maximum of occurrance in the interface layer or just above it (cf. Tables I a, I b, and II). Remarkably enough, records of *V. intermedia* in Sweden are extraordinarily few. The species was recorded only from Lake Vättern in 1915 by EKMAN (SPERBER, 1948).

Three chains of *Chaetogaster cristallinus* were observed in the interface layer (Table I a), each of them consisting of two or three specimens. Budding and fission continue during earlier stages of maturity.

In Norra Björkfjärden on September 27, *Chaetogaster setosus* (SVETLOV) was caught about 10 centimetres up in the water column. This is quite remarkable and possibly a consequence of slight turbulence. Before it was not recorded outside Russia (SPERBER, 1948). The species is very inconspicuous, just 0.6 mm long.

Stylodrilus heringianus, a *Lumbriculidae*, is a characteristic species of soft bottom sediments with some sand elements in the deep waters of Norra Björkfjärden (Table II).

Nematoda are generally concentrated in the upper sediment layers as in Table I a and Table II, but can be found anywhere within a core, often very deep in the sediments. The specimens may be free-swimming, as well (Tables I a and I b).

In the samples from Lilla Ullevifjärden (Table III), the *Nematoda* fauna was dominated by at least two different populations. One, consisting of comparatively big animals, lived about 10 or more centimetres deep in the mud. The other population, consisting of very tiny specimens, was attracted to aggregates of sedimented *Melosira*-filaments in the interface layer.

Hydra sp. and *Tardigrada* are generally typical "mud surface animals", while *Pisidium sp.* may also dwell in deeper layers (cf. BERG, 1938).

Tardigrada are known from terrestrial habitats and the upper littoral, but evidently are frequent at depths of 30 metres. The records of tiny *Tardigrada* specimens limited to the interface region bespeak very little turbulence within the cores (Tables I a and I b).

In Norra Björkfjärden on September 27, the *Tardigarda* were very common on the detritus particles in the interface region, but also a few centimetres up, probably due to slight turbulence within this particular core.

As a rule, specimens of *Plathelmintes* are limited to the interface layer in my cores (cf. Tables I b and II), but they also at times occur high up in the water column.

IV. Acknowledgements

V. Summary

With a new microstratification sampler designed by the author for the purpose (OIKOS 19,1.) further tests have been carried out. At the end of the summer stratification period in 1968 samples were taken parallelly in two basins of Lake Mälaren.

The mud-water cores have been analysed in detail to illustrate what information may be obtained with this technique.

Each core yields samples for, among other things, chemical and zoological analyses. Of special interest are the sites of the different animal species in relation to the mud surface. What is "bottom fauna" in this respect?

The chemical concentrations and the physical factors obtained very close to the mud surface are also of the greatest interest.

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On the Composition and Distribution of Oligochaetes in Lake Vättern 1967–1968

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I. Introduction

.Oligochaetes may be extremely useful in studying water pollution since, amongst other things, they pass their entire life in the sediments and do not move around much. Moreover, the species composition of the oligochaete community and their numbers reflect the long term changes in the overlying water, and the variation in numbers and species composition from year to year are fairly small.

The group of particular interest amongst the oligochaetes for pollution studies are the tubificids, as they may constitute 30 per cent or more of the total biomass and numbers of bottom animals. Since, however, it has only recently become possible to identify them to species with relative ease owing to the systematic revision of the group by Dr. Brinkhurst and Dr. Kennedy, amongst others, our knowledge of this important group of bottom dwelling animals is still incomplete in many respects.

Between 1911 and 1912 Prof. Ekman collected a large amount of oligochaete material from Lake Vättern which he sent to Prof. Piguet for identification. His original specimens were re-examined in the 1960's by Dr. Binkhurst who confirmed and occasionally corrected the specific identifications. Since the present author carried out a sampling programme in 1967 and 1968 for the Lake Vättern Preservation Committee in the same localities using the same methods as Ekman a unique opportunity arose to make a qualitative and quantitative comparison of the oligochaetes over an interval of 55 years. Such a comparison could also reveal whether any changes in the water and sediments of the lake, particularly those resulting from pollution, had taken place during the interval.

The ecological notes on the different tubificids are derived from Brinkhurst, 1964 b: "Observations on the biology of lake-dwelling *Tubificidae*" and from Dr. Kennedy, University of Exeter, England (personal communication).

II. Presentation of material and discussion

The tubificids are frequently found in large numbers in polluted water. The animals are not, as one can be misled into believing, attracted by bottom filth. It is rather the case that certain species have a greater capacity for

withstanding lack of oxygen caused by organic decomposition than other bottom animals have, and their numbers can rapidly increase when predators are lacking.

A small number of tubificid species are very tolerant of unfavourable conditions, and, when most of the other bottom animals, including predators, are greatly reduced in numbers, these tolerant species assume the dominant position among the bottom fauna. This is true, for example, of *Limnodrilus hoffmeisteri, Tubifex tubifex,* and *Euilyodrilus hammoniensis.*

It should be noted that these three species often occur in large numbers even under conditions which are poor in nourishment and rich in oxygen. *T. tubifex,* for instance, is common in oligotrophic lakes at elevations considerably above sea level.

It is accordingly not the occurrence of certain species in a community which reveals the condition of the bottom and consequently the quality of the water, but rather the lack of certain species and great abundance of others.

In a polluted milieu there are generally to be found one or two tolerant species of tubificids but in a milieu relatively poor in nourishment but rich in oxygen there often occur up to ten species.

In corroboration of this statement it can be noted that Ekoln, to the south of Uppsala, and Västeråsfjärden, which are among the most polluted parts of Lake Mälaren, contain *Limnodrilus hoffmeisteri, Tubifex tubifex,* and *Euilyodrilus hammoniensis.* To recapitulate 2 or 3 species constitute 90—100 per cent of the tubificids in the sub-littoral and the profundal and the number of individuals is very high. On the other hand, one can mention that in Norra Björkfjärden one of the least polluted parts of Lake Mälaren, at least 5—6 species are found, as a rule, with *Peloscolex ferox* and *Psammoryctes barbatus* predominating. The quality of the water in Norra Björkfjärden, however, is substantially worse than that of Lake Vättern, where at a depth of 20—40 metres up to 10 species can occur, provided a sufficiently quantity of decomposable material is present in the sediment. If, on the other hand, the organic contents of the sediment are very low, the number of specimens of oligochaetes is accordingly quite insignificant.

This also applies, for example, to the sub-littoral of central Lake Vättern,

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Tables I and II:

The left hand columns indicate area and depth. *S* signifies that the dredgings derive from the Jönköping district or directly north of it; *M* from a line drawn from Hästholmen towards Hjo; and *N* from the inlet of the Motala Ström and westerly towards Karlsborg. "EKMAN's m" is the average for the lake as a whole, which EKMAN calculated for four important species.

Table I. The composition and distribution of oligochaetes on different localities and depths in Lake Vättern.

Specimens/5 dm² Locality, depth	*Peloscolex ferox* (EISEN)	*Limnodrilus helveticus* (PIGUET)	*Psammoryctes barbatus* (GRUBE)	*Tubifex tubifex* (MÜLLER)	*Eutilyodrilus hammoniensis* (MICHAELSEN)	*Limnodrilus hoffmeisteri* (CLAPARÈDE)	*Tubifex ignotus* (STOLC)	*Rhyacodrilus coccineus* (VEJDOVSKY)	*Aulodrilus limnobius* (BRETSCHER)	*Bothrioneurum vejdovskyanum* (STOLC)	*Stylodrilus heringianus* (CLAPARÈDE)	*Arctenais lomondi* (MARTIN)	*Stylaria lacustris* (LINNAEUS)	*Uncinais uncinata* (ØRSTED)	Immature *Limnodrilus* sp.	Immature *T. tubifex* or *E. hammoniensis*	Unidentified enchytraeids	*Rhyacodrilus ekmani* (PIGUET)	*Rhyacodrilus falciformis* (BRETSCHER)	*Rhynchelmis limosella* (HOFFMEISTER)	*Vejdovskyella intermedia* (BRETSCHER)	*Chaetogaster diaphanus* (GRUITHUISEN)	Sum Tot.	4 sp.
S (Jönköping), 100 m	18	31	13	8	—	—	—	—	—	—	28	—	—	—	—	—	—	—	—	—	—	—	98	67
M (Hästholmen), ,, ,,	11	24	9	7	—	—	—	—	—	—	14	—	—	—	—	—	—	—	—	—	—	—	65	41
N (Motala), ,, ,, 15–28 EKMAN's m	15–28	—	4.5–7.0	4.3–7.0	—	—	—	—	—	—	16–30	—	—	—	—	—	—	—	—	—	—	—	39.8–72.0	—
S ,, 50 m	1	—	<1	2	—	—	—	—	—	—	8	—	—	—	—	—	—	—	—	—	—	—	10	10
M ,, ,,	2	6	4	3	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—	—	13	13
N ,, ,, 1.0–2.7 EKMAN's m	1.0–2.7	—	0.3–1.3	0.3–0.7	—	—	—	—	—	—	2.1–7.5	—	—	—	—	—	—	—	—	—	—	—	3.7–12.2	20/14
S ,, 20–40 m	29	2	4	12	4	4	2	2	—	—	3	—	—	—	4	4	—	—	—	—	—	—	67	48
M ,, ,,	<1	—	<1	<1	<1	<1	—	—	<1	—	2	—	<1	—	<1	—	<1	—	—	—	—	—	3	3
N ,, ,, 3.2–9.0 EKMAN's m	8	—	0.2–2.0	0.1–1.2	—	1	—	—	—	—	4	7	—	—	4	—	<1	—	—	—	—	—	28	14
EKMAN's m 3.2–9.0	3.2–9.0	—	2.0	1.2	—	—	—	—	—	—	3.4–3.3	—	—	—	—	—	—	—	—	—	—	—	7.9–15.5	—
S ,, 10–20 m	13	—	10	1	2	3	<1	2	—	—	16	—	—	—	—	—	—	—	—	—	—	—	44	40
M ,, ,,	4	—	2	<1	—	—	—	—	—	—	2	—	—	1	—	—	—	—	—	—	—	—	12	7
N ,, ,,	30	—	2	0	—	<1	<1	—	—	—	<1	—	4	—	—	—	<1	37	—	—	—	—	39	33
EKMAN's m 8.6	8.6	—	—	<1	—	—	—	—	—	—	1.3	—	—	—	—	—	—	—	—	—	—	—	11.9	—

Samples taken by S. EKMAN on the same localities in 1911–1912.

	Peloscolex ferox	*Limnodrilus helveticus*	*Psammoryctes barbatus*	*Tubifex tubifex*	*Eutilyodrilus hammoniensis*	*Limnodrilus hoffmeisteri*	*Tubifex ignotus*	*Rhyacodrilus coccineus*	*Aulodrilus limnobius*	*Bothrioneurum vejdovskyanum*	*Stylodrilus heringianus*	*Arctenais lomondi*	*Stylaria lacustris*	*Uncinais uncinata*	Imm. *Limnodrilus*	Imm. *T. tubifex*	enchytraeids	*Rhyacodrilus ekmani*	*Rhyacodrilus falciformis*	*Rhynchelmis limosella*	*Vejdovskyella intermedia*	*Chaetogaster diaphanus*	Tot.	4 sp.
S 100 m	—	—	—	20	—	—	—	—	—	—	60	—	—	—	—	—	—	—	—	—	—	—	165	146
S 120 m	—	15	13	—	—	—	—	—	—	—	14	—	2	4	—	—	1	—	—	1	—	—	22	15
M 100 m	—	—	13	4	—	—	—	—	—	—	27	—	4	—	—	—	—	—	—	—	—	—	67	67
M 100 m	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	1	—	—	1	9	—	15	3
S 50 m	—	—	—	—	—	—	—	—	—	—	9	—	—	—	—	—	1	—	1	—	5	1	10	9
S 20–40 m	106	—	27	16	21	—	—	—	—	—	31	—	—	—	—	—	23	37	—	—	—	23	243	180
N ,, ,,	7	—	—	—	—	—	—	—	—	—	6	—	—	—	—	—	—	—	—	—	—	—	59	13

where the steep inclination of the bottom is a causative agent in the rapid conveyance of organic material down to greater depths.

Table I provides a graphic illustration of the distribution of oligochaetes calculated per 5 dm² in different parts and at different depths in Lake Vättern during 1911—1912 and 1967—1968. We are concerned in all with twelve definitely identified species of tubificids and eight remaining oligochaetes of which *Stylodrilus heringianus* obviously (see Tables I and II) is of considerable importance in the profundal.

The left hand side of the table is devoted to tubificids which I repeatedly found in my dredgings. From and including *Stylodrilus* the remaining oligochaetes follow as far as up to *Rhyacodrilus ekmani,* where species just found by EKMAN continue.

"EKMAN's m" represents the average value for the lake as a whole, which EKMAN was able to calculate for four important species. His calculations are based on a relatively large number of dredgings. The care with which the calculations were made has been well vouched for, a factor which renders their values usable for subsequent comparisons.

Far-reaching conclusions, however, cannot and should not be drawn from the material.

The lowest portion of the table takes up samples which EKMAN had analysed in their entirety. Because these are of interest from a qualitative viewpoint they have been included; they are, however, hardly of any quantitative significance, because the number of dredgings was too small.

The right hand side of Table I indicates the total number of individuals in each area and deep zone, and also the totals for the four species for which EKMAN calculated the averages.

All figures in the table represent averages and are based on three series of sampling, the whole accounting for a large number of dredgings. (Central Vättern is covered, however, by only one series).

Table II diverges from Table I insofar as the figures have been transformed into percentages in order to display the dominant species within each area and deep zone.

As far as the 100 metre level and deep holes are concerned, it is generally true that a local accumulation of decomposable organic material probably has beneficial effects on the density of the tubificid fauna, provided the oxygen supply is good, which is the case in Lake Vättern. The values for *S* (southern Lake Vättern) are always above EKMAN's average values, which can be explained by effects of pollution from Jönköping—Huskvarna, (cf. Information from "Lake Mälaren Research", Report No. 23, and GRIMÅS, 1969).

The dominant species, according to Tab. II are *Limnodrilus helveticus,* a well-known species of the extreme profundal of deep Swiss lakes — probably a stenothermous coldwater species, also known from rivers — and

Table II. The composition and distribution of oligochaetes on different localities and depths in Lake Vättern. The figures in percentages of totals of oligochaetes.

Locality, depth	*Peloscolex ferox* (EISEN)	*Limnodrilus helveticus* (PIGUET)	*Psammoryctes barbatus* (GRUBE)	*Tubifex tubifex* (MÜLLER)	*Euiliodrilus hammoniensis* (MICHAELSEN)	*Limnodrilus hoffmeisteri* (CLAPARÈDE)	*Tubifex ignotus* (STOLC)	*Rhyacodrilus coccineus* (VEJDOVSKY)	*Aulodrilus limnobius* (BRETSCHER)	*Bothrioneurum vejdovskyanum* (STOLC)	*Stylodrilus heringianus* (CLAPARÈDE)	*Arcteonais lomondi* (MARTIN)	*Stylaria lacustris* (LINNAEUS)	*Uncinais uncinata* (ÖRSTED)	Immature *Limnodrilus* sp.	Immature *T. tubifex* or *E. hammoniensis*	Unidentified enchytraeids	*Rhyacodrilus ekmani* (PIGUET)	*Rhyacodrilus falciformis* (BRETSCHER)	*Rhynchelmis limosella* (HOFFMEISTER)	*Vejdovskyella intermedia* (BRETSCHER)	*Chaetogaster diaphanus* (GRUITHUISEN)	%
S 100 m	18	31	13	8	—	—	—	—	—	—	28	—	2	—	—	—	—	—	—	—	—	—	100
M „	17	37	14	11	—	—	—	—	—	—	21	—	—	—	—	—	—	—	—	—	—	—	100
N „	11	—	4	—	—	—	—	—	—	—	85	—	—	—	—	—	—	—	—	—	—	—	100
S 50 m	15	—	31	15	—	—	—	—	—	—	39	—	—	—	—	—	—	—	—	—	—	—	100
M „	5	30	15	15	—	—	—	—	—	—	35	—	—	—	—	—	—	—	—	—	—	—	100
N „	44	3	6	18	6	0.5	3	3	—	—	4.5	—	—	—	6	6	—	—	—	—	—	—	100
S 20—40 m	17	—	—	—	—	—	—	—	—	—	83	—	—	—	—	—	—	—	—	—	—	—	100
M „	36	—	23	2	—	3	—	—	—	—	36	—	—	—	—	—	0.5	—	—	—	—	—	100
N „	30	—	5	5	4.5	27	4	4.5	3	—	18	—	—	—	1	—	—	—	—	—	—	—	100
S 10—20 m	36	—	—	3	—	—	0.5	—	—	—	18	32	11	—	—	—	—	—	—	—	—	—	100
M „	80	—	1	—	0.5	1.5	1.5	—	—	—	1.5	—	—	10	—	—	1	—	—	—	—	—	100
Samples taken by S. EKMAN on the same localities in 1911—1912.																							
S 100 m	32	9	8	12	—	—	—	—	—	—	36	—	1	—	—	—	—	—	—	1	—	—	100
S 120 m	4.5	—	—	6	—	—	—	—	—	—	64	—	18	—	—	—	—	—	—	9	—	—	100
M 100 m	34	—	20	6	—	—	—	—	—	—	40	—	—	—	—	—	—	—	—	—	—	—	100
M 50 m	—	—	—	—	9	—	—	—	—	—	90	—	—	—	—	—	—	—	—	—	—	—	100
S 20—40 m	44	—	11	—	—	—	—	—	—	—	13	—	—	—	—	—	7	—	10	7	2	6	100
S 20—40 m	12	—	—	—	—	—	—	—	—	—	10	—	—	—	—	—	4.5	15	—	—	60	—	100
N „	—	—	—	—	—	—	—	—	—	—	20	—	—	—	—	—	39	—	—	—	—	39	100

Stylodrilus heringianus, which shows a preference for sandy bottoms in oligotrophic lakes. In addition, *Peloscolex ferox* is common at all depths, and is, as a rule, characteristic of a medium poor in nourishment. *Psammoryctes barbatus* is dependent upon a good supply of oxygen and generally prefers shallower water and a sandy bottom.

On the whole, it is true that the combination of species and number of individuals in the lower profundal (the depths of from 50 to 100 metres accounted for here) are found to be fairly homogeneous throughout Lake Vättern (except for the above mentioned local organic accumulations) and do not differ substantially from the averages EKMAN determined.

For the depth of 20—40 metres, on the other hand, it is obvious that conditions are quite different in the different parts of the lake. *Peloscolex ferox,* which otherwise is characteristic of the parts of Lake Vättern poor in nourishment, and *Rhyacodrilus coccineus* have increased considerably in the southern regions of the lake. This is clearly an effect of fertilizing. The following species have also increased, *Tubifex tubifex, Limnodrilus hoffmeisteri,* and *Euilyodrilus hammoniensis, a*nd these are also the most tolerant species.

The number of species is also at its highest in southern Lake Vättern, which is often characteristic of slightly polluted oligotrofhic waters. It is noteworthy, however, that this effect can already be discerned in EKMAN's material from Jönköping 1911—1912 (see values farthest down in both tables). *The accumulation of alloktonous, organic material has probably been going on for a long time.*

At a depth of 20—40 metres the differences in oligochaete composition between different parts of the lake become more conspicuous. In the Jönköping district (*S*) the number of individuals/ bottom surface unit by far exceed EKMAN's average value. The figures for the Motala district (*N*) are also relatively high while the values for central Lake Vättern (*M*) are below this value.

The Motala district's rather high figures become less difficult to explain if we recall that here is a bottom area with attendant good conditions for rich plankton occurrence, which, along with sedimentation, provide nourishment for the bottom organisms. The nearness to Vadstena and other dense populated districts can also effect the type of fauna.

In the littoral, at a depth of about ten metres, the same tendencies can be recognized that prevail in the sub-littoral. The Jönköping and Motala districts are both well above the average with the distinction that *Peloscolex ferox* — which was mentioned above as a "clean water species" — accounts for 80 per cent of the total number of individuals within the Motala district, compared to only 30 per cent within the Jönköping district.

In Lake Vättern's southern portion the distribution of the tolerant species becomes more obvious from about 40 metres deep and upwards. Separate

dredgings can display a remarkable richness of individuals depending upon the bottom sediment's high contents of alloktonous, organic material (pulp fibre, coarse detritus etc.).

Still, a relatively small proportion of all tubificids in this region are tolerant species such as *Limnodrilus hoffmeisteri, Euilyodrilus hammoniensis* and *Tubifex tubifex*. The high oxygen concentrations all the year round probably promote a diversified oligochaete fauna.

Statistically the dispersion between values obtained from stations within the same area and deep zone have proved very small. Similarly, the changes within the oligochaete fauna from one series of sampling to another within the same locality have been relatively insignificant. A greater number of sampling stations, however, would naturally have given more accurate results.

Up to this time I have found at least five new oligochaetes in Lake Vättern, of which three tubificids — *Tubifex ignotus, Aulodrilus limnobius* and *Bothrioneurum vejdovskyanum* — are also new species for Sweden.

Of greatest interest is the find of an almost sexually mature specimen of *Bothrioneurum vejdovskyanum* (ŠTOLC) at the depth of 21 metres near the inflow of the Motala Ström. (The species has somewhat vague characteristics and this specimen was not entirely "mature". The correctness of the identification, however, has been confirmed by Dr. KENNEY). Another specimen has lately been identified from the Motala district. Of the genus *Bothrioneurum* up to now very little is known. The variations within each species are great and the ecological preferences are entirely or partially unknown.

III. Acknowledgements

IV. Summary

In order to get an opinion of the degree of pollution of Lake Vättern the species composition of the oligochaete community has been studied in 1967 and 1968. Of special interest in this context are the tubificids.

Since a similar investigation was carried out in 1911 and 1912 by Prof. S. EKMAN a unique opportunity for comparisons arose.

The species composition as well as the number of specimens per square metre unit of the profundal are very homogenous in the whole lake and seem

not to have changed very much since EKMAN's investigations. Local differences, however, appear in the littoral and the sublittoral. In the southern part of the lake pollution has evidently been going on for a long time and caused a marked rise in the number of certain tubificid species. This tendency was discernable already in EKMAN's material.

At least five new oligochaetes have been found in Lake Vättern out of which three tubificids are new species for Sweden.

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A Contribution to the Methods of Classification for some *Mysidae* and *Gammarus* Species in the Baltic

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I. Introduction

The methods of biochemical systematics may provide additional taxonomic characters in species groups where morphological similarity is great, *e.g.* among sibling species. Such analysis may also add considerably to the knowledge of genetic changes and may thus help to solve problems of phylogeny. In crustaceans may cases of sibling species occur, *inter alia* in *Gammarus* and *Mysidae,* where, at least in the case of *Gammarus* species, morphological characters overlap. Consequently identification is difficult, having in fact become fairly satisfactory only since works by Spooner (1947), Segerstråle (1947, 1959) and Kinne (1954a).

Interspecific sterile matings are known to occur between *Gammarus* species, which act as a competitive-ecological factor. Males of *G. salinus* and *G. zaddachi* make use of the females of *G. duebeni* (Kinne, 1954b).

In various groups of animals non-specific esterases have proved to be effective tools in the search for species-specific characters (Nyman, 1965a, 1966, Sims McReynolds, 1967, Koehn and Rasmussen, 1967, de Ligny, 1968).

The present communication provides a means of taxonomic classification close to the gene level, where all kinds of variations are almost invariably genetically determined and intraspecific variation, especially, is normally absent or limited (Nyman, 1966).

II. Material and methods

There normally occur five species of *Mysidae* and five species of *Gammarus* in the Baltic. All ten species were obtained in habitats of various exposition and depth at Askö Laboratory in the archipelago of Trosa. The taxonomy and number of the studied specimens of the various species as classified by morphological characters is given in Table 1. No young *Gammarus* specimens were investigated, owing to the difficulties in identification.

Table 1. The number of the studied specimens as classified by morphological characters.

Fam. *Mysidae.*

Mysis mixta LILLJEBORG	29
Mysis relicta LOVÉN	6
Praunus flexuosus (O. F. MÜLLER)	37
Praunus inermis (RATKHE)	15
Neomysis integer LEACH	71
Total	158

Gen. *Gammarus*

Gammarus locusta (LINNÉ)	4
Gammarus zaddachi SEXTON	19
Gammarus oceanicus SEGERSTRÅLE	19
Gammarus salinus SPOONER	4
Gammarus duebeni LILLJEBORG	13
G. ocean.×G. loc.	2
G. salin.×G. zadd.	1
Total	62

Whole-animal homogenates were prepared in a glass tissue grinder, which was cooled in ice-water. All animals were buffered in equal amount of body and buffer (ASHTON and BRADEN 1961) prior to homogenating. After grinding, the tissue-buffer mixture was transferred to the polyethylene tubes of the Beckman Spinco Analytical System and the cell debris was spun down (10 minutes). The supernatant was then transferred to new tubes, which were either stored in a deep freezer at —20°C or used for immediate analysis. The electrophoretic procedures consist of horizontal starch-gel electrophoresis utilizing the discontinuous buffer system suggested by ASHTON and BRADEN (1961) and an enzyme staining method using α-naphthyl acetate in acetone and aq. dest. as substrate and Fast Red T R salt as dye-coupler. The electrophoretic apparatus employed allowed 25 specimens to be analysed simultaneously. The apparatus is cooled by running tap water. Filter papers (Whatman No. 3) 5×8 mm were damped with 20 microlitre of homogenate and inserted into a vertical slit in the gel. Electrophoresis was performed for 100 minutes with a voltage gradient of 12 V/cm. After electrophoresis the gel was sliced horizontally and the bottom layer was calibrated to a thickness of 2 mm. This slice was incubated in a buffer consisting of mono- and dibasic sodium phosphate of pH 7.0. After 15 minutes 20 mg of Fast Red T R salt in 1.0 ml α-naphtyl acetate and 50 ml aq. dest. was added to the incubation buffer. Maximum staining intensity was reached in 2 hours, after which the migration distance from the starting point was measured directly on the gel for each enzyme zone. The zones were permanented by washing the gel with a solution of acetic acid, methonal and aq. dest. in the proportion 1/5/5.
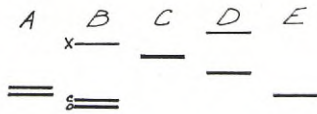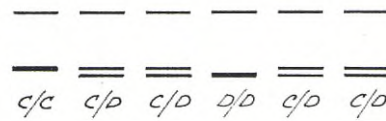
Fig. 1.

Fig. 2.

Fig. 1. Esterase patterns of *Mysidae*. A=*Neomysis integer*, B=*Mysis relicta*, C=*Mysis mixta*, D=*Praunus flexuosus*, E=*Praunus inermis*. In all figures the horizontal line represents the starting line and the arrow indicates the direction of anodic migration.

Fig. 2. Phenotypes and genotypes of the six *Mysis relicta* specimens investigated.

III. Results and discussion

The non-specific esterases of *Mysidae* are no exceptions from the rule of species specificity (Fig. 1). In two of the species only one molecular form of esterase was detected (*Mysis mixta* and *Praunus inermis*), in two of the others (*Neomysis integer* and *Praunus flexuosus*) two zones of enzymatic activity appear. In these four species no zones correspond in distance of migration, nor is there any kind of intraspecific variation. Animals of different sex and age thus have identical enzyme pattern, a circumstance which simplifies classification. In *Mysis relicta,* however, it was recently shown that one of the esterase zones in this species is polymorphic for at least four allelic genes, giving ten different phenotypes (Fürst and Nyman 1968). In two populations there was shown to be an excess of homozygote combinations and the highest frequency was reported for the C-allele. The phenotypes and supposed genotypes of the six specimens of *Mysis relicta* investigated in the present study are presented in Fig. 2. In Fig. 1 the heterozygote combination CD is drawn to indicate the position of both bands found. The fast-migrating band at "x" has no correspondence in *Neomysis integer,* which prevents any confusion with an A B heterozygote of the *Mysis relicta* group.

The situation in the *Gammarus* is much more complicated. In only one (*Gammarus duebeni*) of the five species investigated was intraspecific variation absent (Fig. 3). One zone is common to all species (1), but with a marked difference in staining intensity between *Gammarus locusta,* where it is hardly visible, and the four others, where it is one of the dominating

zones. In order to find out the amount of intraspecific variation, and also to concentrate the homogenates, mass homogenates were prepared. Only one fifth of the buffer proportion mentioned above was used.

The best way of discriminating between these five species is the different position of the slowest-migrating zone, where only one case of intraspecific variation was detected (*Gammarus salinus*). In this species only four specimens were obtained, one of which had a diverging position of the slowest-migrating band. The different mobility does not seem to be connected with hybridization, since its position indicates a slower migration than all other specimens observed.

Gammarus duebeni. A pattern consisting of four bands with no intra-specific variation. The slowest and the fast band (1) stained darkest. Easy to distinguish from all others.

Gammarus salinus. One diverging pattern (see above). The general pattern is rather similar to that of *G. oceanicus,* but the slowest-migrating band in *G. salinus* is about 1 mm slower than the corresponding band in *G. oceanicus,* and moreover the band present at (2) in *G. oceanicus* is absent in *G. salinus.* The position of the bands at (3) and (4) is also different, but since these bands may be very faint in *G. salinus,* they are of minor importance for classification purposes.

Gammarus oceanicus. Somewhat similar to *G. salinus* (see above). Sepa-rated from *G. zaddachi* by a different position of the slowest migrating zone. The band at (2) is absent in *G. zaddachi,* and the bands at (5) stain more faintly than do those located at (4) in *G. oceanicus,* moreover the space between the bands is broader in *G. zaddachi.* A polymorphism is evidently present at (4), where three different patterns have been detected. It may be assumed that the polymorphism is controlled by a pair of allelic genes with a low frequency of the allele controlling the slowest-migrating band of this system. Only one "slow" homozygote and two heterozygote patterns were found.

Gammarus zaddachi. Somewhat smiliar to *G. oceanicus* (see above).

Gammarus locusta. This species is most easily distinguished from the others by the almost complete absence of the band at (1), which stains very strongly in the other four species. The band at (6) is also clearly separated from the slowest-migrating bands in the rest of the species. Some intra-specific variation was noted in the faint bands located between (1) and (6), but these are of no importance for a secure classification.

From a taxonomic standpoint *G. duebeni* and *G. locusta* are the morpho-logical extremes, and they are thus rather easy to distinguish. The other three species overlap somewhat. In the protein patterns it is obvious that *G. duebeni* and *G. locusta* differ distinctly from the others in the general pattern, but also that the other species have the general characteristics in common, the main differences between them located in slight variations in
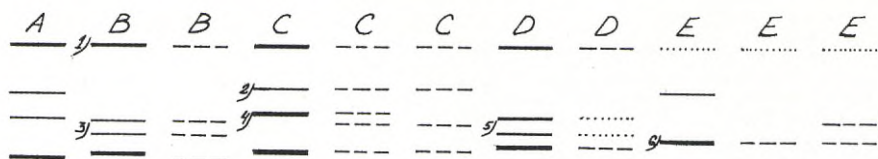
Fig. 3. Esterase patterns of *Gammarus*. A=*Gammarus duebeni*, B=*Gammarus salinus*, C=*Gammarus oceanicus*, D=*Gammarus zaddachi*, E=*Gammarus locusta*. The patterns indicated by short dashes are examples of intraspecific variation.

migration for most bands, *i.e.* slight variations in the net charge of the proteins involved. Consequently it may be justifiable to conclude that *G. salinus*, *G. zaddachi* and *G. oceanicus* are more closely related to one another than are the other two species and that they probably exhibit the most primitive pattern.

Three of the about 400 specimens examined had morphological characteristics of two species (Fig. 4). It would thus seem possible that they were hybrids. This was in fact confirmed in the esterase test, but the mode of segregation was at least partly different from the results found in, for instance, freshwater fish (NYMAN 1965b), where practically all zones in the parental patterns were segregated to the hybrid.

Two of these animals were assumed to be hybrids between *G. locusta* and *G. oceanicus*. The slowest migrating band had an intermediary position compared with the parents and the fast-migrating zone at (1) had the appearance of that of *G. locusta*, *i.e.* it stained very faintly. The diffuse bands between these extremes mostly resembled those of *G. oceanicus*.

The third supposed hybrid had characteristics of *G. salinus* and *G. zaddachi*. Its esterase pattern was almost impossible to distinguish from *G. salinus*, but the bands located at (3) stained more strongly than did those of both parental species. Consequently the mode of segregation in *Gammarus* seems to be an "either/or" rather than the "both/and" expected from other investigations of hybrids. It also seems evident from the present investigation that mating between the closely related *Gammarus* species sometimes gives rise to viable hybrids.

In the hope that a knowledge of the chromosome numbers of gammarids might be of taxonomic value an investigation of nine species was performed
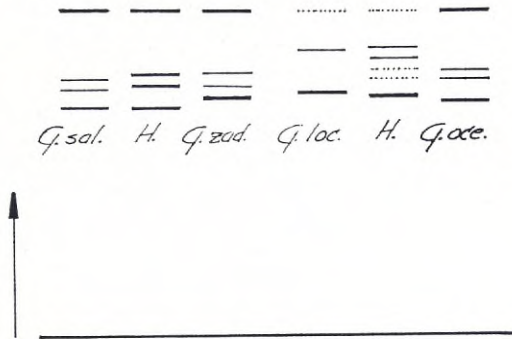
11

Fig. 4.   Fig. 5.

Fig. 4. Esterase patterns of the two types of hybrids found in the material examined.
G. sal.=*Gammarus salinus*, G. zad.=*Gammarus zaddachi*, G. loc.=*Gammarus locusta*,
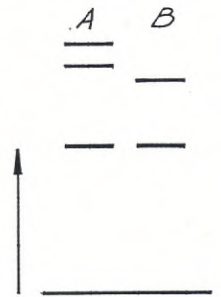G. oce.=*Gammarus oceanicus*, H=hybrid.

Fig. 5. Amido Black dyed proteins of *Mysidae* (A) and *Gammarus* (B).

by ORIAN and CALLAN (1957). Their investigation indicates that it seems most
unlikely, that chromosome studies will be of material assistance to taxo-
nomists working on this group because the chromosomes are generally small
and the numbers overlap from species to species. The authors found, how-
ever, that one of the two extremes (*G. duebeni*) had a haploid number of 27,
while the other (*G. locusta*) had a haploid set of 26 chromosomes. This
number agrees with *G. zaddachi,* one of the three more closely related species
discussed above.

The difference in chromosome numbers between *G. duebeni* and, for
instance, *G. zaddachi* could explain the occurrence of sterile mating, while
mating between *Gammarus* species with the same chromosome numbers
would give rise to viable hybrids. Further examinations in this state of things
are desirable.

Amido Black dyed proteins of *Mysidae* and *Gammarus* species exhibit dis-
crimination at another level of classification (Fig. 5). The distinct bands
here schematically drawn are the same in the five *Mysidae* species investiga-
ted, and the same state of things is present in the five *Gammarus* species.

IV. Summary

An additional character for systematical classification in the family
Mysidae and the genus *Gammarus* is proposed. By employing the methods
of biochemical systematics, the non-specific esterases of whole animal homo-
genates were analysed electrophoretically. Species-specific patterns were
detected, a circumstance which supports the previous morphological classi-
fication, and some of them give information about hybridization and phylo-
genetic trends.

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Blood Protein Systematics of *Cottidae* in the Baltic drainage area

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I. Introduction

The genetic information is located in the molecules of deoxyribonucleic acid which constitute the genes of the chromosomes. Through complicated reactions involving various types of ribonucleic acid this information is translated into protein molecules, which are thus primary translation of the genotype. Use is made of this knowledge in the methodology of biochemical systematics. The methods are founded on a technique for the separation of proteins (electrophoresis) in combination with histochemical staining procedures capable of demonstrating the different proteins as coloured bands in the white or colourless medium. Since the various tissues of the body are almost invariably composed of proteins, and since many proteins are not only species-specific but organ-specific, the investigation of protein systems may be a good source for the study of intraspecific variation, ontogeny, general systematics and phylogeny. A change in electrophoretic mobility is the indication of a change in the net charge of a protein, *i.e.* it indicates a mutation has occurred. From this information it may seem evident that the protein patterns of closely related species are more closely similar than are those of unrelated species. Since blood is a well-defined tissue, easy to obtain and rich in enzymes and other proteins, it has been the major source of electrophoretic investigations. The present communication is a contribution to the systematics of the various species of *Cottidae* living in the fresh and brackish waters of the Baltic drainage area.

In the brackish water of the Baltic five different species of *Cottidae* are present. Within a small region, the coast of the province of Uppland, all five are to be found. Two of the species, *Myoxocephalus scorpius* (L.) and *Myoxocephalus bubalis* (Euphr.), are marine in their distribution, since they are found mainly in the Atlantic Ocean. The northern limit of *Myoxo-*

Note

Synonymous of *Myoxocephalus* (Steller 1811):

M. quadricornis: *Cottus* (Linné 1758) and *Oncocottus* (Gill 1862) subg. ad Myoxocephalus.

M. scorpius: *Cottus* and *Acanthocottus* (Girard 1850) subg. ad *Myoxocephalus*.

M. bubalis: *Cottus, Acanthocottus* and *Taurulus* (Gratzianow 1907) subg. ad *Myoxocephalus*.

Table 1. The numbers of specimens analysed.

Myoxocephalus quadricornis 114
Myoxocephalus bubalis 11
Myoxocephalus scorpius 11
Cottus gobio 13
Cottus g. koshewnikowi 10
Cottus poecilopus 71

Total 230

cephalus bubalis in the Baltic is the Åland Archipelago, whereas *Myoxocephalus scorpius* is found in all parts of the Baltic. The fourhorn sculpin *Myoxocephalus quadricornis* (L.), considered to be a brackish-water form, is found from the northernmost parts of the Gulf of Bothnia down to the latitude of Kalmar. The two remaining species. *Cottus gobio* L. and *Cottus poecilopus* HECKEL, are freshwater species. *Cottus gobio* is reported from the Gulf of Bothnia down to the coast of the province of Småland, while *Cottus poecilopus* is not found south of the Uppland coast. The "Russian sculpin *Cottus gobio koshewnikowi* GRATZIANOW is considered to be geographic form of *Cottus gobio* and is not reported from the Baltic. It was earlier considered to be of full species rank (*Cottus koshewnikowi* GRATIZIANOW), but nowadays it is even questioned (KOLI, personal communication) whether it is a subspecies or not.

II. Material

The material was collected and analysed during the period August 1966 —September 1968. *Cottus gobio* and *Cottus poecilopus* specimens were caught by electrofishing at Kengisfors in the Torne älv river. Further *Cottus poecilopus* individuals were collected from the Mörrumsån river (electrofishing), and some *Cottus gobio* specimens were caught by hand in shallow water at the Askö Laboratory. Two different populations of *Cottus gobio koshewnikowi,* differing in degree of development of the spinefields (KOLI — personal communication) were investigated. The more spiny population was caught in the Vihtijoki å river, the least spiny came from the Sundeå river. Both rivers are in the province of Nyland. The specimens were caught by electrofishing. Fourhorn sculpin (*Myoxocephalus quadricornis*) were obtained from the Baltic, at Tyresö-Brevik and Askö Laboratory, as well as from a number of lakes in southern Sweden. All specimens were caught by gill net. Specimens of *Myoxocephalus bubalis* and *Myoxocephalus scorpius* were obtained from both the east and the west coasts of Sweden (Askö, Tyresö-Brevik, Tjärnö and Kristineberg) by gill net. In Table 1 the numbers of specimens analysed are presented.

III. Methods

Blood sampling procedure.

Blood can be obtained in sufficient quantities from fish down to a length of some 6 cm. An incision is made in the ventral region between the pectoral fins, the mucus and scales first having been removed at the spot of injection, to prevent clotting of the blood. The heart is punctured with a thin scalpel, after which the blood is collected in heparinized glass capillaries and transferred to the tubes of the Beckman Spinco Analytical System and centrifuged. The supernatant serum is then removed (after 30 seconds) and may be used for further analysis. The erythrocyte fraction is treated as follows: the fraction is mixed with 3 parts of a physiological saline (0.923 g NaCl/100 ml water) and the mixture is stirred by means of a glass rod and centrifuged for 1 minute. The supernatant is removed and the procedure is repeated twice. Haemolysation is performed by adding 2 parts of distilled water. After stirring this mixture should be used within a few hours to avoid problems of denaturation. The haemoglobin solution should be kept at $O°C$.

Electrophoresis apparatus.

A somewhat modified version of the apparatus constructed by GAHNE and GEDIN (University of Uppsala, unpublished) is used. Starch is used as supporting medium and the electrodes are of platinum. The starch plate is cooled by means of running tap water. The electrode vessels are detachable to allow them to be cleaned (important when changing buffers) and they each hold some 400 ml. The discontinuous buffer system described by ASHTON and BRADEN (1961) was employed in all cases. For power supply we made use of the Oltronix LS 107 with maximum capacity of 200 mA and 500 V.

Preparation of gel.

200 ml of buffer is brought to boiling point and another 100 ml of the same buffer is mixed with 31 g of starch (Hydrolyzed Starch, Connaught Medical Research Laboratories, University of Toronto, Canada). The starch-buffer solution is mixed with the boiling buffer and is degassed. The gel mixture is then poured onto a framed glass plate measuring some 15×30 cm and is allowed to grow stiff. After 15 minutes the plate is put in a cold chamber at $+5°C$. The gel may be used in one hour.

Electrophoresis.

For haemoglobin electrophoresis a special slit is made for each specimen to prevent interaction. For all other proteins a vertical slit is made in the gel parallel to the longest side.

Filter papers (5×8 mm) are damped with 20 microlitres of serum and are placed in the gel with an intermediate space of 2 mm. The gel is covered with a thin plastic film (Marathon Menasha, Wis., USA) and the electrodes are connected to the gel. A constant voltage of 400 V is used, the current strength varying between 100 and 200 mA. The electrophoresis is performed for 105 minutes. The filter papers are removed after 15 minutes. After the completion of the electrophoresis the gel is sliced horizontally by means of a device ensuring discs of a standard thickness. Up to 25 specimens may be analysed simultaneously, a great advantage in population studies.

Staining methods.

Serum proteins and haemoglobin (Amido Black).

The following stock solution is prepared: 300 ml methanol, 300 ml distilled water (aq. dest.), 60 ml glacial acetic acid, 150 ml glycerine, 5 g Amido Black and 2 g nigrosine. A small amount of the solution is poured onto the gel. After 3 minutes the excess dye is removed by washing the gel with a solution of 5 parts methanol, 5 parts aq. dest. and 1 part glacial acetic acid. After a few hours the proteins appear as distinct blue bands.

Serum esterases.

Non-specific esterases are visualized as follows. α-naphthyl acetate is diluted to a concentration of 1 per cent in equal amounts of aq. dest. and acetone. One ml of this solution and 100 mg Fast Red T R salt are diluted further in 50 ml of aq. dest. and are mixed with an incubation buffer of mono- and dibasic sodium phosphate (pH=7.0). After 1 hour the esterase zones usually reach their maximum intensity.

Serum peroxidases.

A solution containing 1 g benzidine, 9 ml glacial acetic acid and 36 ml aq. dest. is mixed with a solution of 45 ml 1 per cent hydrogen peroxide in aq. dest. After a few minutes distinct blue bands appear. These patterns should be photographed or measured immediately as they rapidly grow weak and disappear.

Serum ceruloplasmin. 0.5 ml paraphenylene diamine dihydrochloride is dissolved in acetate buffer (pH=5.0). After 2—3 hours diffuse bands appear. Agitation necessary.

Nitroso-R reagent. The following stock solution is prepared. 100 ml aq. dest., 1 g hydroxylamine hydrochloride, 2.7 g sodium acetate (3H$_2$O), 1.5 ml glacial acetic acid, 0.5 g Nitroso-R salt (1-hydroxy-2 nitrosonaphtalene-3.6-disulphinic acid). Pour the solution over the gel and leave for 15 minutes. The gel should be washed throughly with several changes of the

wash solution mentioned above. Transferrins will show after a few hours as faint green bands. The green can be intensified by adding 5 microgrammes of ferric ammonium sulphate per ml of serum. Other proteins usually stain yellowish-brown.

IV. Results and discussion

During the last few years many papers have been published on the protein taxonomy of fishes. The majority of them deal with analysis of blood proteins, for instance haemoglobin (SICK, WESTERGAARD and FRYDENBERG 1962, NYMAN 1965, TSUYUKI, ROBERTS and VANSTONE 1965, TSUYUKI and ROBERTS 1965, WILKINS 1966) and various serum proteins (MØLLER and NAEVDAL 1966, NYMAN 1966, ODENSE, ALLEN and LEUNG 1966, LUKYANENKO, ERMOLIN, SEDOV and POPOV 1967, KOEHN and RASMUSSEN 1967, DE LIGNY 1968). These studies have shown the necessity of using the classical systematics as a base for the investigation and also the importance of studying variations caused by polymorphisms, ontogeny, disease or type of food. In this study, to avoid problems of ontogeny only the proteins of adult fish have been used.

Serum esterase.

Serum esterase patterns seem to be good indicators of species and family (NYMAN 1966). This is also the case among the *Cottidae* (Fig. 1). The normal esterase pattern is only one molecular form, *i.e.* one band in each species. One exception was found, the polymorphic esterase of *Myoxocephalus bubalis,* with three phenotypically different patterns, the segregation of which seems to be due to the action of two autosomal codominant allelic genes. The small number of specimens found prevented chi-square test of the correlation between observed and expected numbers. This type of intraspecific variation is, however, very common in fish (KOEHN and RASMUSSEN 1967, NYMAN 1966, NYMAN and WESTIN 1968, unpublished) and is found to be due to the type of inheritance mentioned above. The subspecies status of *Cottus g. koshewnikowi* is further indicated by its esterase pattern, which is identical with that of *Cottus gobio.* The differing mobility of the esterases of *Cottus gobio* and *Cottus poecilopus* may be used as a convenient character for systematic purposes, and the hybrids found by this method (NYMAN and WESTIN 1968) have not earlier been detected by employing classical methods. STARMACH (1967), however, has checked the chromosome numbers in *Cottus gobio* and *Cottus poecilopus* and found that they differed, *C. gobio* having 2n=52 and *C. poecilopus* having 2n=48. The karyotype comprised of 8 metacentric and 40 acrocentric chromosomes, while that of *C. gobio* consisted of 6 metacentric and 46 acrocentric chromosomes.
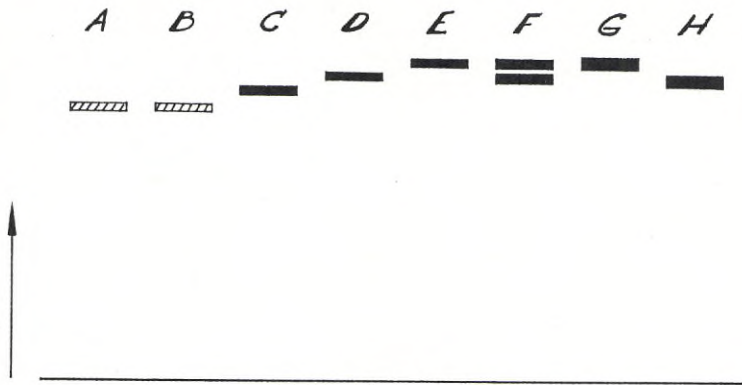
Fig. 1. Blood serum esterase patterns in *Cottidae*. A=*Cottus gobio koshewnikowi*, B=*Cottus gobio*, C=*Cottus poecilopus*, D=*Myoxocephalus scorpius*, E=*Myoxocephalus quadricornis*, F, G, and H=*Myoxocephalus bubalis* (polymorphism). — These letters are used in all figures to designate the same species. In all figures the horizontal line represents the starting line and the arrow indicates the anodic migration.

Despite identical mobility of the two bands of *Myoxocephalus bubalis* and the single bands of *Myoxocephalus scorpius* and *Myoxocephalus quadricornis,* these three species can be separated, since the zones of *Myoxocephalus bubalis* are darker (higher enzyme activity) and "thicker".

Serum proteins (Amido Black).

The serum proteins as revealed by staining with Amido Black indicate that intraspecific variation occurs in most species. A few bands are, however, present in all specimens, and may be used as characters for species discrimination. These species-specific bands are drawn black in the schematic figure (Fig. 2), while bands that may show any degree of variation are marked with dots. The good concordance between the *Cottus* species is striking. *Myoxocephalus bubalis* differs from the other species of this genus by a very fast-migrating prealbumin (a) and by the absence of bands in the "b-region". No distinct differences between *Myoxocephalus bubalis* or *Myoxocephalus scorpius* specimens from the east and from the west coast of Sweden could be detected. A minor difference between *Cottus gobio* and *Cottus g. koshewnikowi* at "c" might be significant.

Haemoglobin.

The multiple haemoglobins of all *Cottidae* species investigated show a lesser degree of intraspecific variation than did the serum proteins (Fig. 3). One drastic exception was, however, noted. In *Myoxocephalus scorpius* very few bands were found to exist in all specimens, and the size of the specimens examined showed that the variation could not be ontogeny-
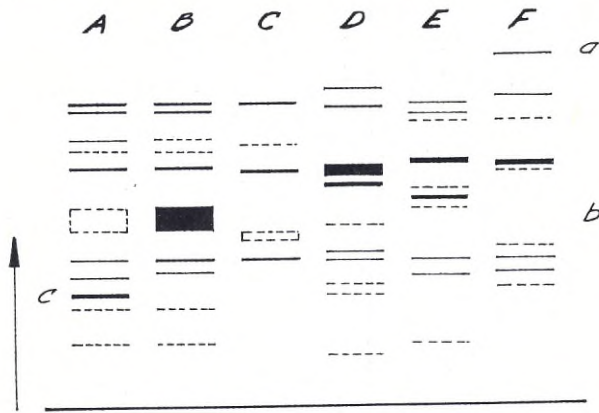
Fig. 2. Blood serum proteins (Amido Black). (a) the fast prealbumin in *Myoxocephalus bubalis*, (b) lack of bands in the b-region of *Myoxocephalus bubalis*, (c) divergent protein band in *Cottus g. koshewnikowi*.

correlated. The intraspecific variation is marked in the same way as mentioned above. Patterns from the west and the east coasts were also identical. Note the concordance of the *Cottus* species. No differences between *Cottus g. koshewnikowi* and *Cottus gobio* were found, nor could any differences whatsoever between the two populations of *Cottus g. koshewnikowi* be detected. The precense of the 'koshewnikowi-type' (A) haemoglobin pattern in young *C. gobio* specimens indicates that the difference shown below between band is ontogeny correlated.

Serum peroxidase.

These enzymes seem to be poor characters for systematic purposes (Fig. 4). Not only is interspecific variation slight but intraspecific variation is considerable and is influenced by a variation in the degree of haemolysis, the haemoglobin fraction having a very high peroxidase activity.

Myoxocephalus bubalis differs from the others in usually having a very strong band at "a". The fast-migrating zone in *Myoxocephalus quadricornis* has been described as polymorphic in a previous paper (NYMAN and WESTIN 1968). The various populations of sculpin examined seemed to prevent any discrimination between two supposed sibling species of fourhorn sculpin, and further studies by WESTIN (1968) have shown that the difference in roe colour found in different populations is due only to the type of diet.
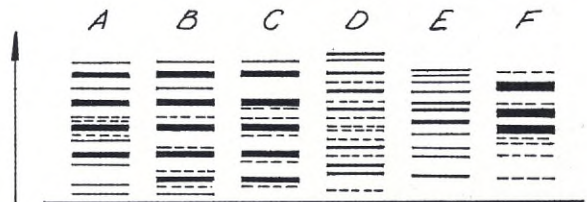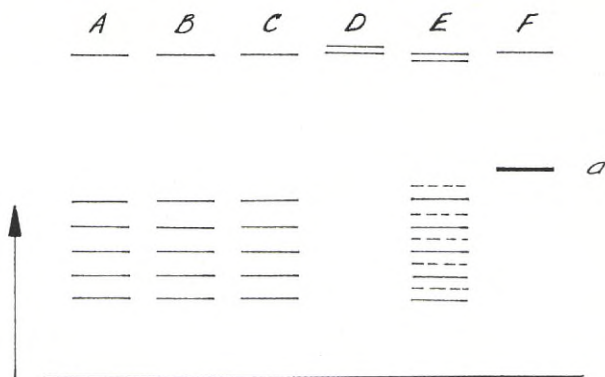

Fig. 3. Haemoglobin patterns.

Fig. 4. Serum peroxidase patterns. (a) the diverging band in *Myoxocephalus bubalis*.

Nitroso-R reagent.

This dye should give protein patterns similar to those obtained by Amido Black, this stain, too, being non-specific. In order to mark the transferrin zones a few microgrammes of ferric ammonium sulphate were added. No intraspecific variation could be detected in these intensely green bands, but the general patterns of *Myoxocephalus bubalis* differed. As only two specimens were obtained from the east coast it cannot be determined whether this is a true geographic variation or merely a case of intraspecific variation due to, for instance, type of food. The two general patterns are schematically presented in Fig. 5, the possible transferrin zone being marked with an x.

Ceruloplasmin.

With the staining method described in this study the ceruloplasmin fraction stains rather diffusely. Only one zone of enzyme activity was detectable in each species (Fig. 6). The *Myoxocephalus* species had the same mobility in common, and so had the *Cottus*. It was not possible to discriminate between species within the two genera.
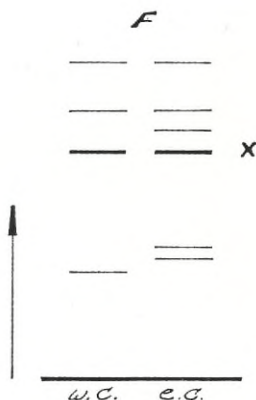

Fig. 5. Possible geographic variation in Nitroso-R-stained serum proteins of *Myoxocephalus bubalis*. w.c.=west coast, e.c.=east coast, x=possible transferrin band.
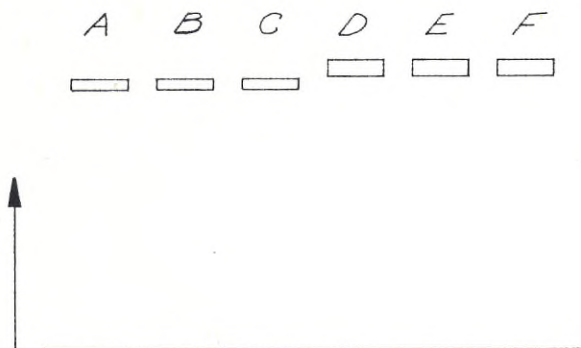
Fig. 6. Ceruloplasmin patterns. Homogeneity within the two genera.

"*Copper fraction*".

When sera of *Myoxocephalus bubalis* and *Myoxocephalus scorpius* are analysed electrophoretically the blue colour of the sera migrates as a distinctly visible band, faster than all other proteins examined. This protein, which obviously contains copper is species-specific (Fig. 7), *Myoxocephalus scorpius* having a monomorphic type, *Myoxocephalus bubalis* a polymorphic one, indicated by three phenotypically different patterns, evidently with a mode of segregation similar to that described in the esterases. The sera of the other *Cottidae* examined are colourless, when not subjected to haemolysis.

V. Survey of the results

(1) *Cottus*. The morphological sibling species. *Cottus gobio* and *C. poecilopus* show a high degree of agreement in the protein patterns, but, distinctly species-specific bands are present in both species. *Cottus gobio koshewni-*

Fig. 7. "Copper fraction" proteins in the serum of *Myoxocephalus scorpius* and *Myoxocephalus bubalis* (polymorphism).

kowi, is indistinguishable from *C. gobio* except for the Amido Black dyed serum proteins, but this difference may be due to non-genetic circumstances, since the patterns obtained with this stain are often influenced by polymorphisms, type of food, disease or ontogeny correlated variation in haemoglobin patterns. No differences were detected between the two populations of "Russian sculpin" differing in degree of spine field development, nor were there any differences between populations of *C. gobio* or *C. poecilopus* of different geographic origin.

(2) *Myoxocephalus.* Members of this genus are less closely related than are those of the *Cottus* group.

(A) *Myoxocephalus quadricornis.* Almost every protein zone is species-specific. Polymorphism in the serum peroxidases. The protein patterns of *M. quadricornis* remained constant although samples were obtained from both fresh and brackish water.

(B) *Myoxocephalus scorpius.* Almost every protein zone is species-specific. The very complex haemoglobin pattern differs markedly from the other *Cottidae* examined. Blueish serum, the protein involved being species-specific (see below). No differences between populations from the east (brackish) and west (marine) coasts of Sweden.

(C) *Myoxocephalus bubalis.* Almost every protein zone is species-specific. Blueish colour of the serum and the presence of polymorphic serum esterase and protein. A minor difference is noted between populations from the east and west coasts of Sweden. *M. bubalis* differs distinctly from all other Cottidae examined and may evidently be placed in a separate genus where protein patterns are concerned. This proposed division of the *Myoxocephalus* genus is also supported by the fact that *Myoxocephalus bubalis* differs from all other *Cottidae* examined in having internal fertilization. However, it still seems unwise to apply generic or subgeneric names until the whole genus is reviewed.

VI. Summary

The five species and one subspecies of *Cottidae* occurring in the Baltic drainage area were investigated by employing the methods of biochemical systematics. Various blood proteins were analysed by horizontal starch gel electrophoresis in combination with histochemical staining methods. On the basis of the protein patterns the separation of the investigated *Cottidae* into two genera seems justifiable. The *Cottus* species are much more closely related than are those of the genus *Myoxocephalus.* *Myoxocephalus bubalis* (Euphr.) differs distinctly in three of the systems investigated from the other *Cottidae,* and should perhaps be regarded as a separate genus.

VII. Acknowledgements

The help of Dr. ÖSTEN KARLSTRÖM of the Fishery Board, Luleå, in obtaining all the *Cottus poecilopus* and *Cottus gobio* specimens from Kengisfors and the River Mörrumsån is gratefully acknowledged, as also is the help of Dr. LAURI KOLI of the Zoological Museum at the University of Helsinki, who supplied the "Russian sculpin" and commented on the problems involved.

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The Mode of Fertilization, Parental Behaviour and Time of Egg Development in Fourhorn Sculpin, *Myoxocephalus quadricornis* (L.)

By

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I. Introduction

In ovaries of fourhorn sculpin, *Myoxocephalus quadricornis* (L.) and sea-scorpion, *Myoxocephalus scorpius* (L.), the well-known ichthyologist O. Nordqvist (1899) discovered eggs with well-developed embryos. He considered this observation to be a proof that internal fertilization occurred to this species.

The information in the recent literature about the fourhorn sculpin is founded on Nordqvist's discovery, as far as fertilization is concerned.

Earlier, however, that excellent observer Ekström (1831) believed that external fertilization occurred in fourhorn sculpin, since both sexes appeared in shallow water during the spawning time. As regards the sea-scorpion, he assumed that internal fertilization occurred in the depths and after that the females alone appeared in shallower water where they deposited their eggs.

According to the literature, the male fourhorn sculpin protects the roe. This statement has its origin in the observation of Gisler (1748), that to protect the roe the sculpin "gave its life, if necessary".

In order to calculate the time of development, Cederström (1855) fertilized eggs of ten different species. He was successful with all these species except fourhorn sculpin, but estimated the time for these at 100 days (Cedeström, 1855).

The following investigation was carried out to ascertain whether internal or external fertilization occurs, whether the fourhorn sculpin male protects the roe, and if so, what is the duration of this protection. The question regarding the time of egg development could be solved at the same time.

II. Material and methods

This investigation was performed at the Askö Laboratory south of Stockholm during two spawning seasons. This period lasts from the middle of December to the end of January.

Fourhorn sculpins were trapped in gill-nets in the vicinity of the laboratory, where the fish occurs frequently.

Two aquaria with a capacity of 610 and 1000 litres respectively were supplied with running Baltic water from a depth of 10 metres, in order to keep the temperature and salinity at the same level as that of the habitat. The salinity in this part of the Baltic is 6—7 promille S.

Zones with different bottom material, ranging from fine sand to fairly big stones, were arranged in the aquaria.

The diurnal variation of light influenced the two aquaria. At night, however, the 1000-litre aquarium was illuminated in order to enable photographs to be taken. In the other aquarium only a very weak light was allowed, but after the observer's eyes had become adapted to the dark observations could be made.

The temperature in the aquaria in the spawning time at the turn of the year was 1.5—2°C.

III. Results

The results are based on observations of sculpins in the 610 litres aquarium. In the brightly lit bigger aquarium no spawning at all occurred. The females finally shed their roe but no male showed any interest. The roe was immediately consumed by the other sculpins.

In daytime the sculpins normally rested on the bottom but when the light decreased the level of activity rose. No aggressiveness between the sculpins had been observed earlier but at the beginning of December two males behaved threateningly towards the other sculpins in the aquarium. These two males mostly remained within their own territories. When an intruder came too close, the aggressive male turned round on the same spot so he was facing the intruder. He then advanced in quick short darts and finally stopped 3—5 cm short of the intruder with the anterior dorsal fin and the large pectoral fins erected. In this position the two fishes seemed only to watch each other. After a short time, however, the intruder usually lowered his anterior dorsal fin and either swam away or turned round on the same spot. In this latter position he was ignored by the aggressive male. If neither of these two actions was taken, he was bitten and sometimes also held.

It was surprising that an intruder practically always swam away even though no visual threat display except the darting approach could be observed.

There was, however, an acoustic threat display. When the threatening male stopped in front of his antagonist he gave off a dull vibrating sound which could easily be heard through a stethoscope lowered into the aquarium. An analysis of the sound showed a frequency of 125 cycles per second.

Other species of the family *Cottidae* are known to have the capacity to

generate sound. The sound is produced by muscles in the gills. (DUNCKER, 1929.)

The females began, 2—3 days before the spawning, a restless swimming interrupted by short periods of resting on the bottom in different parts of the aquarium.

When the females swam over the bottom-dwelling males, the latter vigorously undulated the body laterally and displayed the anterior dorsal fin with its white spot. The white spot at the posterior end of the fin could easily be seen in the darkness, and must have been clearly visible to the females.

When the female had landed on the bottom in the vicinity of a male, he moved closer to her in short darts in a mouse-like manner. Finally he lay by her side, with his tail turned under hers. In this position the male performed rhythmical movements with the tail at intervals of one per second. The tail movements were always directed towards the side where the female was situated. The female remained passive during the spawning. The eggs were extruded in one portion and were adhesive, forming a clump. The female disappeared soon after the spawning and showed no interest in the eggs. The male, however, guarded the egg mass and violently attacked any sculpins who came within a radius of 30 cm of the egg mass. The displays took the same form as those of a male guarding a territory.

The male protected the roe not only against other fourhorn sculpins but also against fishes of other species. Some of these were fishes ten times as heavy as himself. When finally he was confronted by a pike of 2 kg he lay motionless, but when the pike moved away he swam after it in an aggressive manner.

In spite of the male's protection, the egg mass survived in no case more than six hours, presumably owing to the overcrowding in the aquarium.

On one occasion a spawning took place just on the boundary of one of the two territories guarded by an aggressive male. Owing to the violent movements of the male during the spawning, the egg mass found its way into that territory. The male that had spawned was frightened by photo flashes and disappeared (Fig. 1), but the aggressive male placed himself on the egg mass and defended it from predators. This protection was, however, only of short duration.

To get an idea of the duration of the protection, a male that had just spawned was left alone with the roe in another aquarium. The male remained with the eggs until they hatched. During this time he often lay on them or close beside them (Fig. 2) and ventilated and aereted them by fanning his large pectoral fins. The current produced by this fanning action was so strong that small particles could be seen being carried away by it. From time to time the male cleaned the egg mass, and dead eggs were removed and spat away.

12

Fig. 1. The male, frightened by photo flashes, is leaving the half-spent female. The egg mass is behind the female to the left (dotted lines). White eggs are dead.

If the male was irritated, he pushed away the egg mass with his snout or drove the clump along the bottom, having tucked it in the angle between his head and his pectoral fin. On rare occasions he took the roe in his mouth and swam away, then spat it out and placed himself on the eggs. If the human observer took the eggs from him, the male followed them to and fro, and if the clump of eggs was divided into two parts he always took care of the larger one (Fig. 2).

Faced with a choice between his own egg mass, which he had guarded for 70 days, and a new clump, which was three times as large and moreover was of different colour, he chose the new one without any hesitation. When after 24 hours the new egg mass was taken away from him, he immedietly returned to the original one. After the eggs had hatched, no interest in the fry could be observed. The development of the eggs lasted in this instance for 97 days with an average value of 1.5°C. In a higher temperature the duration of the development was shorter. A mean value of 2.0°C diminished the time of development to 74 days and 4.7°C to 55 days.

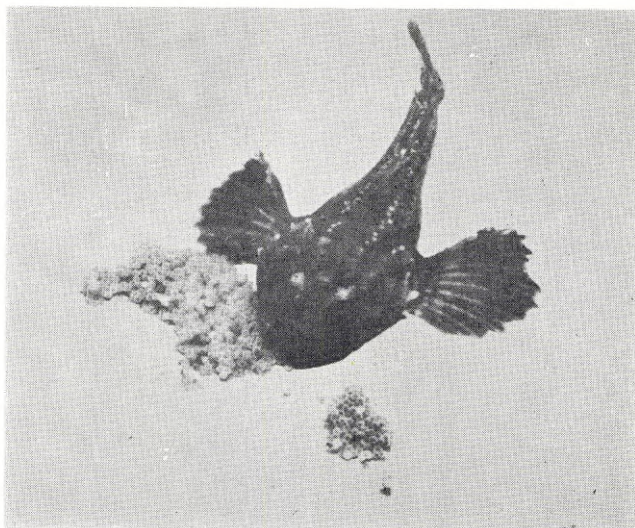In a temperature of 10.5°C no fry at all hatched.

Fig. 2. The egg mass is divided into two parts. The male takes care of the larger fraction. The position of the protected egg mass tucked into the angle between head and pectoral fin, is typical.

IV. Discussion

Fertilization

After the observation of fertilized eggs in the ovary of *Myoxocephalus quadricornis* it was stated that the species had an internal mode of fertilization.

The occurrence of fertilized eggs in the sculpin's ovary could be explained by the hypothesis that spermatozoa find their way through the oviduct to the ovary and there fertilize the remaining eggs.

In material collected from Tyresö-Brevik in March and April 1967 fifty per cent of the fourhorn sculpin females still had a small number of eggs in the ovary. About 20 per cent of these females had fertilized eggs. In an ovary with fertilized eggs all of them were fertilized.

The embryonal development was in all cases the same, irrespective of whether the material was from the beginning or from the end of the period of investigation. This indicates that the development of the eggs in the ovary probably can proceed up to a certain stage only, and then becomes arrested. In comparison with the other four species of the family *Cottidae* present in the Baltic, the mode of fertilization is known to be external in *Cottus gobio* L. and *Cottus poecilopus*, HECKEL. As regards *Myoxocephalus scorpius*, which also was thought to have an internal fertilization (NORDQVIST, 1899), it was recently proved that internal fertilization does not take place in this species (LAMP, 1966). In one species, however, *Myoxocephalus (Cottus) bubalis*, (EUPHRASÉN), (syn. *Taurulus bubalis*), internal fertilization has been shown to occur (LAMP, 1966).
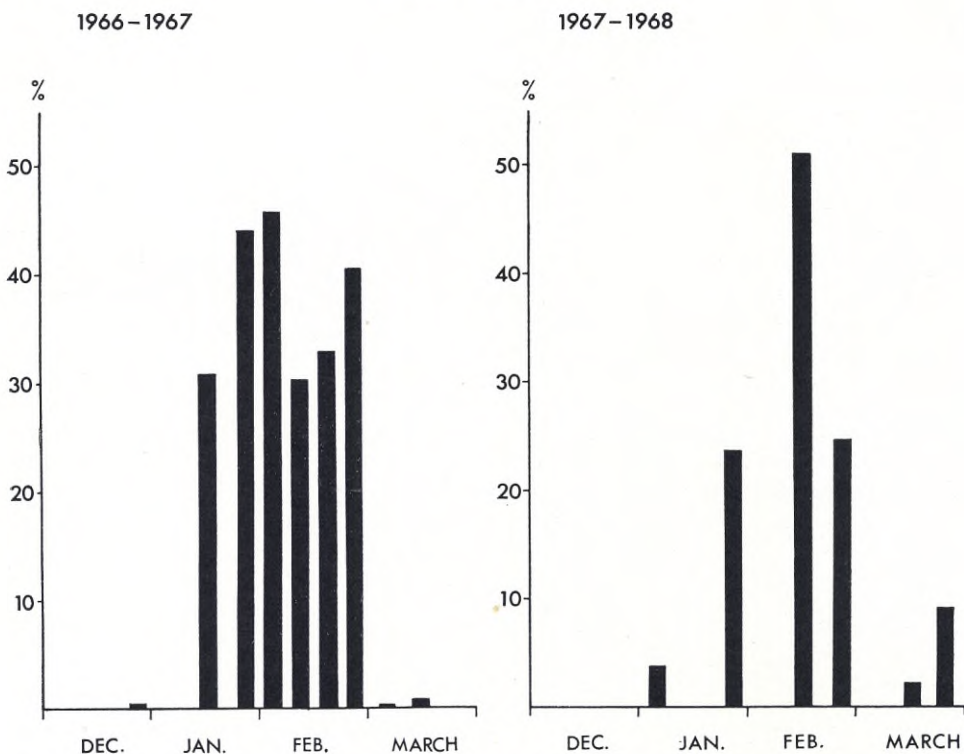
Fig. 3. The presence of species-specific roe as a percentage of stomach contents of 864 fourhorn sculpins during two consecutive periods of egg devlopment (end of December to end of March).

Parental behaviour

In this species there is a long parental cycle, lasting for approximately three months, presumably owing to the low temperature during the time of development. During this time the male alone guarded, cleaned and aerated the eggs. The spent female left the spawning place once the eggs had been laid and fertilized, and she did not appear to take any further part in the parental behaviour.

The fertilized egg masses from nine fourhorn sculpin females were held unguarded by a cleaning and aerating male. All the egg masses developed fungus and only 60 fry hatched from the 25,000 eggs. The males' care of eggs is obviously of great significance for the survival of the species.

In the aquarium an intense predation on the roe was carried out in spite of the male's guarding, by sculpins which had not taken part in that particular spawning. The same seems to occur in the natural habitat too. During two years of study, analyses of the stomach contents from 2000 fourhorn sculpins were made. A considerable part of the stomach contents during the period of egg development consisted of species-specific roe, which appears in

the stomach at the end of December and culminates in January and February when up to 50 per cent of the total stomach contents consists of the sculpin's eggs (Fig. 3).

During this period the females exceeded the males in number in gill-net catches, presumably owing to the males remaining with the roe. Since the fourhorn sculpin has a relatively small number of large eggs and these eggs are stuck together in a clump, are not hidden and besides need a long time to hatch, the roe is highly vulnerable to predation, and the males guarding serves to compensate for this vulnerability.

In the cases of *C. gobio, C. poecilopus* and *M. scorpius* the adhesive eggs are stuck onto same convenient surface and guarded by the male. In *C. gobio* and *C. poecilopus* a hole is dug out underneath some solid object resting on the bottom and the eggs are usually stuck to the inside wall of the cavity (MORRIS 1954, STARMACH 1962).

V. Summary

In a study of the mode of fertilization in fourhorn sculpin *Myoxocephalus quadricornis* (L.) external fertilization was proved to occur, although internal fertilization is reported in the previous literature. After the spawning the females take no further part in the parental behaviour. The male alone takes care of the egg mass and warns enemies by a dull vibrating sound before attacking them violently. The male remains with the eggs during the time of egg development, which lasts about 100 days.

A predation on species-specific roe is shown to occur and the importance of the parental behaviour is discussed.

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Addendum

Accompanied by the head of the Askö Laboratory Dr. B.-O. JANSSON and Mr. B. NYQVIST, who first found a spawning locality, several dives were performed during the spawning season 1968—69.

The direct observations confirmed the information from GISLER (1748), who observed that the fourhorn sculpin dug a hole in the bottom in which the eggs were deposited and protected.

At a depth of 15—20 metres seven fourhorn sculpin males were found, everyone in a hole of its own. The holes had a diameter of 20—25 cm and a depth of 10—15 cm presumedly corresponding to the size of the fish.

The edges were absolutely cylindrical except for the slightly concave bottom. The occurrence of the hole explained a peculiar behaviour observed but not discussed above. Before the spawning both sexes performed a rythmical movement with their large pectoral fins on the same spot for hours which resulted in a hole in the soft bottom.

Crustacea, especially *Lepidurus arcticus* Pallas, as brown trout food in Norwegian mountain reservoirs

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I. Introduction

Efficient fishing management of the mountain reservoirs requires a knowledge of which food organisms the fish makes use of. In this respect the short-term effects of the impoundments on the food fauna are of less importance. Proper planning of fish management in these lakes presupposes information on the long-term effect of the impoundments and the adjustment of the fish populations to the new and stabilized conditions. If some fish food organisms tolerate the effects of impoundment, which often include an increased predation pressure by fish, this should have consequences for the management of the reservoirs.

Most of the Scandinavian investigations about the effects of impoundments on the fish food fauna have been carried out in lakes situated within or below the subarctic region. The impoundments quickly reduce the quantity of the true bottom animals, especially big and available species such as *Gammarus lacustris* Sars and *Asellus aquaticus* L. (Dahl 1926, 1932, 1933, Huitfeldt-Kaas 1935, Stube 1958, Grimås 1961, 1962). Nevertheless the importance of crustaceans as fish food increases during the first years, owing to the increased abundance of semibenthic forms, especially *Eurycercus lamellatus* Müll, as a short-term effect of the impoundment. (Dahl, Huitfeldt-Kaas, Grimås, *op. cit.*, Runnström 1955, Nilsson 1955, 1961, Axelsson 1961). This positive effect is more accentuated in reservoirs established by damming, incorporating surrounding areas, but it also occurs in reservoirs which are regulated only by a draw-down of the water level. This effect may be due to a larger amount of nutrition in the contact zone bottom water owing to the redeposition of the sediment and the breaking down of submerged vegetation (Grimås 1965). The length of this short-term effect depends mainly on the topography of the basin and the annual amplitude of the water level. Thus after a few years the semibenthic *Cladocera* will be reduced to a concentration which often seems to be lower than the original one (Grimås, op. cit.).

The long-term effect on the utilization of crustaceans as fish food is, however, known to a lesser degree. The investigations have mostly been carried
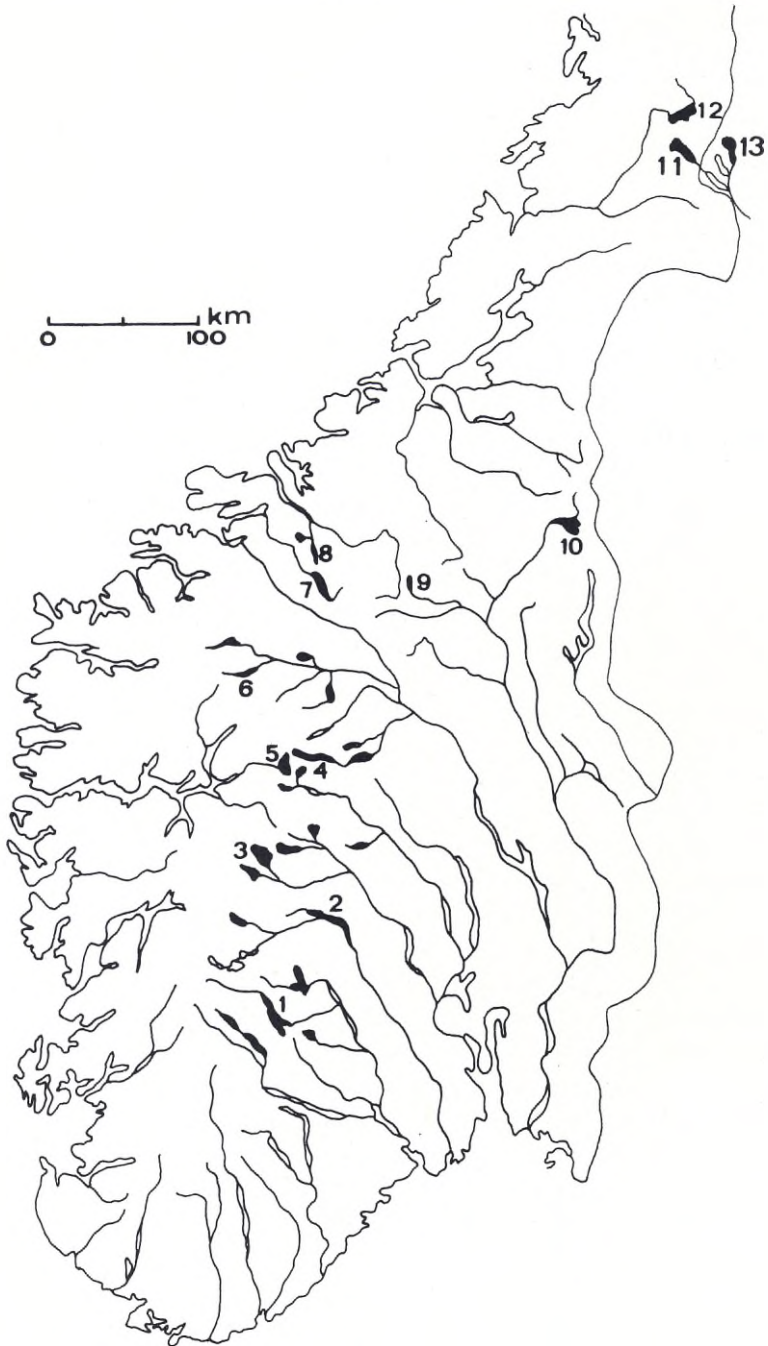
Fig. 1. The position of the investigated reservoirs. Figures indicate reservoirs referred to in the text. 1. Møsvatn, 2. Pålsbufjord-Tunhovdfjord, 3. Strandavatn, 4. Steinbusjøen, 5. Tyin, 6. Raudalsvatn, 7. Aursjø (Lesja), 8. Osbuvatn, 9. Elgsjø, 10. Aursunden, 11. Limingen, 12. Namsvatn, 13. Blåsjön.

out before they could really be known. In the following rough survey on the feeding habits of brown trout (*Salmo trutta* L.) in impounded lakes, some of the oldest reservoirs in Norway are included. Arctic lakes, which constitute a great part of the Norwegian fresh-water area, are also treated in the present paper.

II. Material

The position of the reservoirs examined appears from Fig. 1. They are mostly situated in the central south Norwegian mountain range between Rauland, county of Telemark, and Sunndal, county of Møre & Romsdal. Lake Elgsjø and Lake Aursunden are situated in the upper courses of the Folla and Glomma rivers, county of Sør-Trøndelag. The lowest and highest impoundments are 2.2 and 35.0 metres respectively: these are rather small or moderate heights by present standards. Year of starting and particulars of the impoundments appear from Table 1, where the reservoirs are listed according to their position from south to north. The material originates from 38 lakes, some of them constituting parts of big, complex reservoirs when the water level rises towards its maximum. In all 1,303 brown trout from the reservoirs, caught in the years 1949—68, were examined. The fish was of the size 25 cm or more. To this may be added several hundred trout from neighbouring lakes, investigated in order to help explain the development of the food fauna in the reservoirs. The catch was made by gill nets and, with exception of Lake Pålsbufjord, in August—September when the fishing is best and the crustacean fauna richest. The material from Lake Pålsbufjord partly originates from July because in this lake the crustaceans attain their maximum rather early.

The number of fish from each lake is small and does not admit of a detailed food analysis. The stomach content of each fish was divided into a few comprehensive food groups. Only the crustaceans were examined in more detail. In summing up their relative importance, both the frequency and the volume of each group were considered, each fish being given equal weight. The main food groups from each reservoir are listed in Table 2 in order of their importance. This shows the food consumed in a short period only, and tells nothing of the feeding habits of the trout in other seasons of the year. But in mountain lakes a big proportion of the yearly food consumption is concentrated to late summer and early autumn. For some reservoirs the table may also give a wrong impression of the general food trend in August—September, owing to a mass occurrence of some terrestrial food animal. For example, *Oporinia autumnata* Bкн. appeared in large numbers in 1964—65 in parts of southern Norway. In lakes surrounded by subalpine birch forest, the trout concentrated its feeding on this species (cf. Lakes Kalhovdfjord and Tunhovdfjord in Table 2) and showed little interest in aquatic organisms.

III. Results and discussion

With few exceptions crustaceans of the suborders *Euphyllopoda* and *Cladocera* were found to be the predominant food item of the trout at time of fishing. Nothing else was found in stomach samples from Lakes Halnefjord, Strandavatn, Buvatn, Djup, Flævatn, Gyrinosvatn and Steinbusjøen. When not the predominating food object, crustaceans came second in importance, apart from Lake Raudalsvatn where crustaceans were not found in the samples. Even if insect larvae and imagines had been put together, instead of being divided among different orders, this would not have altered the main impression of benthic and semibenthic crustaceans being the predominating food group. Next to crustaceans, *Chironomidae* larvae and pupae, *Trichoptera* larvae, terrestrial *Diptera* and *Tipulidae* imagines were of greatest importance.

In some of the reservoirs investigated, one or more fish species are found together with the trout. Char (*Salvelinus alpinus* L.) is most frequent, but grayling (*Thymallus thymallus* L.), whitefish (*Coregonus sp.*) and perch (*Perca fluviatilis* L.) also occur. To a great extent these species, too, live on crustaceans. This has not prevented trout from its mainly crustacean feeding, but it may well have influenced the composition of the group in the food. Probably the grayling offers the strongest food competition, and in the Aursjø (Lesja) reservoir, for instance, it has developed into an *Eurycercus* specialist. The food segregation between trout on the one hand and char and whitefish on the other is more pronounced. In reservoirs the char and whitefish tend to utilize *Daphnia sp.*, *Bosmina coregoni* BAIRD and *Holopedium gibberum* ZADDACH to a much higher degree than do the trout.

A more detailed survey of the crustaceans in the food of trout is found in Table 3. All the reservoirs investigated are situated below the local upper limit of *Gammarus*. Before the impoundments the species was the most important food object in many of the lakes. This significance has been lost in all the lakes — a further confirmation of the intolerance of *Gammarus* even in case of small impoundments. It will be seen from Table 3 that *Gammarus* has been found in Lakes Nedre Heimdalsvatn, Buvatn, Vinstervatn, Halnefjord, Elgsjø, Øyvatn, Møsvatn and Strandavatn, with impoundments of 2.2, 3.6, 4.0, 4.0, 5.0, 5.9, 18.5 and 28 metres respectively. In seasons not covered by this survey, *Gammarus* has been found in Lakes Tunhovdfjord and Pålsbufjord with amplitudes of 18 and 24.5 metres. Greater numbers of *Gammarus* were only found in Lakes Nedre Heimdalsvatn, Vinstervatn, Halnefjord and Buvatn, i.e. when impoundments exceed about 5 metres the populations of *Gammarus* become too small to be of importance as fish food. This limit also applies to the northern reservoirs of Blåsjön and Limingen on the Swedish-Norwegian border (NILSSON 1961, AASS 1963).

Cladocera have become the staple food of trout in nearly all subarctic

reservoirs, *Eurycercus* and *Bythotrephes longimanus* LEYD. being the most important species. *Daphnia sp.* often occurred in the stomachs, but only in small quantities. The material was, however, collected after their maximum. But the trout commonly feeds on *Daphnia* ephippies to late autumn, often until the freezing up. Only a few trout stomachs contained *Bosmina* and *Holopedium*, but the latter may appear more frequent during its maximum in July. *Copepoda* were of little importance to trout of the sizes examined. The great consumption of *Cladocera* must be interpreted as a long-term effect of impoundment. Trout populations, allopatric included, feeding to a great extent on *Cladocera* are not common in Norwegian mountain lakes, except where the other bottom fauna is for one reason or another scarce.

In the majority of the arctic reservoirs, *Cladocera* were not the predominating crustaceans in the food of trout. This was due to the fish having fed heavily on *Lepidurus arcticus* PALLAS. This big euphyllopod is distributed, with many interruptions, through the central mountain range of southern Norway. Without the presence of *Lepidurus* many arctic lakes could not harbour a valuable trout population. *Lepidurus* appears in greater number only for one or two months a year, but still forms the basis of the trout's metabolism and growth. The quality of trout feeding on *Lepidurus* is excellent. When *Lepidurus* lakes are converted into reservoirs, the food situation of the fish does evidently not alter to the same degree as in subarctic lakes. *Lepidurus* still constituted an essential part of the trout food (Table 3). At times it was the only food organism occurring in the stomachs, cf. Lake Steinbusjøen. Even after impoundments, up to 250—300 specimens have been found in a single stomach.

In the subarctic lakes the trout changes its main diet from a varied bottom fauna to semibenthic *Cladocera*. No corresponding change takes place in arctic lakes with *Lepidurus*. The species is not known to have disappeared as a consequence of water fluctuations only. On the contrary, *Lepidurus* apparently has established itself in reservoirs where it was not found in the original lakes. In some lakes where *Lepidurus* was known to exist but was rarely seen, their number increased markedly after impoundment.

The fish food resources of the river system of Numedalslågen were studied thoroughly for several years by DAHL (1926, 1930, 1932). In Lake Tunhovdfjord *Lepidurus* was not found before the impoundment, but ten years afterwards it appeared well established, being found in 11 per cent of the trout stomachs (HUITFELDT-KAAS 1935). Nor was *Lepidurus* known to exist in Lake Pålsbufjord, situated close to Tunhovdfjord. The year after the first impoundment was carried out it was found by DAHL (1932). At 736 and 750 metres above sea level, both lakes are situated far below the local pine-forest line. According to the vertical distribution line indicated by S. SØMME (1934) they are the lowest localities of *Lepidurus* known. To these lakes *Lepidurus* has access by River Numedalslågen, where previously it had been

Table 1. The examined reservoirs and information about the impoundments.

Name of lake	Start of impoundment	Damming in metres	Lowering in metres	Annual amplitude in metres	Regulated levels metres above sea level		Areas in sq.km	
					Minimum	Maximum	Minimum	Maximum
Vindsjåen	1958	15.00	—	15.00	956.00	971.00	3.05	4.65
Totak	1958	1.20	6.10	7.30	680.00	687.30	34.00	40.00
Songa	1962	34.50	0.50	35.00	939.00	974.00	7.80	29.20
Mosvatn	1906	9.80	0.20	—	—	—	—	—
	1909	12.30	2.20	—	—	—	—	—
	1943	16.30	—	18.50	900.00	918.50	28.30	77.70
Kalhovdfjord	1917	5.40	1.40	—	—	—	—	—
Gjeitebufjord	1947	8.40	2.70	11.10	1,075.50	1,086.60	17.10	31.10
Gøystdalsvatn	1947	8.30	2.80	11.10	1,075.50	1,086.60		
Mårvatn	1917	6.70	0.30	—	—	—	—	—
	1947	—	1.30	—	—	—	—	—
	1959	—	14.60	21.30	1,100.00	1,121.30	10.70	20.20
Tunhovdfjord	1919	18.00	—	18.00	718.00	736.00	14.25	25.35
Pålsbufjord	1927	—	9.00	—	—	—	—	—
	1946	12.50	—	—	—	—	—	—
Halnefjord	1958	—	12.00	24.50	726.00	750.50	5.25	19.50
Strandavatn	1942	2.00	—	—	—	—	—	—
	1956	—	2.00	4.00	1,125.25	1,129.25	9.00	14.50
Buvatn	1942	3.00	—	—	—	—	—	—
	1955	24.00	—	—	—	—	—	—
	1957	28.00	—	28.00	950.00	978.00	15.10	24.30
Djup	1948	2.10	1.50	3.60	1,087.40	1,091.00	3.10	4.25
	1948	6.50	4.50	11.00	1,080.00	1,091.00	4.60	8.65

Reservoir	Year							
Flævatn	1960	19.90	0.10	20.00	1,088.50	1,108.50	⎱ 7.80	16.55
Gyrinosvatn	1960	12.90	—	12.90	1,095.60	1,108.50	⎰	5.05
Vavatn	1957	—	8.00	8.00	1,116.30	1,124.30	3.45	—
Tisleifjord	1950	10.00	1.50	11.50	808.85	820.35	8.00	13.70
Utrovatn	1962	1.00	4.50	5.50	965.50	971.00	2.00	2.50
Steinbusjøen	1959	2.30	3.00	5.30	1,205.70	1,211.00	6.60	8.20
Tyin	1951, 1945, 1961	5.00	5.35	10.35	1,072.50	1,082.85	20.00	—
Bygdin	1933*, 1934	0.90	8.25	9.15	1,048.25	1,057.40	33.90	35.00
Vinstervatn	1942*	1.50	2.50	4.00	1,027.50	1,031.50	23.20	38.90
Øyvatn	1953	5.50	0.40	5.90	1,013.10	1,019.00	3.30	—
Nedre Heimdalsvatn	1955	2.20	—	2.20	1,050.00	1,052.20	6.00	28.00
Tessevatn	1957	0.80	11.60	12.40	841.50	853.90	9.30	4.90
Aursjø (Skjåk)	1959, 1943*, 1919	2.50	10.00	12.50	1,085.00	1,097.50	2.00	7.40
Raudalsvatn	1965	23.30	7.00	30.30	886.00	916.30	3.20	12.10
Breidalsvatn	1952*	2.50	10.50	13.00	895.00	908.00	3.90	—
Gautsjø	1944*	12.50	—	12.50	843.50	856.00	3.10	7.40
Grunningen	1955	18.50	—	18.50	837.50	856.00	⎱ 5.50	7.40
Aursjø (Lesja)	1955	25.00	3.00	28.00	828.00	856.00	⎰	6.70
Reinsvatn	1955	12.30	5.30	17.60	874.40	892.00	1.30	10.00
Sandvatn	1964	12.60	10.40	23.00	825.80	848.80	23.30	26.30
Langvatn	1958	16.50	8.00	24.50	824.30	848.80	—	4.00
Osbuvatn	1958	23.10	7.90	31.00	817.80	848.80	—	1.50
Elgsjø	1958, 1912	5.35	—	5.35	1,128.00	1,133.35	—	1.50
Aursunden	1923	1.40	4.50	5.90	684.10	690.00	—	44.00

* Small provisional impoundments were accomplished in the year(s) prior to the permanent impoundment.

Table 2. Food of trout in August—September with the exception of Lake Pålsbufjord (July—August).

Name of lake	Year	Number of fish	Predominant food	Other food items listed in order of importance
Vindsjåen	1966	39	*Crustacea*	*Chironomidae* l., *Diptera* terr., *Hymenoptera*
"	1967	41	"	*Chironomidae* l., *Diptera* terr., *Coleoptera* l.
Totak	1968	36	*Crustacea*	*Chironomidae* l., *Diptera* terr., *Coleoptera* terr.
"	1965	26	*Crustacea*	*Chironomidae* l., *Diptera* terr.
Songa	1964	23	*Crustacea*	*Chironomidae* l.
Mosvatn	1964	33	*Lepidoptera*	*Crustacea*, *Pisces*, *Diptera* terr.
"	1965	14	*Chironomidae* l.	*Diptera* terr., *Hymenoptera*
Kalhovdfjord	1964	15	*Crustacea*	*Lepidoptera*, *Chironomidae* l., *Diptera* terr.
"	1965	6	"	*Chironomidae* l., *Pisidium*
Gjeitebufjord	1964	20	"	*Trichoptera* l.
Gøystdalsvatn	1964	20	"	*Chironomidae* l., *Trichoptera* l., *Lepidoptera*
"	1965	12	"	*Chironomidae* l., *Pisidium*
Mårvatn	1964	30	"	*Chironomidae* l., *Tipulidae* l., *Pisces*
Tunhovdfjord	1965	37	*Lepidoptera*	*Crustacea*, *Planorbis*
Pålsbufjord	1949	80	*Pisces*	*Crustacea*, *Chironomidae* l., *Ephemeroptera* l.
"	1952	52	*Crustacea*	*Diptera* terr., *Ephemeroptera* l., *Chironomidae* l.
"	1963	121	"	*Ephemeroptera* l., *Tipulidae* i., *Planorbis*
Halnefjord	1964	11	*Crustacea*	*Lymnaea*, *Coleoptera* l.
"	1967	19	"	
"	1968	8		
Strandavatn	1964	12	*Crustacea*	
Buvatn	1964	19	*Crustacea*	
Djup	1964	11	"	

Locality	Year	%				
Flævatn	1964	23	Crustacea		Lymnaea	Chironomidae l.
Gyrinosvatn	1964	21	"		Diptera terr.	Hymenoptera
Vavatn	1966	36	Crustacea	Tipulidae l.		
Tisleifjord	1965	23	Crustacea	Tipulidae l.		
Utrovatn	1966	29	Crustacea	Trichoptera l.		Tipulidae i.
"	1967	20	"	Chironomidae l.		
Steinbusjøen	1964	13	Crustacea	Chironomidae p.		
Tyin	1964	26	Crustacea			
Bygdin west	1964	10	Chironomidae l. & p.	Chironomidae l. & p.	Chironomidae l.	
east	1967	17	Crustacea	Chironomidae l. & p.	Trichoptera l.	
Vinstervatn	1965	24	Crustacea		Planorbis	Chironomidae l.
Øyvatn	1965	32	Crustacea	Lymnaea	Coleoptera l.	Lymnaea
Nedre Heimdalsvatn	1965	45	Crustacea	Trichoptera l.	Ephemtroptera l.	Lymnaea
Tessevatn	1965	20	Crustacea	Trichoptera l.	Diptera terr.	Coleoptera terr.
Aursjø (Skjåk)	1967	26	Crustacea	Chironomidae l.	Hymenoptera	Coleoptera l.
Raudalsvatn	1965	28	Crustacea	Ephemeroptera l.	Hymenoptera	Coleoptera terr.
Breidalsvatn	1967	15	Diptera terr.	Chironomidae l.	Pisidium	Hydracarina
Gautsjø	1965	12	Crustacea	Chironomidae l.	Chironomidae l.	Coleoptera terr.
Grunningen	1965	21	Crustacea	Diptera terr.	Diptera terr.	Plecoptera l.
"	1968	26	"	Tipulidae l.		
Aursjø (Lesja)	1965	37	Crustacea	Chironomidae l.	Tipulidae i.	Diptera terr.
Reinsvatn	1967	20	"	Chironomidae l. & p.	Trichoptera l.	Lymnaea
"	1968	21	"			Planorbis
Sandvatn / Langvatn / Osbuvatn	1967	20	Crustacea	Chironomidae l. & p.	Ephemeroptera l.	Trichoptera i.
Elgsjø	1964	11	Tipulidae i.	Crustacea	Tipulidae l.	Trichoptera l.
Aursunden west	1967	19	Trichoptera l.	Planorbis	Lymnaea	Pisces
east	1967	23	"	Crustacea		Trichoptera i.

Table 3. *Crustacea* in the food of trout in August—September with the exception of Lake Pålsbufjord (July—August).

Name of lake	Year	Predominant *Crustacea*	Other *Crustacea* listed in order of importance
Vindsjøen	1966	*Bythotrephes*	*Daphnia*, *Eurycercus*, *Diaptomus*, *Bosmina*
,,	1967	,,	,, , ,, , *Diaptomus*, *Bosmina*
,,	1968		
Totak	1965	*Eurycercus*	*Daphnia*, *Bythotrephes*, *Lepidurus*, *Gammarus*
Songa	1964	*Bythotrephes*	*Daphnia*, *Diaptomus*, *Lepidurus*
Mosvatn	1964	*Eurycercus*	*Holopedium*, *Bosmina*
,,	1965	,,	
Kalhovdfjord	1964	*Eurycercus*	*Daphnia*, *Bythotrephes*, *Daphnia*
,,	1965	*Daphnia*	*Lepidurus*, *Eurycercus*, *Lepidurus*
Gjeitebufjord	1964	*Eurycercus*	*Eurycercus*, *Daphnia*, *Holopedium*
Goystdalsvatn	1964	*Lepidurus*	*Daphnia*, *Bythotrephes*, *Bythotrephes*
Mårvatn	1965	*Lepidurus*	*Eurycercus*, *Lepidurus*, *Daphnia*
,,	1964	*Eurycercus*	*Eurycercus*, *Bythotrephes*, *Lepidurus*
Tunhovdfjord	1965	*Bythotrephes*	*Bythotrephes*, *Eurycercus*, *Lepidurus*
Pålsbufjord	1949	*Eurycercus*	*Eurycercus*, *Lepidurus*
,,	1952	*Lepidurus*	
,,	1963	,,	
Halnefjord	1964	*Lepidurus*	*Gammarus*, *Daphnia*, *Bythotrephes*, *Eurycercus*
,,	1967	,,	*Gammarus*, *Bythotrephes*, *Daphnia*
,,	1968	,,	
Strandavatn	1964	*Bythotrephes*	*Lepidurus*, *Gammarus*, *Bythotrephes*
Buvatn	1964	*Lepidurus*	*Gammarus*, *Bythotrephes*, *Eurycercus*
Djup [1]	1964	,,	*Bythotrephes*
Flævatn	1965	,,	*Eurycercus*

Locality	Year				
Gyrinosvatn	1965	Lepidurus	Bythotrephes	Daphnia	Diaptomus
Vavatn	1966	Eurycercus	Lepidurus	Eurycercus	
Tisleifjord	1965	Bythotrephes	Daphnia	Daphnia	
Utrovatn	1966	Eurycercus	Bythotrephes	Bythotrephes	
"	1967		Daphnia	Daphnia	
Steinbusjøen	1964	Lepidurus			
Tyin	1964	Bythotrephes	Daphnia	Lepidurus	Eurycercus
Bygdin west	1964	Daphnia	Eurycercus	Eurycercus	
" east	1967	Lepidurus	Daphnia	Daphnia	Bythotrephes
Vinstervatn	1965	Lepidurus	Eurycercus	Eurycercus	Eurycercus
Øyvatn	1965	Lepidurus	Daphnia	Bythotrephes	Daphnia
Nedre Heimdalsvatn	1965	Lepidurus	Gammarus	Eurycercus	Diaptomus
Tessevatn	1965	Daphnia	Bosmina	Holopedium	
Aursjø (Skjåk)	1967	Eurycercus	Lepidurus	Bythotrephes	Daphnia
Raudalsvatn	1965				Eurycercus
Breidalsvatn	1967	Eurycercus	Lepidurus	Bythotrephes	Eurycercus
Gautsjø	1965	Eurycercus	Lepidurus	Bythotrephes	Holopedium
Grunningen	1965	Bythotrephes	Lepidurus	Daphnia	Bythotrephes
"	1968	Eurycercus	Bythotrephes	Lepidurus	
Aursjø (Lesja)	1965	Lepidurus	Eurycercus	Daphnia	Daphnia
Reinsvatn	1967	Eurycercus	Lepidurus	Bythotrephes	
"	1968	"	"	"	
Sandvatn / Langvatn / Osbuvatn	1967	Eurycercus	Lepidurus	Bythotrephes	
Elgsjø [2]	1964	Bythotrephes	Eurycercus	Gammarus	
Aursunden west	1967	Eurycercus		Polyphemus	
" east	1967	Eurycercus			

[1] Buvatn and Djup are two of 15—20 lakes and tarns constituting a complex reservoir. Lepidurus occurs in all.

[2] Lepidurus found in trout stomachs 1967. ING. KORSEN, pers. comm.

found down to Lake Ossjøen situated 951 metres above sea level. Thus its vertical altitude in the watershed has been lowered by 215 metres. *Lepidurus* might also have entered by River Skurdalselv, the other main tributary to Lake Pålsbufjord. In that case its lowering has been 430 metres.

The food fauna of the three lakes now constituting the Aursjø reservoir, Gautsjø-Grunningen-Aursjø, was investigated during the three years preceding the impoundment in 1955. (EVENSEN 1956). *Lepidurus* was found neither in bottom samples nor in fish stomachs. The first find of *Lepidurus* in stomachs was made in 1960 (OLAF HEITKÖTTER, pers. comm.), and it now forms an important part of the diet of the trout. Most probably the *Lepidurus* has descended from Lake Skjelbreia, which drains into the reservoir (Fig. 2). The lake is situated 85 metres above Aursjø and contains *Lepidurus* in great numbers. But the *Lepidurus* may also have descended from Lake Svartdalsvatn situated 149 metres above the reservoir. The old outlet of Lake Aursjøen has been closed by a dam, and since 1955 the water has been transferred to the Litledalen valley. In this valley three lakes, Sandvatn-Langvatn-Osbuvatn, form a joint reservoir after an impoundment of 31 metres in 1958. Before the impoundment *Lepidurus* had never been seen in any of the separate lakes. The lakes in the catchment area of the Osbu reservoir have also been searched for *Lepidurus,* but without positive result. In 1964 the first specimen was found in a trout stomach (HÅKER 1964), and a few years later *Lepidurus* appeared quite regularly in the stomach contents. After establishing itself in the Aursjø reservoir, *Lepidurus* has evidently passed through the tunnel and having been given the opportunity after the impoundment has also settled in the Osbu reservoir. Between the two complex reservoirs is situated the little Lake Torbuvatn. It is fed from the same mountain ridge as the Aursjø reservoirs and discharges into Lake Sandvatn, but has not been impounded. *Lepidurus* has been found neither in its catchment area nor in the lake itself, which is bypassed by the tunnel. Lying beside the migration route, *Lepidurus* has not had access to the lake, and as a result of its absence the quality of the trout is poorer than in the nearby reservoirs.

The development in Lake Namsvatn exemplifies the expansion of existing populations after impoundments. *Lepidurus* increased considerably in numbers after the impoundment of 14 metres and now constitutes a bigger proportion of the food of trout than ever before (SIVERTSEN 1962). According to RUNNSTRÖM and MÄÄR (1950) the neighbouring great lakes of the Faxälven river system harboured tiny populations of *Lepidurus*. In Lake Blåsjön out of 304 char and 361 trout examined in the years 1945—49, *Lepidurus* was found in 1945 in the stomach of one char. Between the years 1947 and 1958 the lake was impounded in three steps, with a resulting annual amplitude of 13 metres. In 1966—67 a considerable number of trout stomachs examined contained *Lepidurus* (NILS-ARVID NILSSON, pers. comm.).

In Lake Vavatn *Lepidurus* was found only once before impoundment. In Lake Tyin it was observed neither before the first step of impoundment, nor in the years preceding the final damming-up (DAHL 1943, LEIV ROSSELAND, pers. comm.) *Lepidurus* is now found regularly in both lakes. The expansion is no doubt real, but both lakes are situated in districts where *Lepidurus* occurs commonly, and the pre-impoundment material may give a too favourable impression of the development. Living *Lepidurus* is rarely observed, and its frequency and number in stomachs may vary considerably owing to different factors. Its yearly maximum varies according to the water temperature, and the topography and substrate of the bottom affect its density locally.

The predominant feeding by the trout on *Cladocera* in impounded subarctic lakes is obviously not a short-term effect, since many of the examined reservoirs have been in use for from 20 to 60 years. After the pioneering investigations in Lake Tunhovdfjord by DAHL (1926) and HUITFELDT-KAAS (1935) it might have been expected that *Eurycercus* would be of greatest importance to the trout during the early years of impoundment and then be replaced by *Bythotrephes*. However, no such correspondence between age of reservoirs and the feeding habits of the trout can be derived from the present material. The great consumption of *Cladocera* in older reservoirs is not due to a permanent increase in their number, but to a proportionately greater reduction of the bottom fauna. This compels the trout to change its diet. I. SØMME (1941) called attention to the fact that this change leads to a reduction of the quality of food. The annual maximum of *Cladocera* appears late in the feeding season, and it requires much energy to chase the prey. It is a general experience that the *Cladocera* feeding predominant in older reservoirs results in a reduced growth rate.

The survival of *Lepidurus* in arctic reservoirs early became apparent (DAHL 1932, HUITFELDT-KAAS 1935) but its expansion in subarctic and boreal reservoirs could only be regarded as probable. Its increasing importance as fish food below the arctic region is, however, no transitory effect, corresponding to the flourishing of *Cladocera*. In fact there may be a considerable time lag between impoundment and the reaching of maximum size by the *Lepidurus* population. The observation by DAHL (1932) of the quick response of *Lepidurus* to the first impoundment of Lake Pålsbufjord is rather exceptional. The annual amplitude was later raised from 9 to 21.5 and 24.5 metres in 1946 and 1958. In 1949 and 1952 only 1 and 2 per cent of the trout stomachs contained *Lepidurus,* but by 1963 the percentage had risen to 32. Before impoundment *Lepidurus* was a not uncommon food item of the trout of Lake Namsvatn. In 1950—52 the lake was impounded with an amplitude of 14 metres, and in the years immediately following *Lepidurus* was not observed. It reappeared in the stomach contents in 1956, and afterwards the

Fig. 2. Map of Aursjø and Osbu reservoirs with catchment areas. Figures indicate height
in metres above sea level.

consumption soared. In August of the three years 1959—61 37, 56 and 23
per cent of the trout stomachs, respectively, contained *Lepidurus* (SIVERTSEN
1962). The time lag in Lake Blåsjön was even longer. After impoundments
between 1947 and 1958, increased numbers of *Lepidurus* were not noticed
until 1966—67. It is possible that in the upper subarctic reservoirs less time
elapses between impoundment and an observable expansion of the *Lepidurus*
population; only 5 years after impoundment it had become of substantial
importance as fish food in the Aursjø reservoir.

In the central part of southern Norway dense *Lepidurus* populations are mostly found in lakes situated between 1100—1300 metres above sea level. These lakes are distinguished by trout being the only fish species present, and generally the populations are sparse owing to restricted spawning grounds. In such cases a concentration of food consumption on *Lepidurus* has no observable effect on the number of this species. If for one reason or another a fish population becomes numerous, *Lepidurus* decreases in number or disappears. Situated in the mountains between the valleys of Hemsedal and Lærdal are many arctic lakes with excellent trout populations mainly feeding on *Lepidurus*. Interspaced between these lakes there are a few with trout of poor quality. Repeated investigations of such lakes have not produced any *Lepidurus*. One of them, Lake Vesle Øljusjø, is fed by snow-drifts only, and the great trout population was due to an unduly large planting of fry. When this was not repeated the trout population decreased and *Lepidurus* turned up in the stomach contents after an absence of nearly ten years. In neighbouring lakes with permanent crowding due to an abundance of spawning sites, *Lepidurus* has not been observed. Lake Møsvatn, at 918 metres above sea level, was regulated in several steps from 1906 and onwards. *Lepidurus* gained in number and importance and in the summer and autumn of 1930 it was the leading food item by quantity (HUITFELDT-KAAS 1935). In the early thirties char migrated into the lake and now forms a numerous population, while *Lepidurus* is rarely seen. The examples may be multiplied and predation is obviously a factor affecting the abundance and distribution of *Lepidurus*.

The establishment or expansion of *Lepidurus* in reservoirs is not, however, a consequence of reduced predation. If predation had been the limiting factor for its distribution, *Lepidurus* should easily have been observed in the lakes before impoundment. The stocks of trout have certainly been reduced, but in total the fish populations have increased. Char or grayling have more than made up for the loss of trout, and these species feed on *Lepidurus* just as trout do. The char invaded Lake Pålsbufjord some years before the first impoundement in 1927 and descended to Lake Tunhovd-fjord just after this reservoir was established. The number has steadily increased and char now form enormous populations of dwarfed individuals in both lakes. In the Aursjø reservoir the existing population of grayling has increased markedly after impoundment. Through the tunnel grayling entered the Osbu reservoir, where previously trout was the only fish species present. In lake Namsvatn the impoundment favoured the reproduction of the existing char population, resulting in an exceptional rise in number. *Lepidurus* has expanded in spite of the fact that the fish populations have simultaneously become more numerous and more complex. In these cases the former absence of *Lepidurus* cannot be attributed to predation. Locally this factor may possibly contribute to the absence of *Lepidurus* in some mountain reservoirs

in spite of its occurrence in their catchment areas, e.g. Lakes Stuesjø, Aur-
sunden and Tisleifjord. But not only are these reservoirs situated below the
local *Lepidurus* limit, but they also contain complex fish populations in-
cluding besides trout one or several of the following species: char, whitefish,
grayling, perch and burbot (*Lota lota* L.).

In Norwegian hydroelectric reservoirs a draining and lowering of the
water level is carried out during the winter, as a result of which wide areas
are laid dry (Table 1). If the drying and freezing of winter eggs alone caused
the expansion of *Lepidurus,* this should take place in all reservoirs with
Lepidurus higher up in the catchment area, if rendered possible by the fish
populations, and not be restricted to the subarctic ones. The existence of
Lepidurus in a multitude of lakes not impounded would also be difficult to
explain. It is likely that the draining also contributes to the distribution of
Lepidurus through its effect on the temperature standard of the water. The
drained bottoms freeze and the drawdown allows the cooling effect of the
ice sheet to be felt below its former limit. The trend is further strengthened
by the circumstance that the winter discharge mainly consists of the warmer
bottom layers. The reservoirs are mostly filled in early spring and the
warming of the bottoms consumes heat from the water. This all contributes
to a reduced temperature budget in winter and spring as compared with a
natural lake. The arctification of the reservoirs is evident from the changed
composition of the chironomid fauna after impoundment (GRIMÅS 1961, 1962).

As indicated by the specific name, the distribution of *Lepidurus arcticus*
depends on the prevalence on arctic conditions. Climatic changes should
influence its distribution, and THIENEMANN (1950) connects the decline of
Lepidurus in the Abisko district with the improved climate. Possibly in dif-
ferent stages of its life cycle it reacts differently to temperature changes.
Facts suggest that at least the late larval and adult stages tolerate and are
even favoured by rather high temperatures. The adult animals gather in
shallow water, which on sunny days may be considerably heated. When
Lepidurus is established in reservoirs situated below its original habitat the
development is accelerated. In Lakes Pålsbufjord and Tunhovdfjord the adult
maximum occurs in July compared with August-September in the arctic lakes
in the upper reaches of the water course. It can hardly be the increasing sum-
mer temperatures that prevent its downstream extension. In southern Norway
Lepidurus does not occur above c. 1,400 metres above sea level, so tempera-
ture may determine its upper limit too. The greatest changes in reservoirs
take place in winter, and the winter egg stage must be affected above all.
The effect of the prolonged and severe winter conditions must be a favour-
able one, since *Lepidurus* reacts by extending its distribution. This suggests
that the winter temperature is the limiting factor of *Lepidurus,* and that the
winter eggs are unable to tolerate higher winter temperature standards than
those which occur in arctic lakes.

IV. Fish management applications

The increased consumption of *Cladocera* by trout in reservoirs results in decreased populations and stunted individual growth. The yield of trout flesh is severly reduced. As is shown by the great populations of char and whitefish in many reservoirs, a plankton feeder by nature would exploit the fish food production better. The superiority of the trout, both for sport and consumption, however makes the introduction of other fish species into trout lakes undesirable if this will lead to a further reduction of the trout population. It should only be done if means of increasing the yield of trout to a satisfactory level cannot be found. The effects of introduction on the fish stocks of the unimpounded lakes in the watercourses must also be taken into consideration. One possibility is a higher utilisation of *Cladocera* by introducing the mainly *Cladocera*-feeding trout living into several lakes in western Norway. Introductory experiments are in progress with the trout of Lake Jølstervatn. This population consumes *Cladocera* most of the year, and it also spawns in the lake itself (KLEMETSEN 1967, JENSEN and SENSTAD 1962). This adds to its importance, as impoundments often destroy the possibilities of spawning in running water.

The supplementation of the *Cladocera* food is another task of current interest. The successful introductions of *Mysis relicta* LOVÉN into Swedish reservoirs (FÜRST 1968) will undoubtly become of the greatest importance to the fish management of Scandinavian boreal and subarctic reservoirs. It remains to be proved that *Mysis* will thrive in arctic reservoirs as well. The fact that *Lepidurus* forms the basis of the trout fishery in many arctic reservoirs and establishes populations in the subarctic and even high boreal reservoirs should also be exploited. The impoundments have made it possible for *Lepidurus* to extend its present range, but in many cases separating valleys prevent its access to the reservoirs. The first experimental introductions of *Lepidurus* were carried out in 1967—68, and further studies on its biology have been undertaken.

V. Summary

1. The food of brown trout in 38 arctic and subarctic reservoirs in southern Norway was investigated.
2. In August—September, the main feeding season of the mountain trout, crustaceans were the predominant food. In subarctic reservoirs the trout turn to semibenthic *Cladocera* as their main food.
3. Introductions into subarctic reservoirs of trout populations mainly feeding on *Cladocera* were started in 1968.
4. The trout living in arctic reservoirs feed to a great extent on *Lepidurus arcticus* PALLAS, which survives annual amplitudes of at least 35 metres.

5. Impoundments create conditions favourable to *Lepidurus*. The species has extended its downstream distribution by invading subarctic and high boreal lakes. Populations already existing in subarctic lakes have flourished after impoundments.
6. Introductions of *Lepidurus* into reservoirs where it has no natural access were started in 1967.

VI. Acknowledgements

VII. References

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Resistance to the Crayfish Plague in some American, Japanese and European Crayfishes

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Introduction

The crayfish plague fungus, *Aphanomyces astaci* SCHIKORA is probably not endemic to Europe where the disease appeared about one hundred years ago in Italy (SELIGO 1895). Neither the disease nor the parasite has been found outside Europe.

The disease apparently causes one hundred per cent mortality in attacked populations of the European crayfish *Astacus astacus* (L.) and no resistant strains of the species have ever, as far as known, developed in the hundred years during which the disease swept through Europe. In Sweden, inspite of protective regulations, the plague has eradicated close on fifty per cent of the crayfish since it arrived from Finland in 1907 (HANSEN & JOBSON 1965, and available information from the Fishery Board of Sweden). The disease has "existed" for 50 years in some Swedish waters with continuous immigration of crayfish into infected areas without giving rise to resistant populations of *Astacus astacus*. Therefore, there is no natural "balance" between the host and the parasite.

It is already known that apart from *Astacus astacus* other European species are also susceptible to the disease (SCHIKORA 1906, 1916) and that *Orconectes limosus,* introduced in Europe from America, is highly resistant (SCHÄPER-CLAUS 1935, SCHIKORA 1916).

The present study was conducted in order to increase our knowledge of the host range in the hope that such information might help to trace the origin of the disease.

Materials and methods

The test animals of *Astacus astacus* (L.) were received from lake Ullvättern, Värmland, Sweden, the specimens of *A. leptodactylus* ESCHSCHOLTZ from an unknown water in Poland and *Austropotamobius pallipes* (LER.) from Ireland. The American species were collected in the following waters:

Cambarus bartoni bartoni (FABRICIUS), Swift Run, Va., and Savannah drainage, Ga.; *C. acuminatus* FAXON, Swift Run, Va., *C. longulus longulus* GIRARD, Swift Run, Va.; *C. latimanus* (LE CONTE), Saluda River drainage, S.C.; an undescribed species closely related to *C. extranius* (HAGEN), Coosa River systeme, Ga.; *Orconectes erichsonianus* (FAXON), Tennessee River drainage, Ala., and Coosa River, drainage, Ga.; *O. virilis* (HAGEN), creek close to Madison, Wisc.; *O. propinquus* (GIRARD), creek close to Madison, Wisc.; *Procambarus clarcii* (GIRARD), Lake Temescal, Oakland Calif.; *P. hayi* FAXON, Tombigbee River drainage, Miss.; *Faxonella clypeta* (HAY) unknown water, Louisiana. *Cambaroides japonicus* (DE HAAN) was received from Okotampe-ko, Hokkaido, Japan.

The plankton crustaceans used and *Asplanchna priodonta* GOSSE were collected from Lake Erken, Uppland, Sweden in the month of June.

The stock crayfish were kept in non-chlorinated tap or pond water at 8—12°C and were fed weekly with carrots and deep frozen fish. The experiments were carried out in 14 l plastic aquaria, each containing 4 l. glass double distilled water. A concentrated parasite zoospore suspension was added and, 4—12 (depending upon the size in each species) middle sized crayfish were placed in each aquarium. Heavy air bubbling from compressed air (air stones) was used to supply oxygen and to make the water circulate. In certain cases some crayfish had to be separated into smaller plastic containers placed in the aquarium water, due to cannibalistic behavior in some species. These containers were well perforated to allow for good water circulation. Two weeks after the start of the experiment the animals were fed weekly with carrots which were again removed if not eaten after one or two days. The experiments were run at 16—18°C for at least 70 days with daily observation. Every experiment was repeated several times, each time with 3—5 specimens per crayfish species. In all experiments *Astacus astacus* was used as a control.

Crayfish dying during the course of the experiments were examined carefully and only if *Aphanomyces* hyphae were found in an animal, *i.e.* in some part of the exoskeleton or in the nervous system, it was considered as infected (cf. SCHÄPERCLAUS 1935, NYBELIN 1936, UNESTAM and WEISS 1969). At the end of the experiment all living crayfish were also examined carefully to reveal possible "unsuccessful" plague attacks.

The plankton animals were tested at 10°C immediately after collection in June (surface water temperature, 15°). The crustaceans were kept in the mixture in one-liter beakers containing the original lake water at 10°C. Zoospores of *Aphanomyces astaci* (30 % swimming, strain J_1) were added to the 10 mm deep water and the animals were daily, for six days, observed briefly in an inverted microscope. On the sixth day all animals were carefully examined to reveal any fungal attack on individual specimens.

Several hundred crustaceans were kept in each beaker containing a great

number (> 50) of each of *Eudiaptomus graciloides, Mesocyclops leuckarti* (copepodids), *Bosmina* (*coregoni?*) and lower numbers (20—50) of *Cyclops strenuus* group (copepodids), *Daphnia hyalina, Leptodora hyalina, Chydorus sphaericus,* and *Bytotrephes longimanus. Asplanchna priodonta* was kept separately, about 10 specimens per container, and were treated like the plankton crustaceans.

Zoospores of *A. astaci* were produced according to a method described elsewhere (UNESTAM 1966, UNESTAM and WEISS 1969).

Results and discussion

Table 1 summarizes results of experiments concerning resistance and susceptibility of the crayfish plague in a number of crustaceans. All the American crayfish species showed very high resistance as compared to the European and the Japanese species. Only occasionally have fungal attacks been established in the animals collected in the USA. Thus, in *Cambaroides longulus* one individual died from the disease in one out of a great number of experiments, where 10^4 spores were used per ml. In *Pacifastacus leniusculus* only a few specimens have been infected out of a several hundred tested, and this only in very high concentrations of spores (UNESTAM and WEISS 1969). No fungal attack was observed on *P. leniusculus* whether under natural conditions in waters with heavy acute plague or in aquaria in the presence of some infected *Astacus astacus* but test specimens of *A. astacus* always had 100 % mortality in the same waters or aquaria. In the few observed attacks in *C. longulus* and *P. leniusculus* (Table 1) subabdominal, intersegmental membranes were heavily penetrated and at least partly stained brown from melanine. Some penetration was also found in one specimen of *O. virilis.*

SCHIKORA (1906, 1916) has earlier reported that *Astacus leptodactylus* as well as *Austropotamobius torrentium* were very susceptible to the plague. BENISCH (1940) reported successful transfer of the disease from *Astacus astacus* (infected in nature) to the wool hand crab, *Eriocheir sinensis.* The attacks were very often lethal to the crabs but the pace of the course of disease was slower than in *A. astacus.*

Using 10^4 spores per ml, strain D_1, in an aquarium containing three crayfish species, all the animals of *Astacus astacus* and *Cambaroides japonicus* died within 11 days with fungal attacks in intersegmental membranes and/or in joints (Table 2). However, only three out of five *A. leptodactylus* died, all showing only very weak fungal attacks with many heavily melanized hyphae (cf. UNESTAM and WEISS 1969). The two remaining ones also showed melanized mycelial attacks when sacrificed after 75 days. In another experiment two out of two available specimens of *Austropotamobius pallipes* were heavily attacked after 6 days under the same conditions. Therefore, this

Table 1. Plague resistance and susceptibility in some crustaceans tested under laboratory conditions. High resistance: infections very seldom or never found. Low resistance: all animals infected. The number of spores added per ml of aquarium water is given.

Crustacean	Collected in	Inoculum	Parasite strain	Resistance	
Crayfish					
Procambarus clarcii	Calif., USA	10^4 sp/ml	D1	high	
P. hayi	Mississ., USA	„ —	D1	high	
Cambarus bartoni ..	Georgia, USA	„ —	D1	high	
C. bartoni	Virginia, USA	„ —	D1, J1	high	
C. sp. (close to extranius)	Georgia, USA	„ —	D1	high	
C. latimanus	S. Carol., USA	„ —	D1	high	Heavy infection in one case with strain D1
C. longulus	Virginia, USA	„ —	D1, J1	high	
C. acuminatus	Virginia, USA	„ —	D1, J1	high	
Orconectes propinquus ..	Wisconsin, USA	„ —	D1, J1	high	
O. erichsonianus	Alabama, USA	„ —	D1	high	
O. virilis	Wisconsin, USA	„ —	D1, J1	high	Penetration in one case with strain D1 SCHIKORA 1916, SCHÄPERCLAUS 1935
O. limosus	Originally from eastern USA	unknown	—	high	
Faxonella clypeta	Louisiana, USA	10^4 sp/ml	D1	high	
Cambaroides japonicus .	Hokkaido, Japan	„ —	D1	low	
Pacifastacus leniusculus .	Calif., USA	10^5 sp/ml	D1	high	{Heavy infection in a few cases, UNESTAM and WEISS 1969
Astacus astacus	Calif., USA	infected *A. astacus*	—	high	
„ —	—	„ —	—	low	{SCHIKORA 1906, NYBELIN 1936, SCHÄPERCLAUS 1935
„ —	Värmland, Sweden	aseptic mycelium	D1	low	NYBELIN 1936
„ —	—	3×10^2 sp/ml	J1, G1, L1	low	
A. leptodactylus	„ Poland	10^4 sp/ml	D1	low	
	?	unknown	—	moderate	SCHIKORA 1906, 1916
Crab					
Eriocheir sinensis	originally from China	infected *A. astacus*	—	moderate	moderate BENISCH 1940
Plancton crustaceans	Uppland, Sweden	10^4 sp/ml	J1	probably high	

Table 2. Infectivity of the plague fungus in three susceptible crayfish species. 10^4 spores (strain D_1) per ml added to the aquarium water.

Crayfish	Dead/Total	Time of death, days after inoculation
Astacus astacus	5/5	5, 6, 6, 7, 7
A. leptodactylus	3/5	14, 15, 17
Cambaroides japonicus..	4/4	8, 8, 9, 11

species should probably also be included among the other, very susceptible European crayfishes.

The plankton crustaceans were tested in the present study because of earlier findings (BARTHELMES 1962a, SCOTT 1958) that in this group attacks by *Aphanomyces* are repeatedly found and also because they sometimes have been considered as possbile vectors or a possible "hiding place" for the crayfish disease after it has eradicated the crayfish population in a lake SCHÄPERCLAUS 1954, SCHIKORA 1906). PROWSE (1954) succeeded in infecting *Daphnia hyalina* by using zoospores from agar grown *Aphanomyces daphniae* but attempts to infect other plankton crustaceans were not successful. *Aphanomyces* attacks have also been observed in the rotatorian species *Asplanchna priodonta* (BARTHELMES 1962 b) and *Hydatina senta* (VALKANOV 1931).

Although a high spore density was used no heavy fungal attack could be seen in any species. Most animals were still alive after 3 days and many after 6 days, both in inoculated and in control beakers. At six days it was found in the inoculated water that *Aphanomyces*-like hyphae had penetrated the bodies of two out of more than 200 *Bosmina* specimens present. But, since both animals were dead a saprophytic attack cannot be excluded. In the controls and in the other species no such phenomena were seen. Nor was *Asplanchna priodonta* attacked, which survived about two days during the test. In drops of zoospore suspension on a glass slide living specimens of the rotifer showed no chemotactical attraction on the swimming spores.

The value of the results with the plankton animals in this study is only limited, however. Although a great number of animals were tested, using several duplicate containers, the length of the experiment was very limited since it was not possible to keep the animals alive for longer periods under the experimental conditions. Nevertheless, the resistance in these plankton species is probably considerable since all control animals of *A. astaci* showed heavy, widely spread attacks after 6 days in the same spore concentration and penetration of the crayfish cuticle is normally seen already after 1 or 2 days (UNESTAM and WEISS 1969). *Aphanomyces daphniae* has been shown to infect and kill *Daphnia* in 8 hours (PROWSE 1954).

The possibility of such plankton crustaceans being carriers of the plague fungus in the absence of crayfish is still valid, however. A population with high resistance is probably more able to house the fungus than one where the host-parasite relationship is permanently out of balance as in *Astacus astacus - A. astaci.* Temporary epidemics, like the one caused by *Leptolegnia baltica* on the plankton crustacean *Eurytemora* (HÖNK and VALLIN 1953) may be explained by suddenly changed environmental conditions or the introduction of new parasite strains but is overcome by the host, e.g. by natural selection. The present results do not suggest any permanent instability like that in *A. astaci,* in the relationship between the used strain of the plague parasite and the tested plankton animals.

A balanced relationship ought to exist in a natural host-parasite system (PERSON 1967) with a restored equilibrium after any temporary advantage of one of the participants. Such a relationship is very likely to exist where the parasite only occasionally kills its host, i.e. as it does in American crayfish.

The findings that European crayfish showed an "abnormally" high susceptibility to the plague parasite, and that a crab from China (only occasionally living in fresh water) as well as a Japanese crayfish, not closely related to the European species, were also very or extremely susceptible, suggests that these animals occupy geographical areas where the plague fungus does not belong. The fungus seems to be adapted to fresh water arthropods and does not survive in sea water (UNESTAM 1969). In America, on the other hand, all tested crayfish show high but not always total resistance, whether they are closely related to the European ones or not. The distribution of high and low resistance in crayfish is, therefore, probably dependant more on geographical barriers than on taxonomical relationships. The present data do not prove that the plague was imported from America to Europe a hundred years ago but this is a possibility suggested by our present knowledge in the field. Work in the future, *e.g..* on crayfish and crustaceans from different parts of the world, may help clarify the matter.

Interestingly, on six occasions where *Pacifastacus leniusculus* were introduced in waters in Finland and Sweden (1960—1967) the original population of *Astacus astacus* disappeared within a year, in three cases without known reason. In the three remaining ones crayfish plague was established in the dying *A. astacus* population. Therefore, upon introduction the American species may have carried the pathogen. Small scale laboratory experiments with mixed populations have not yet confirmed such a possibility, however.

Only weak plague attacks were found in *Astacus leptodactylus* in aquaria while earlier (SCHIKORA 1906, 1916), this species was easily infected. It is

therefore interesting to speculate that resistance in this species might have developed in Poland where the plague first arrived more than 70 years ago. At least, this possibility merits further attention.

Acknowledgements

Summary

Thirteen crayfish species from the USA, three from Europe, and one from Japan were tested with respect to their resistance to the crayfish plague fungus, *Aphanomyces astaci*. As earlier known the European species, *Astacus astacus,* was susceptible while another species from Europe, *A. leptodactylus,* (earlier shown to be very susceptible) showed moderate resistance. The third tested European species is probably also very susceptible. An American species, closely related to *Astacus,* as well as all more distant American relatives were highly resistant. A Japanese crayfish not closely related to *Astacus* was, however, very susceptible. A number of plankton crustaceans were tested but no attacks were observed.

Since the plague fungus is probably not endemic to Europe the results were discussed with respect to their significance to indicate the origin of the plague.

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